Ionic Currents Activated During Hyperpolarization of Single Right Atrial Myocytes From Cat Heart

Jianyi Wu, Johan Vereecke, Edward Carmeliet, and Stephen L. Lipsius

Whole-cell recording techniques were used on single right atrial myocytes to study the ionic currents that may be responsible for the diverse diastolic voltage characteristics of atrial tissue. Ionic currents were activated by hyperpolarizing voltage pulses negative to −30 mV. In general, four different types of cells were identified based primarily on the ionic currents elicited during hyperpolarization. The first cell type exhibited an inward current that decayed with time at more negative voltages, reversed near the potassium equilibrium potential, inwardly rectified at more positive voltages, increased in elevated extracellular potassium, and was blocked by 3 mM barium or 10 mM cesium. This current was identified as the potassium current iK₁. A second cell type exhibited a time-dependent inward current that increased at more negative voltages, had an activation range between −50 and −110 mV, had a reversal potential of −26 mV, and was blocked by 3 mM cesium. This current was identified as an iL current. A third cell type exhibited an inward current that initially decayed and then became more inward with time. Barium (3 mM) abolished the initial inward current and revealed a time-dependent increasing inward current that was blocked by 3 mM cesium. This current was composed of both the iK₁ and iL currents. A fourth cell type exhibited only small time-independent leak currents in response to hyperpolarization. These results indicate that individual cells within the right atrium are electrophysiologically heterogeneous with respect to the types of ionic channels present in their sarcolemmal membranes. This specialization in ionic currents partially explains the diverse diastolic voltage characteristics and functional properties of atrial tissue. (Circulation Research 1991;68:1059–1069)

Atrial tissues exhibit a variety of electrophysiological features that are manifest primarily in their diastolic voltage characteristics. In other words, action potential recordings from atrial tissues can exhibit flat resting membrane potentials, variable degrees of diastolic slope, or diastolic depolarizations large enough to reach threshold and generate pacemaker activity.

Voltage-clamp studies have also recorded a variety of ionic currents from atrial preparations. Thus, studies of single atrial myocytes have found background potassium currents (iK₁) believed to be responsible for the resting membrane potential. Other studies on sheep atrial trabeculae and human atrial appendage have recorded time-dependent inward currents (iL) that may participate in pacemaker function. In addition, reports have indicated that atrial myocytes lack significant time-dependent currents in the negative voltage range. Moreover, studies on human atrial myocytes have found that hyperpolarization elicits a variety of current responses from different atrial cells, although an analysis of the different current systems was not performed. Collectively, these studies suggest that atrial myocytes may be electrophysiologically heterogeneous. However, a systematic investigation of this possibility is lacking.

Therefore, the primary aim of the present work was to voltage-clamp single right atrial cells (excluding the sinoatrial SA node region) to study the ionic currents activated in the negative voltage range, thereby determining whether there is a heterogeneity of ionic currents.
currents in individual atrial myocytes. Our results demonstrate that there are at least four different types of single right atrial cells from cat heart; those that exhibit 1) a relatively pure iKt current, 2) a prominent iKt current but lack a substantial iKr current, 3) both an iKt and iKr current, and 4) time-independent leak currents in response to hyperpolarization.

Materials and Methods

Ten adult cats of either sex weighing 1.8–4.5 kg were anesthetized with sodium pentobarbital (30–50 mg/kg i.p.). Atrial myocytes were isolated by methods modified from Silver et al.,8 Lee,9 and Bechem et al.10 After a midsternal thoracotomy, the heart was quickly removed and mounted on a Langendorff perfusion apparatus. The heart was perfused for 5 minutes with a Tyrode’s solution containing (mM) NaCl 137, KCl 5.4, CaCl2 1.8, MgCl2 1.0, NaHCO3 12, NaH2PO4 0.6, and glucose 11 and saturated with 95% O2–5% CO2 to yield a pH of 7.4. This procedure was followed by a 5-minute perfusion with a nominally Ca2+-free Tyrode’s solution and a final perfusion for 50–60 minutes with a Tyrode’s solution containing 36 μM Ca2+, 0.1% albumin, and 0.06% collagenase (type II, Worthington Biochemical Corp., Freehold, N.J.; 163 units/mg). The enzyme perfusate was recirculated with a pump. During the enzyme perfusion phase, 27 μM Ca2+ was added to the perfusate every 10 minutes to yield a final Ca2+ concentration of approximately 200 μM. Both atria were removed from the heart, and the right atrium was separated from the left by cutting through the septal region of the atria. The right atrium was opened to expose the endocardium of the free wall. In the manner of our previous studies of the electrophysiology and ultrastructure of the cat SA node,11 the SA node region, including a margin of surrounding atrial muscle, was excised. The SA node region was removed by a cut made perpendicular to and through the midportion of the crista terminalis caudal to the SA node region and into the atrial free wall. A second cut was made parallel with the crista terminalis to the rostral margin of the right atrium, separating the SA node region and superior vena cava from the right atrium. To avoid contamination from SA nodal tissue, the remaining right atrial tissue was transferred to another dish and cut into small pieces (≤0.5 cm²). This tissue was incubated in fresh enzyme solution for 15 minutes at 36°C while being agitated in a shaking water bath. In addition to collagenase, the incubation media contained 0.01% protease (type XIV, Sigma Chemical Co., St. Louis). The tissue suspension was filtered through a nylon mesh (210 μm) using Tyrode’s solution containing 200 μM Ca2+ and 0.1% albumin. After the cells settled for about 30 minutes, the solution was suctioned off and gradually replaced with a HEPES-buffered solution containing (mM) NaCl 137, KCl 5.4, MgCl2 1.0, CaCl2 1.8, HEPES 10, glucose 11, and 0.1% albumin and titrated with NaOH to pH 7.4. Cells were stored in this solution at room temperature until use.

Cells used for recordings were transferred to a small tissue bath (0.5 ml) mounted on the stage of an inverted microscope (Nikon Diaphot, Nikon, Garden City, N.Y.) and superfused at 35±0.5°C with a HEPES-buffered solution containing (mM) NaCl 137, KCl 5.4, MgCl2 1.0, CaCl2 1.8, HEPES 10, and glucose 11 and titrated with NaOH to pH 7.4. Action potentials and ionic currents were recorded in the whole-cell configuration with suction pipettes as described by Hamill et al.12 Only cells that appeared elongated, relaxed, and with sharp borders were used for recordings. Some cells studied exhibited rhythmic spontaneous activity associated with discrete twitches, which was characteristic of normal pacemaker function. Cells described as spontaneously active were observed to be so both before and during the recordings. Pipettes had inner diameters of 1.5–2.0 μm and when filled with internal solution had resistances of 2–3 MΩ. The pipette internal solution contained (mM) potassium glutamate 130, MgCl2 1.0, HEPES 5, EGTA 2, K2ATP 5, and NaCl 7 and was titrated with KOH to pH 7.2. With the pipette tip in the control superfusate the reference potential was adjusted to zero, and the pipette capacitance was neutralized in the constant-current mode. All records were corrected for electrode tip junction potentials (about 10 mV). Once the suction pipette made a gigohm seal (3–5 GΩ) with the cell, the membrane was ruptured by applying additional suction.

Resting cell input resistance (Rin) and membrane capacitance (Cm) were measured in every cell studied. Rin was measured by delivering a small hyperpolarizing current pulse (−0.01 nA) through the pipette and dividing the resulting change in resting membrane potential by the current amplitude. Cm was measured by delivering a ramp voltage pulse (dV/dt=4 V/sec) and dividing the half-amplitude of current jump at the turning point of the ramp pulse by the slope of the ramp.13 Resting and action potential recordings were performed first and were followed within 5 minutes by voltage-clamp protocols. Once a cell exhibiting iKt current was identified under control conditions, the iKr current was analyzed by changing to an external solution containing 3 mM nickel (Sigma) to block interference from slow inward calcium currents and sodium/calcium exchange.13 2 mM barium (Sigma) to block potassium currents,14,15 and 2 mM 4-aminopyridine (Sigma) to block transient outward currents.7,16 Data for the voltage dependence of iKt activation were fitted by a curve calculated using the Boltzmann function

\[ I_t/I_{max} = \frac{1 + \exp \left[ (V_t - V_{0.5})/k \right]}{1} \]

where I_t is the peak tail current, I_{max} the maximum tail current, V_t the hyperpolarizing test voltage pulse, V_{0.5} the half-maximum voltage, and k the slope factor. Peak and steady-state iKt currents were measured in reference to zero current. The iKt tail current amplitudes were measured in reference to the steady-state...
current achieved at each voltage level tested. Background leak currents were not subtracted.

An Axoclamp-2A (Axon Instruments, Inc., Foster City, Calif.) amplifier was used to record resting and action potentials (bridge mode) and ionic currents (discontinuous single electrode voltage clamp). In the voltage-clamp mode, the amplifier sample rate was 8–10 kHz. Computer software (PCLAMP program; Axon Instruments, Inc.) was used to generate voltage-clamp protocols as well as to acquire and analyze voltage and current signals. Currents were sampled at 4 kHz by a 12-bit resolution A/D converter (Tecmar Labmaster, Tecmar Inc., Cleveland, Ohio) with the use of a Compaq 286 computer. Data were stored on hard disk and videotape for later analysis. Signals were filtered at 0.3–1.0 kHz. Voltage-clamp protocols were applied at >5-second intervals.

Data are presented as mean ± SEM. Values among groups (Table 1) were analyzed for significance by use of a one-way analysis of variance for independent events and Student-Newman-Keuls test. Spontaneous cycle lengths were compared using Student's t test. Data with a value of p < 0.05 were considered statistically significant.

Results

Figure 1 shows action potentials (upper traces) and the corresponding ionic currents (lower traces) recorded from a single atrial myocyte. The cell was initially quiescent and had a resting membrane potential of −66 mV. Electrical stimulation at 1 Hz elicited action potentials, with an overshoot potential of 21 mV and a flat diastolic potential. After the voltage recordings, the cell was clamped to a holding potential of −30 mV. Hyperpolarization to −50 mV elicited a small net inward current that remained constant during the clamping. Steps to more negative voltages elicited larger instantaneous inward currents that decayed with time. To determine if this current corresponded to the potassium current iK1, cells exhibiting this current were exposed to 3 mM barium (Ba2+). Figure 2A shows control inward currents elicited by 2-second hyperpolarizing steps from a holding potential of −30 mV. As described above, voltage steps less negative than −90 mV elicited inward currents that remained constant during the clamp, whereas voltage steps to −90 mV or more negative elicited larger inward currents that decayed with time. At −100 mV, the decay of current was best fit by a biexponential, an initial fast component (τ1 = 1.5 msec) followed by a secondary slow component (τ2 = 353 msec). The return of the voltage to the holding potential (−30 mV) resulted in a large and
fast-decaying inward current, which is probably an uncontrolled fast sodium current. Panel B shows that in the presence of 3 mM Ba\(^{2+}\) the holding current became more inward, and the same hyperpolarizing clamp steps elicited only small time-independent inward currents. Panel C shows the Ba\(^{2+}\)-sensitive currents obtained by subtracting the currents recorded in the presence of Ba\(^{2+}\) (panel B) from control (panel A). Note that the Ba\(^{2+}\)-sensitive current does not contain the large inward current elicited upon return to the holding potential. Panel D shows the peak inward (○) and steady-state (●) Ba\(^{2+}\)-sensitive currents plotted as a function of voltage. The peak inward current increased almost linearly, whereas the steady-state current became less inward at more negative voltages. In addition, the reversal potential was approximately −80 mV, and the current exhibited inward rectification between −90 and −50 mV. Similar results were obtained in a total of 12 cells. In five additional cells, 10 mM Cs\(^{+}\) instead of Ba\(^{2+}\) blocked this current. Figure 3 shows results obtained from another cell in which the steady-state current-voltage (I-V) relation of the Ba\(^{2+}\)-sensitive current was determined in 5.4 and 10.8 mM extracellular potassium. Raising potassium shifted the reversal potential from −84 to −63 mV and increased both outward and inward current amplitudes, resulting in crossover of the I-V relations. The increase in the inward current was more pronounced than the change in the outward current. Similar results were found in a total of seven cells. These findings support the conclusion that this current is similar to the current reported in ventricular,\(^1\)−\(^19\) Purkinje,\(^20\)−\(^21\) and human atrial cells,\(^3\) which is believed to be responsible for the resting membrane potential. As shown in Table 1, the iK\(_{\text{s}}\) current was found in 37 of the 62 cells studied (60%), all 37 of which were quiescent. In addition, these cells had a mean Rin of 1.0 ± 0.1 GΩ and Cm of 87.9 ± 5.3 pF (n = 37).

Figure 4 shows action potentials and the corresponding ionic currents recorded from a second type of atrial cell. This cell exhibited rhythmic, spontaneous activity at a cycle length of 1,050 msec. Action potentials had a pacemaker voltage range between −70 and −55 mV and an overshoot potential of 27 mV. The diastolic depolarization was biphasic, an initial steeper slope followed by a more gradual slope. After these voltage recordings, the cell was clamped at a holding potential of −30 mV. Hyperpolarizing voltage steps elicited a time-dependent net inward current that increased in amplitude and activated more rapidly at more negative voltages. At −100 mV, the current activated with a single exponential time constant of 156 msec. To determine whether this current corresponded to the i\(_{\text{f}}\) current reported in the SA node\(^22\)−\(^29\) and Purkinje fibers,\(^30\)−\(^32\) a series of experiments was performed to characterize its properties. Figure 5A shows a typical experiment in which the voltage activation range of this current was determined. From a holding potential of −30 mV, the cell was hyperpolarized to test voltages between −40 and −110 mV for 3 seconds and then clamped to 20 mV for 900 msec. The lower portion of panel A shows that hyperpolarizing steps elicited

Table 1. Characteristics of Different Atrial Cell Types

<table>
<thead>
<tr>
<th>Cell type</th>
<th>iK(_{\text{s}})</th>
<th>i(_{\text{f}})</th>
<th>iK(<em>{\text{s}})+i(</em>{\text{f}})</th>
<th>iL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell diameter (μm)</td>
<td>15.3±0.6*</td>
<td>6.4±1.2†</td>
<td>15.0±1.6*</td>
<td>9.1±1.5†</td>
</tr>
<tr>
<td>Cell length (μm)</td>
<td>161±6.8*</td>
<td>88±8.5†</td>
<td>141±24.0‡</td>
<td>110±13.3§</td>
</tr>
<tr>
<td>Total capacitance (pF)</td>
<td>87.9±5.3*</td>
<td>36.4±5.2†</td>
<td>86.1±19.4*</td>
<td>39.0±5.4†</td>
</tr>
<tr>
<td>Total input resistance (GΩ)</td>
<td>1.0±0.1*</td>
<td>1.9±0.2</td>
<td></td>
<td>1.1±0.1È</td>
</tr>
<tr>
<td>Spontaneous cycle length (msec)</td>
<td>...</td>
<td>726±53</td>
<td>...</td>
<td>1,166±146‡</td>
</tr>
<tr>
<td>Occurrence (%)</td>
<td>59.6</td>
<td>12.9</td>
<td>8.1</td>
<td>19.4</td>
</tr>
<tr>
<td>Number of cells (total, 62)</td>
<td>37</td>
<td>8</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

Cell diameters and cell lengths measured for iK\(_{\text{s}}\) and iL cell types are based on 30 and 10 cells, respectively. iK\(_{\text{s}}\), background potassium current; i\(_{\text{f}}\), time-dependent inward current; iL, time-independent leak current.

*Significantly different from i\(_{\text{f}}\) and iL cell types.
†Significantly different from iK\(_{\text{s}}\) and iK\(_{\text{s}}\)+i\(_{\text{f}}\) cell types.
‡Significantly different from i\(_{\text{f}}\) cell type only.
§Significantly different from iK\(_{\text{s}}\) cell type only.
ÈSignificantly different from all other cell types.
characteristic time-dependent inward currents that increased at more negative voltages. Clamp steps to 20 mV elicited outward tail currents that reached peak amplitude after test pulses to -110 mV. Peak outward tail currents were normalized in relation to maximum tail current amplitude and plotted against the imposed hyperpolarizing test voltages. Panel B shows the voltage activation range determined from four cells. The current is activated at voltages more negative than -50 mV and reaches maximum activation at -110 mV. Calculations with the Boltzmann function (see "Materials and Methods") estimated the half-maximum activation voltage and slope at $-83.1 \pm 1.6$ mV and $7.0 \pm 0.2$, respectively. Panel C shows that exposure to 3 mM cesium completely blocked the currents activated during hyperpolarization and most of the tail current amplitude elicited at 20 mV. Similar results were obtained in a total of six cells. In Figure 6, the fully activated I-V relation of this current was determined by the method of DiFrancesco. From a holding potential of -30 mV, clamp steps were imposed to voltages at which the current was either fully activated (-110 mV) or fully inactivated (0 mV) for 1.5 seconds. From these levels, the membrane was clamped to identical voltages ranging from 20 to -100 mV. To determine the fully activated I-V relation, the peak tail current amplitudes obtained at any given voltage after a prepulse to -110 and 0 mV were subtracted from each other, and the difference current was plotted against the imposed voltage. Panel A shows selected traces of ionic currents obtained during clamp steps to 0 mV (fully inactivated) and -110 mV (fully activated), and the tail currents obtained by clamp steps to 0 mV and -100 mV. Panel B shows that the fully activated I-V relation is essentially linear between -100 and 20 mV, and the current reverses at approximately -27 mV. In five cells, the mean reversal potential was $-26 \pm 1.2$ mV. These findings are consistent with the properties of the $i_{r}$ current found in other cardiac pacemaker tissues. As shown in Table 1, $i_{r}$ currents were found in a total of eight of 62 cells studied (12.9%), all of which exhibited sponta-

**Figure 4.** Action potentials (top) and membrane currents (bottom) recorded from a single atrial myocyte. Action potentials are spontaneously active. Membrane currents were elicited by hyperpolarizing clamp steps between -40 and -100 mV from a holding potential of -30 mV (cell W0427C11).

**Figure 5.** Voltage protocol and membrane currents used to determine the activation voltage range of $i_{r}$ current. Panel A: The cell was hyperpolarized between -40 and -110 mV for 3 seconds from a holding potential of -30 mV and then clamped to -20 mV. The dashed arrows (<-->) point to corresponding currents recorded during clamp to -110 mV and then +20 mV. The external solution contained 3 mM nickel, 2 mM barium, and 2 mM 4-aminopyridine. Panel B: Normalized tail currents are plotted against the voltage imposed during hyperpolarization (n=4). The solid line was determined by calculations with the Boltzmann function (see "Materials and Methods"). Panel C: Effect of 3 mM cesium on membrane currents elicited by hyperpolarization between -40 and -110 mV from a holding potential of -30 mV (cell W0514C10).
neous activity. Mean spontaneous cycle length was 726±53 ms (n=6), Rin was 1.9±0.2 GΩ, and Cm was 36.4±5.2 pF (n=8).

Figure 7 illustrates a third type of cell that exhibited action potentials and ionic currents different from either of the cell types described above. Action potentials were elicited by electrical stimulation at 0.75 Hz and exhibited a maximum diastolic potential of −76 mV and an overshoot potential of 23 mV. The maximum diastolic potential was more negative than the takeoff potential, and there was a gradual diastolic slope. Following the voltage recordings, the membrane potential was held at −30 mV and clamped for 2 seconds to more negative voltages. Hyperpolarizing steps to voltages less negative than −90 mV elicited net inward currents that were time independent. Clamping to −90 mV elicited a relatively large inward current that showed a small, initial time-dependent decay. At −110 mV the instantaneous inward current was increased, and the initial decay of inward current was followed by a secondary increase in inward current. This type of response was more evident at −140 mV, where there was a large initial decay of inward current followed by a large secondary increase in inward current. This current appeared to result from activation of both an iK1 and iK current in the same cell. This was tested in the experiment shown in Figure 8, where Ba²⁺ and Cs⁺ were used to separate the two currents. Panel A shows selected control recordings similar to those in Figure 7 (i.e., 2-second hyperpolarizing steps to voltages more negative than −80 mV elicited a relatively large instantaneous inward current that initially decayed and then became more inward again). At the end of each hyperpolarizing clamp, returning to the holding potential (−30 mV) resulted in a large, uncontrolled, transient inward current. Panel B shows that 3 mM Ba²⁺ shifted the holding current slightly more inward. In addition, at each potential, Ba²⁺ essentially eliminated the instantaneous inward current and decreased the steady-state current elicited during hyperpolarization. As a result, Ba²⁺ revealed a time-dependent inward current that increased in amplitude and rate of activation at more

**Figure 6.** Determination of fully activated current–voltage relation for iᵢ current (see “Materials and Methods”). Panel A: Selected membrane currents elicited from a holding potential of −30 mV to 0 mV (fully inactivated) and −100 mV (fully activated) followed by clamp steps to 0 and −100 mV. The difference between the peak tail currents (arrows) was plotted against the voltage at which the tail current was elicited. Panel B: Fully activated iᵢ current–voltage relation was linear between +20 and −110 mV and reversed at −27 mV (cell W0514C10).

**Figure 7.** Action potentials (top) and membrane currents (bottom) recorded from a single atrial myocyte. Action potentials were elicited by external stimulation at 0.75 Hz. Membrane currents were elicited by hyperpolarizing voltage steps between −40 and −140 mV from a holding potential of −30 mV (cell W0425C02).
negative voltages. This current appeared very similar to the Cs⁺-sensitive current described above. In panel C, addition of 3 mM Cs⁺ to the Ba²⁺-containing solution abolished the time-dependent inward current. To determine the Ba²⁺-sensitive current, the currents that remained in Ba²⁺ (panel B) were subtracted from control records (panel A). The records in panel D are the difference (Ba²⁺-sensitive currents). These currents are similar to those described above as iK₁ current. They are relatively large inward currents that partially decay with time and decay more at increasingly negative voltages. To determine the Cs⁺-sensitive current, the records obtained in the presence of Ba²⁺ and Cs⁺ (panel C) were subtracted from the records obtained in the presence of Ba²⁺ alone (panel B). The Cs⁺-sensitive currents (panel E) are similar to the iK₁ current described above. This current was an increasing inward current that became larger in amplitude at more negative voltages. Panel F shows the steady-state I-V relation for the total current (●), the Ba²⁺-sensitive current (○), and the Cs⁺-sensitive current (▼). Although both the Ba²⁺-sensitive and Cs⁺-sensitive currents increased at more negative voltages, the Ba²⁺-sensitive current rectified at more negative voltages and began to show a negative slope. Similar results were found in a total of four cells. These results indicate that an iK₁ current and an iK₁ current were both present in this cell type. As shown in Table 1, this mixed current was found in five of 62 cells (8.1%) that had a mean Rin of 1.1±0.1 GΩ and Cm of 86.1±19.4 pF (n=5). Four cells were quiescent, and only one cell (shown in Figure 7) exhibited intermittent spontaneous activity.

Figure 9 shows action potentials and ionic currents recorded from a fourth type of atrial cell. Two separate experiments are illustrated in panels A and B. In panel A the cell was spontaneously active, and in panel B the cell was quiescent until stimulated. In panel A, the spontaneous action potentials had a pacemaker voltage range between −66 and −43 mV.
an overshoot potential of 32 mV, and a spontaneous cycle length of 1,450 msec. Diastolic depolarization was biphasic. In panel B, external stimulation at 0.75 Hz elicited action potentials, with a maximum diastolic potential of −68 mV and an overshoot potential of 22 mV. In addition, the maximum diastolic potential was 13 mV more negative than the resting diastolic voltage (−55 mV), resulting in afterhyperpolarization. After the voltage recordings, each cell in panels A and B was clamped to a holding potential of −50 mV. In both experiments, hyperpolarizing and depolarizing pulses were delivered for 2 seconds between 30 and −120 mV. In both cells, hyperpolarization elicited only small time-independent inward leak currents. Because time-independent currents alone cannot readily account for the diastolic voltage responses recorded from these cells, it seemed likely that other currents activated in the positive voltage range may have an influence. An obvious candidate was the iK current. It is apparent that in both cells depolarizing pulses elicited relatively large time-dependent outward currents that increased at more positive voltages. At 30 mV, the time course of outward current activation was best fit as a biexponential process ($\tau_1=114$ msec and $\tau_2=1,062$ msec, cell in panel B). Clamping the membrane potential back to the holding potential elicited outward tail currents that decayed with time. Figures 10A and 10B show the steady-state I-V relations of the spontaneously active cell in Figure 9A and the quiescent cell in Figure 9B, respectively. In Figure 10A, the I-V relation shows net outward currents at voltages more positive than −30 mV that increased linearly at more positive voltages. Small net inward currents were measured at voltages more negative than −30 mV. In panel B, increasing outward currents were activated at voltages more positive than −30 mV and increased linearly at more positive voltages, whereas only small currents were recorded between −30 and −120 mV. The current was net inward at voltages more negative than −80 mV. Panel C shows currents recorded from the cell in Figure 9B after the outward current had decreased slightly. These records show more clearly the tail currents elicited by repolarization after iK activation. The cell was held at −50 mV and clamped to 30 mV for 500 msec or 1.8 seconds and then repolarized to −40, −60, −80, and −100 mV. The increasing outward currents elicited during depolarization are superimposed. Repolarization elicited outward tail currents at −40 and −60 mV that decayed as a single exponential and reversed at −80 mV (<--->). After a 500-msec depolarizing clamp, the time constant of current decay was 67 msec at −60 mV. Tail currents elicited after 500-msec clamps were slightly smaller and decayed slightly slower than those elicited after 1.8-second clamps. In panel D, peak tail currents elicited after the 1.8-second depolarizing clamps were plotted against the voltage imposed during repolarization between −40 and −110 mV. The I-V relation was linear over the voltage range tested, and the reversal potential was −80 mV. These findings are consistent with the iK current described in other cardiac preparations.23,27,34–38 As shown in Table 1, a total of 12 of 62 cells (19.4%) studied exhibited only small time-independent leak currents in response to hyperpolarization. These cells had a mean Rin of 1.5±0.1 GΩ and Cm of 39.0±5.4 pF (n=12). All 12 cells showed iK currents in response to depolarization. Nine of the 12 cells exhibited spontaneous pacemaker activity, and three other cells exhibited afterhyperpolarizations when stimulated. Mean pacemaker cycle length was 1,167±146 msec (n=6), which is significantly longer than the pacemaker cycle length of cell types exhibiting predominantly iK current (p<0.05).
We should mention that iK currents were also found to some extent in virtually every atrial cell studied. Therefore, it is likely that iK contributes to some extent to the diastolic voltage characteristics in all atrial cells. However, the main focus of the present work was to demonstrate the heterogeneity of ionic currents in atrial myocytes rather than to conduct a complete analysis of the ionic currents that contribute to the diastolic potentials. The presence of iK was demonstrated in the fourth cell type because its contribution may be more important in cells with no other time-dependent currents in the negative voltage range, and its presence helps account for the diastolic voltage responses recorded from these cells.

**Discussion**

The purpose of the present study was to determine whether cat atrial myocytes are electrophysiologically heterogeneous in the negative voltage range in an effort to better understand the mechanisms responsible for the diverse diastolic voltage characteristics of atrial tissue. These experiments focused on right atrial cells outside of the SA node region. The present results indicate that in terms of ionic currents there are at least four different types of single atrial myocytes. Experiments were performed on each cell type to characterize each current system.

The majority of cells studied responded to hyperpolarization by activation of an inward current that was blocked by 3 mM barium or 10 mM cesium, exhibited a reversal potential consistent with the potassium equilibrium potential, inwardly rectified at more positive voltages, and exhibited a crossover of the I-V relation in high potassium. In addition, the current displayed a time-dependent decay that became greater at more negative voltages. At voltages more negative than about −120 mV, the current began to show a negative slope conductance. These findings are consistent with iK^+ currents described in ventricular, Purkinje, and human atrial cells. However, work on guinea pig and rabbit atrial cells has found that background currents are present, although they show no time-dependent decay. Other studies on rabbit atrial cells from the crista terminalis have found little if any background current in the negative voltage range. The present findings also show that cells exhibiting predominantly iK^+ currents were always quiescent, generated action potentials with flat diastolic potentials, and exhibited a relatively low input resistance. This is consistent with the presence of a relatively large potassium conductance holding the resting membrane potential constant. From their functional characteristics, relatively large size, and high frequency of occurrence (60%), we conclude that this cell type is a typical working atrial muscle cell.

A second cell type responded to hyperpolarization with a time-dependent increasing inward current that had an activation range between −50 and −110 mV, a reversal potential of about −26 mV, and was blocked by relatively low concentrations of cesium. These properties are similar to those of the i_d pacemaker current reported in the SA node, Purkinje fibers, and the sinus node. In addition, this cell type exhibited little background potassium current, resulting in a relatively high input resistance. These findings are similar to other cardiac pacemaker myocytes, which almost completely lack the inwardly rectifying potassium current. In addition, cells that exhibited predominantly i_d current also showed pacemaker action potentials and spontaneous cycle length similar to those recorded from multicellular tissues exhibiting subsidiary atrial pacemaker activity. Moreover, the activation range of the i_d current was within the pacemaker voltage range of these cells. Therefore, we conclude that these atrial myocytes function as subsidiary pacemakers and that the i_d current contributes to their pacemaker function. This finding agrees with those in multicellular preparations isolated from cat right atria that cesium inhibits subsidiary pacemaker function.

In addition to cells that exhibited either a relatively pure iK^+ or a relatively pure i_d current, evidence was presented for a third cell type that responded to hyperpolarization with activation of both iK^+ and i_d currents. This was determined by finding that barium blocked the instantaneous inward current component and revealed a time-dependent increasing inward current that was in turn blocked by cesium. In addition, the Ba^2+-sensitive current showed a negative slope region at more negative potentials. The simultaneous activation of a decreasing inward current and an increasing inward current would account for the relatively flat total currents recorded during hyperpolarization under control conditions. A similar mixed-current response was recorded from human atrial myocytes, although the two current components were not analyzed. Action potentials recorded from these cells showed only very gradual diastolic slopes and input resistances comparable to those cell types exhibiting predominantly iK^+ current. These findings suggest that iK^+ and not i_d is the predominant current influencing the diastolic membrane potential. This cell type was the least common (8%) of the four cell types found. One can only speculate on its functional importance. It may be a transitional cell that is found near true pacemaker cells. Perhaps conditions that reduce iK^+ and/or enhance the i_d current may stimulate these cells to participate in pacemaker function.

Finally, in a fourth cell type hyperpolarization elicited only small time-independent inward leak currents. These cells apparently lack both iK^+ and i_d current channels, which accounts for their relatively high input resistances. However, action potentials recorded from these cells showed diastolic potential changes that resulted in either spontaneous pacemaker activity (nine cells) or the appearance of prominent afterhyperpolarizations (three cells). In an effort to account for these observations, we investigated the possible contribution of iK current. De-
activation of iK current has been proposed as an important component of primary pacemaker function. 23,27,36-38 Although iK current was found to some extent in every atrial cell studied, iK may be more important in this particular cell type because of their lack of other time-dependent currents in the negative voltage range. In the present study, depolarizing voltage pulses elicited prominent time-dependent outward currents that were deactivated by repolarization. Repolarization into the diastolic voltage range elicited outward tail currents that decayed with a relatively short time constant (60–100 msec). Because of the small slope conductance of these cells, relatively small changes in net current flow could exert a prominent influence on the diastolic membrane potential. Thus, the spontaneous activity recorded from these cells can be explained to a large extent by a decaying iK tail current associated with a small inward leak current (see Figure 10A) and coupled with a relatively low background potassium conductance. This cell type represents a second type of atrial subsidiary pacemaker. Cells isolated from the rabbit crista terminalis and bullfrog sinus venosus37,38 also show a lack of time-dependent current in the negative voltage range and exhibit pacemaker activity. However, unlike the fourth cell type reported here, these cells are significantly larger in size and total membrane capacitance.

Of the 62 cells studied, 44 (71%) were quiescent. Forty-one of these 44 cells (93%) belonged to two cell types, those exhibiting predominantly iK, current (37 cells, 84%) and those exhibiting both iK, and i, currents (four cells, 9%). Both cell types have several features in common. They are essentially the same in size, total capacitance, and input resistance. Perhaps the most important functional similarity is the presence of a relatively large iK, conductance. This feature is probably responsible for their function as nonpacemaker cells and their relatively low input resistance.

On the other hand, 18 of the 62 cells studied (29%) exhibited spontaneous pacemaker activity. Seventeen of these 18 cells (94%) belonged to the two other cell types, those exhibiting predominantly i, current (eight cells, 44%) and those exhibiting only time-independent leak currents (nine cells, 50%). Clearly, cells exhibiting the i, current displayed the most consistent pacemaker activity and the shortest spontaneous cycle lengths. This finding reinforces the idea that the i, current contributes significantly to this type of subsidiary pacemaker function. The second most automatic group of cells were those exhibiting no time-dependent currents in response to hyperpolarization. These findings suggest that at least two different types of subsidiary pacemakers may exist within the right atrium. Because both pacemaker cell types lack a significant background potassium current, one primary difference between them is the presence of i, current. Therefore, there may be one type of atrial pacemaker that is more sensitive to cesium than another. As mentioned above, a common feature of both pacemaker cell types is the lack of any significant background potassium conductance (iK,), which is reflected in their relatively high input resistances. Evidently, the absence of iK, is a primary determinant of atrial pacemaker function. In addition, both pacemaker cell types were similar to one another in cell size (<10-μm diameter) and total membrane capacitance (<40 pF), and these findings are similar to those reported for SA node pacemaker cells.29,43 Although both pacemaker cell types were similar in size, those exhibiting predominantly i, currents exhibited a more tortuous appearance with less apparent myofibrillar material, which is similar to pacemaker cells isolated from rabbit SA node.45 This observation is consistent with morphological studies showing that cells believed responsible for SA node pacemaker activity (i.e., P cells) are also present in specific extranodal regions of the right atrium.11,44 In fact, the i, pacemaker cell types reported here have now been isolated specifically from the eustachian ridge (close to the opening of the inferior vena cava) of the cat right atrium.45

These studies indicate that cells exhibiting predominantly iK, currents are far the most commonly found (60%). This finding seems reasonable because most cells are expected to be nonpacemaker working atrial muscle. Cells exhibiting little iK, current and displaying some form of pacemaker activity seem to be the second most common (32%), and cells exhibiting a combination of iK, and i, currents were the least common (8%). Although the frequency of occurrence of each cell type was quantitated, there are reasons to consider these values cautiously. First, the population sample was extremely small in relation to the total population of right atrial cells. In addition, although an attempt was made to randomly select the cells studied, it became apparent that one could predict that a rhythmically active cell would exhibit one of the two types of pacemaker current systems.

The present experiments demonstrate that individual atrial myocytes are heterogeneous in size, passive membrane properties, and the types of ionic currents present in their sarcolemmal membranes. These specializations may partially account for the diversity in diastolic voltage characteristics and the functional properties exhibited by atrial tissues.

References

1. Hume JR, Uehara A: Ionic basis of the different action potential configurations of single guinea-pig atrial and ventricular myocytes. J Physiol (Lond) 1985;368:525–544
25. Shibata EF, Giles WR: Ionic currents that generate the spontaneous diastolic depolarization in individual cardiac pacemaker cells. Proc Natl Acad Sci USA 1985;82:7796–7800

Key Words: voltage-clamp • pacemaker current • subsidiary pacemakers • barium • potassium current • cesium
Ionic currents activated during hyperpolarization of single right atrial myocytes from cat heart.
J Y Wu, J Vereecke, E Carmeliet and S L Lipsius

doi: 10.1161/01.RES.68.4.1059

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/68/4/1059

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/