Sustained Depolarization-Induced Outward Current in Human Atrial Myocytes

Evidence for a Novel Delayed Rectifier K⁺ Current Similar to Kv1.5 Cloned Channel Currents

Zhiguo Wang, Bernard Fermini, Stanley Nattel

Depolarization of human atrial myocytes activates a transient outward current that rapidly inactivates, leaving a sustained outward current after continued depolarization. To evaluate the ionic mechanism underlying this sustained current (I_{sust}), we applied whole-cell voltage-clamp techniques to single myocytes isolated from right atrial specimens obtained from patients undergoing coronary bypass surgery. The magnitude of I_{sust} was constant for up to 10 seconds at +30 mV and was unaffected by 40 mmol/L tetraethylammonium, 100 mmol/L dendrotoxin, 1 mmol/L Ba²⁺, 0.1 μmol/L atropine, or removal of Cl⁻ in the superfusate. I_{sust} could be distinguished from the 4-aminopyridine (4AP)-sensitive transient outward current (I_{tau}) by differences in voltage-dependent inactivation (1000-millisecond prepulse to −20 mV reduced I_{sust} by 91.7±0.1% [mean±SEM], P<.001, versus 9.4±4% reduction of I_{tau}) and 4AP sensitivity (IC₅₀ for block of I_{sust}, 1.96 mmol/L; for I_{tau}, 49 μmol/L). I_{sust} activation had a voltage threshold near −30 mV, a half-activation voltage of −4.3 mV, and a slope factor of 8.0 mV. I_{sust} was not inactivated by 1000-millisecond prepulses but was reduced by 16±8% (P<.05) at a holding potential of −20 mV relative to values at a holding potential of −80 mV. I_{sust} activated very rapidly, with time constants (τ) at 25°C ranging from 18.2±1.8 to 2.1±0.2 milliseconds at −10 to +50 mV, two orders of magnitude faster than previously described kinetics of the rapid component of the delayed rectifier K⁺ current. At 16°C, I_{sust} activation was greatly slowed (τ at +10 mV, 46.7±4.1 milliseconds; τ at 25°C, 7.1±0.8 milliseconds; P<.01), and the envelope of tails test was satisfied. The reversal potential of I_{sust} tail currents changed linearly with log [K⁺]ᵣ (slope, 55.3±2.9 mV per decade), and the fully activated current-voltage relation showed substantial outward rectification. Selective inhibition of I_{sust} with 50 μmol/L 4AP increased human atrial action potential duration by 66±11% (P<.01). In conclusion, I_{sust} in human atrial myocytes is due to a very rapidly activating delayed rectifier K⁺ current, which shows limited slow inactivation, is insensitive to tetraethylammonium, Ba²⁺, and dendrotoxin, and is highly sensitive to 4AP. These properties resemble the characteristics of channels encoded by the Kv1.5 group of cardiac cDNAs and may represent a physiologically significant manifestation of such channels in human atrium. (Circ Res. 1993;73:1061-1076.)

KEY WORDS • K⁺ currents • repolarization • arrhythmias, cardiac • electrocardiography • action potential

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Action potential duration is a major determinant of the refractory period in cardiac tissue.¹,² The likelihood of reentrant cardiac arrhythmias is greatly influenced by the properties of tissue refractoriness,¹,² and action potential duration is therefore a major determinant of a number of clinically important arrhythmias. The duration of the cardiac action potential is governed by the balance between a variety of inward and outward currents that flow during the depolarized, and particularly the plateau, phase of the action potential.

Several outward K⁺ currents have been identified in human atrial cardiomyocytes. The transient outward current (I_{sust}) is prominent in human atrial myocytes.³⁻⁵ I_{sust} has two components, a longer-lasting one, which is sensitive to 4-aminopyridine (4AP), and a briefer Ca²⁺-dependent component, which is blocked by caffeine or Co²⁺.³ These components have been identified in other systems and are commonly referred to as I_{hol} and I_{hol'2}, respectively.⁶ Both I_{hol} and I_{hol'}2 inactive rapidly,³,⁶ and both are likely to be fully inactivated before the onset of phase 3 repolarization at physiological temperatures in human atrial cells, which have an action potential duration at 37°C in the order of 300 milliseconds.⁷ The inward rectifier current (I_{ki}) and acetylcholine-activated current (I_{KAC}) are present in human atrium, but both show strong inward rectification,⁸,⁹ and basal I_{KAC} activity (in the absence of muscarinic agonists) is small.⁹ When intracellular ATP is depleted, a substantial ATP-sensitive current (I_{KATP}) is recorded; however, in the absence of ATP depletion, the open probability of I_{KATP} is low. The classic type of delayed rectifier current (I_{dr}), as originally described by Noble and Tsien,¹⁰ has been

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considered to be minor or absent in human atrial cells. We have recently found, however, that typical delayed rectifier currents, with activation time constants in the range of 100 to 350 milliseconds at 37°C, can be recorded in the majority of human atrial cells.11

Several lines of evidence point toward the existence of another, potentially important, repolarizing current in human atrium. Depolarizing pulses positive to +20 mV are always associated with an instantaneous outward current “jump,” even in cells that lack I_{in}.11 After the inactivation of I_{in}, a residual outward current is seen,3-5,11 even in cells lacking I_{in}.11 Similar phenomena are observed in rabbit atrial myocytes, in which they are due to a background Cl⁻ current.12 The purpose of the present experiments was to characterize the properties of the sustained depolarization-induced outward current (I_{out}) in human atrial myocytes. The results suggest that this current is carried by an ultrarapidly activating delayed rectifier, which has many similarities to currents carried by channels identified by cDNA cloning from human13-17 heart libraries.

Materials and Methods
Isolation of Single Atrial Cells
Specimens of human right atrial appendage were obtained from the hearts of 39 patients with ages ranging from 37 to 74 (61±1 [mean±SEM]) years undergoing aortocoronary bypass surgery. All patients had normal P waves on electrocardiography, and no patient had a history of supraventricular arrhythmias. All atrial specimens were grossly normal at the time of excision. On excision, the samples were immediately placed in oxygenated Tyrode’s solution for transport to the laboratory. The time between excision and the beginning of laboratory processing was approximately 5 minutes. The procedure for obtaining the tissue was approved by the Ethics Committee of the Montreal Heart Institute.

The samples obtained were quickly immersed in nominally Ca^{2+}-free Tyrode’s solution (100% O₂ at 37°C) of the following composition (mmol/L): NaCl, 126; KCl, 5.4; MgCl₂, 1; NaH₂PO₄, 0.33; dextrose, 10; and HEPES (Sigma Chemical Co, St Louis, Mo), 10; pH adjusted to 7.4 with NaOH. The cell isolation procedure was based on a technique described by Escande et al.3 Myocardial specimens were chopped with scissors into cubic chunks (approximately 1 mm³) and placed in a 25-mL flask containing 10 mL of the Ca^{2+}-free Tyrode’s solution. The tissue was gently agitation by continuous bubbling with 100% O₂ and stirred with a magnetic bar. After an initial 5 minutes in this solution, the chunks were reincubated in a similar solution containing 390 U/mL collagenase (CLS II, Worthington Biochemical Corp, Freehold, NJ) and 4 U/mL protease (type XXIV, Sigma). The first supernatant was removed after 45 minutes and discarded. The chunks were then reincubated in a fresh enzyme-containing solution. Microscopic examination of the medium was performed every 15 minutes to determine the number and quality of the isolated cells. When the yield appeared to be maximal, the chunks were suspended in a solution of the following composition (mmol/L): KCl, 20; KH₂PO₄, 10; glucose, 10; glutamic acid, 70; β-hydroxybutyric acid, 10; taurine, 10; EGTA, 10; and albumin 1%; pH adjusted to 7.4 with KOH. Then the solution was gently pipetted.

Only quiescent rod-shaped cells showing clear cross striations were used. A small aliquot of the solution containing the isolated cells was placed in a 1-mL chamber mounted on the stage of an inverted microscope. Five minutes was allowed for cell adhesion to the bottom of the chamber, and then the cells were superfused at 3 mL/min with a solution containing (mmol/L) NaCl, 126; KCl, 5.4; MgCl₂, 0.8; CaCl₂, 1; NaH₂PO₄, 0.33; HEPES, 10; and glucose, 5.5; pH adjusted to 7.4 with NaOH. In some experiments, the composition of the Tyrode’s solution was modified as specified. Experiments were conducted at room temperature (approximately 25°C), at 16°C, or at 36°C. The higher temperature (36°C) was maintained by a thermostatically controlled heating element (N.B. Datyner, Stony Brook, NY); the lower temperature (16°C) was obtained and maintained with a Pelletier-effect device (N.B. Datyner).

Data Acquisition and Analysis
The whole-cell patch-clamp technique was used to record ionic currents and action potentials in the voltage- and current-clamp mode, respectively. Borosilicate glass electrodes (outer diameter, 1.0 mm) were used, with tip resistances of 2.5 to 4 MΩ (3.1±0.5 MΩ, n=100) when filled with (mM) potassium aspartate, 110; KCl, 20; MgCl₂, 1; HEPES, 10; EGTA, 5; Mg-ATP, 5; and Na⁺-creatinine phosphate, 5 (pH adjusted to 7.4 with KOH). The electrodes were connected to a patch-clamp amplifier (Axopatch 1-D, Axon Instruments, Foster City, Calif). The reference electrode was in contact with the bath solution via a 3-mol/L KCl-agar bridge to minimize changes in junction potentials after changing the electrolyte content of bath solutions. Command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments). Recordings were low-pass-filtered at 1 kHz, and data were acquired by analog-to-digital conversion (model TM 125, Scientific Solutions, Solon, Ohio) and stored on the hard disk of an IBM AT–compatible computer. The maximum sampling rate possible was 100 kHz, but because a maximum of 2048 points can be obtained with pCLAMP for each data record, acquisition rates in the present experiments varied from just over 20 kHz (for 100-millisecond data sets) to 0.2 kHz (for 1-second data sets). In some experiments (eg, when current activation was studied), a dual clock option available in pCLAMP was used to allow for sampling of the initial 40 milliseconds of data at 25 kHz, with slower sampling for the remainder of the data set. A nonlinear least-squares curve-fitting program using the Marquardt procedure (CLAMPFIT in PCLAMP) was used to fit current activation and deactivation to single exponential functions.

In all of the cells studied, junction potentials (4 to 17 mV) were zeroed before formation of the membrane-pipette seal in 1 mmol/L Ca^{2+} Tyrode’s solution. Mean seal resistance as recorded in 34 cells averaged 44.1±5.7 GΩ, and seals with resistances of <10 GΩ were rejected. Several minutes after seal formation, the membrane was ruptured by gentle suction to establish the whole-cell configuration for voltage clamping. The series resistance was electrically compensated to minimize
the duration of the capacitive surge on the current recording and the voltage drop produced across the clamped cell membrane. Typically, over 60% series resistance compensation was achieved. The series resistance along the clamp circuit was estimated by dividing the time constant obtained by fitting the decay of the capacitive transient by the calculated cell membrane capacitance (the time integral of the capacitive surge measured in response to 5-mV hyperpolarizing steps from a holding potential of −60 mV).\textsuperscript{18,19} Before series resistance compensation, the decay of the capacitive surge was expressed by a single exponential having a time constant of 402±50 microseconds (cell capacitance, 74.3±7.6 pF; n=34). After compensation, this value was reduced to 127±7 microseconds (cell capacitance, 66.1±3.3 pF). The initial series resistance was 5.4±1.4 MΩ and was reduced to 1.9±0.5 MΩ after compensation. Currents recorded during this study rarely exceeded 1.5 nA, and maximum total I_{st} after I_{o} activation was <800 pA. The voltage drop across series resistance therefore did not exceed 3 mV. Cells with significant leak currents at −60 mV were rejected. Residual leak currents were compensated when present by subtracting a current linearly scaled and opposite in polarity to the current response to a sequence of 5-mV hyperpolarizing pulses from −60 mV, through the use of software routines incorporated in PCLAMP. To assess the linearity of leakage currents carried by ions other than K\textsuperscript{+}, we studied 10 cells dialyzed with pipettes containing Cs\textsuperscript{+} in place of K\textsuperscript{+}. In the absence of any compensation, leakage currents were linear and reversed at 0 mV. Although I_{K1} can provide a substantial nonlinear leakage current, we and others have found that I_{K1} is relatively small in human atrial cells, and I_{K1} was suppressed by adding 1 mmol/L BaCl\textsubscript{2} to the superfusate in experiments characterizing I_{st}, after verifying that I_{st} was not altered by the addition of Ba\textsuperscript{2+} as described below. In addition, in some experiments, leak currents were minimal, and no correction for leakage was performed. Results from these experiments were the same as from those in which leakage correction was applied.

The length of single cells ranged from 60 to 115 μm (88.0±6.1 μm, n=14), and the diameter ranged from 5 to 11 μm (9.2±0.6 μm): the estimated cell surface area was therefore 2.7±0.3×10\textsuperscript{3} cm\textsuperscript{2} on the basis of assumed right cylinder geometry. The input resistance was determined by the application of four consecutive 5-mV hyperpolarizing steps from a holding potential (HP) of −60 mV. Since no time-dependent current was activated with these small steps, the resulting change in current was used to calculate input resistance.\textsuperscript{20} Mean input resistance as estimated in 10 cells was 1.9±0.1 GΩ. The resting space constant was calculated on the basis of the following equation: \textit{sc} = \sqrt{\textit{r} \cdot \textit{Rm}/(2 \textit{Ri})}, where \textit{sc} is the space constant, \textit{r} is the cell radius, \textit{Rm} is specific membrane resistance, and \textit{Ri} is internal resistivity. \textit{Rm} was estimated from the product of input resistance and surface area, providing a mean value of 50.1±4.9 KΩ·cm\textsuperscript{2}, and \textit{Ri} was assumed to be 100 to 200 Ω·cm.\textsuperscript{20-23} The mean resting \textit{sc} is 3.4±0.3 mm when \textit{Ri} is 100 Ω·cm and 2.4±0.2 mm when \textit{Ri} is 200 Ω·cm. Both values are over 25 times the cell length. During maximum current flow, the corresponding \textit{sc} estimates become 620 and 880 μm, in the range of 10 times the cell length. These may be underestimates, since membrane infolding results in a true surface area larger than that of a right cylinder. The surface area estimated on the basis of a specific capacitance of 1 μF/cm\textsuperscript{2} is about twice as large as the value given above, in agreement with histological studies of the cell surface in rabbit atrium.\textsuperscript{24}

The amplitude of peak I_{st} was measured as the difference between the peak transient outward current and the sustained current level at the end of the pulse (Fig 1). When I_{st} was present, I_{st} was measured as the amplitude of the current remaining at the end of the test pulse relative to the zero current level. When I_{st} was studied in the absence of I_{st} (through the use of inactivating prepulses or selective inhibition with 4AP), I_{st} was measured as the maximal outward current level. Concentration-dependent effects of 4AP (Sigma) on both I_{st} and I_{st} were evaluated with a series of concentrations of 4AP ranging from 10\textsuperscript{-6} to 10\textsuperscript{-2} mol/L. 4AP was prepared as a 1 mol/L stock solution in distilled water, with pH adjusted to 7.4 with the addition of HCl. Small quantities of the stock solution were added to the superfusate to produce the desired 4AP concentration. The following chemicals were used to block potential contaminating currents: tetraethylammonium chloride (TEA, 10 mmol/L, Sigma, to inhibit I_{K1}), BaCl\textsubscript{2} (1 mmol/L, Sigma, to inhibit I_{K1}), and atropine (0.1 μmol/L, Sigma, to inhibit I_{KACl} and choline-activated K\textsuperscript{+} current\textsuperscript{26}). CaCl\textsubscript{2} (2 mmol/L, Sigma) was used to block Ca\textsuperscript{2+} current and I_{lo} in all experiments except for those involving action potential recordings. In experiments studying the ionic selectivity of I_{st}, [K\textsuperscript{+}]o was varied, with equivalent and opposite changes in the concentra-

\begin{figure}[h]
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\caption{Recordings of depolarization-induced transient outward current (I_{st}) from a representative myocyte. Currents were elicited by depolarization to +30 mV for 0.1 second (left tracing), 1 second (middle tracing), and 10 seconds (right tracing) from a holding potential of −80 mV at room temperature. A sustained outward current (I_{s}) remains after complete inactivation of I_{st}. Note that this current stays constant to the end of the pulse even with prolonged depolarization. The method used to measure I_{st} and I_{s} is shown at the right. Similar results were obtained from the eight other cells studied.}
\end{figure}
tion of Na⁺ or choline to maintain constant osmolarity and ionic strength. 4,4'-Disothiocyanatostilbene-2,2'-disulfonic acid (DIDS, 150 μmol/L, Sigma) was used to inhibit Cl⁻ current, and all DIDS-containing solutions were protected from direct light. Dendrotoxin (α-dendrotoxin) was purchased from Alomone Labs, Jerusalem, Israel. Na⁺ current was minimized by equimolar replacement of NaCl in the superfusate by choline chloride (126 mmol/L, Sigma) or by an HP positive to −50 mV.

To explore the possible role of I<sub>n</sub> in membrane repolarization, action potentials were recorded in the current-clamp mode at 36°C in normal Tyrode’s solution free of channel blockers. Action potentials were evoked by clamping the membrane at −80 mV (normal resting potential of human atrial tissue<sup>6</sup>) and then delivering a train of 3-millisecond suprathreshold stimuli at a frequency of 1 Hz. Changes in the time course of repolarization were assessed by measuring the action potential duration to 20%, 50%, and 90% of repolarization.

**Statistical Analysis**

Comparisons among multiple groups were performed by ANOVA with Scheffé contrasts.<sup>27</sup> Baseline and drug data were compared by Student’s t test, and two-tailed P<.05 was taken to indicate statistical significance. Group data are presented as mean±SEM. For analysis of current kinetics, data points were fitted by CLAMPFIT in rCLAMP. For other curve-fitting procedures, a non-linear curve-fitting technique (Marquardt’s procedure) was performed using TABLECURVE software (Jandel Scientific).

**Results**

**Distinction of I<sub>n</sub> From Other Outward Currents**

We initially sought to determine whether I<sub>n</sub> could be explained by the properties of a variety of known outward currents. The first possibility considered was that a slowly inactivating component of I<sub>tol</sub> underlies I<sub>n</sub>. Fig. 1 shows current tracings elicited by voltage steps of various durations to +30 mV from an HP of −80 mV at room temperature. On depolarization, I<sub>tol</sub> activates rapidly and then decays quickly to a steady-state level. The inactivation of I<sub>n</sub> was fit by a single exponential process with a time constant averaging 55±2 milliseconds at 25°C. The average amplitude of I<sub>n</sub> was independent of pulse duration, averaging 408±59 pA for 0.1-second pulses, 406±61 pA for 1-second pulses, and 392±58 pA for 10-second pulses (P=NS, n=6). These results indicate that I<sub>n</sub> cannot be due to slow inactivation of I<sub>tol</sub> unless the inactivation time constant is in the order of hundreds of seconds.

A second possibility is that I<sub>n</sub> represents an I<sub>tol</sub> window current. To evaluate this, we determined the voltage dependence of I<sub>n</sub> activation and inactivation. Voltage dependence of inactivation was evaluated in 10 myocytes with the two-pulse protocol shown in Fig 2A. Activation was analyzed in two ways: (1) on the basis of tail currents on repolarization to −40 mV after a 5-millisecond pulse to a range of test potentials (six cells, Fig 2B), and (2) on the basis of peak currents at a variety of test potentials, assuming a reversal potential of −80 mV (seven cells). Preliminary experiments showed that no I<sub>n</sub> was elicited by test pulses to potential negative to −40 mV. Mean activation and inactivation curves from all experiments are shown in Fig 2C. Crossover between activation and inactivation curves occurs at −20 mV with a value of 0.07, indicating that the maximum I<sub>tol</sub> window current expected would be ≤7% of fully available I<sub>tol</sub> at −20 mV, or approximately 2.5 pA. Positive to 0 mV, I<sub>n</sub> is fully inactivated, while I<sub>n</sub> increases becomes increasingly larger (see Fig 8). Thus, the substantial sustained current present on maintained depolarization of human atrial myocytes cannot be explained on the basis of a slowly inactivating or window component of I<sub>tol</sub>.

We then turned our attention to the possible role of the inwardly rectifying currents I<sub>k</sub>, and I<sub>Kah</sub>. The addition of 1 mmol/L Ba²⁺ to the superfusate reduced the inward current elicited by hyperpolarization from −80 to −140 mV by 92±13% (P<.001, n=3). However, I<sub>n</sub> observed on depolarization from −80 to +30 mV was not altered by Ba²⁺ (301±55 pA before and 286±56 pA after Ba²⁺, n=4, P=NS). Similarly, the addition of 0.1 μmol/L atropine did not affect I<sub>n</sub> (361±28 pA before and 358±30 pA after atropine, n=3, P=NS).

To exclude a contribution of the classic type of I<sub>K</sub><sup>10,11</sup> to the I<sub>n</sub> under study, we selected cells lacking a slowly activating current and a tail current of >50 pA on return to an HP of −30 mV after ≥1 second at +30 mV. We have previously shown that 10 mmol/L TEA reduces the classic type of I<sub>K</sub> activated by a 300-millisecond pulse from −70 to +20 mV by a mean of 76% in human atrial myocytes.<sup>28</sup> Twenty minutes of exposure did not alter I<sub>n</sub> at any test voltage, with either 10 mmol/L (mean change at +30 mV, −2.4±0.8%, three cells, P=NS) or 40 mmol/L (−5.6±2.9%, four cells, P=NS) TEA. After showing that I<sub>n</sub> is manifest in the absence of I<sub>k</sub>, I<sub>k1</sub>, and I<sub>Kah</sub>, we elected to add TEA (10 mmol/L), BaCl (1 mmol/L), and atropine (0.1 μmol/L) to all solutions, unless otherwise indicated, to avoid contamination by these currents.

The above experiments indicate that a sustained depolarization-induced outward current exists in human atrial myocytes independent of I<sub>k</sub>, I<sub>k1</sub>, I<sub>Kah</sub>, and I<sub>k</sub>. Since 2 mmol/L Co²⁺ was present in the superfusate for all the above experiments, I<sub>n</sub> is manifest under conditions in which I<sub>Cl</sub> is fully suppressed.<sup>6</sup> In rabbit atrium, a depolarization-induced I<sub>n</sub> is carried by Cl⁻ ions and is inhibited by the Cl⁻ transport blocker DIDS (150 μmol/L) or by substitution of methanesulfonate for extracellular Cl⁻. The contribution of Cl⁻ substitution and DIDS on I<sub>n</sub>. Complete replacement of superfusate Cl⁻ by methanesulfonate (Fig 3A) did not alter I<sub>n</sub> in four cells, with I<sub>n</sub> at +30 mV averaging 292±51 pA before and 298±68 pA after Cl⁻ replacement (P=NS). DIDS (Fig 3B) had a small and variable effect, with I<sub>n</sub> decreasing slightly after exposure to 150 μmol/L DIDS in four cells and increasing slightly in three. Overall, I<sub>n</sub> at +30 mV averaged 332±69 pA before and 277±61 pA after DIDS (n=7, P=NS), and DIDS did not significantly alter I<sub>n</sub> at any test voltage. These results suggest that I<sub>n</sub> is neither a Cl⁻ current nor one of the well-characterized K⁺ currents evaluated above. Two lines of evidence suggested that I<sub>n</sub> is a K⁺ current. First, we found I<sub>n</sub> to be exclusively sensitive to the K⁺ channel blocker 4AP. As shown in Fig 4A, 50 μmol/L 4AP substantially reduced I<sub>n</sub> without affecting the inactivating component of I<sub>tol</sub>. Second, replacement
of intracellular K+ by dialysis with Cs+ eliminated I\textsubscript{nat}. Fig 4B shows a typical I\textsubscript{nat} decaying to a sustained level elicited by a depolarizing clamp step immediately after membrane rupture with a pipette containing 130 mmol/L Cs+ (CsCl) in place of K+. The outward current elicited by depolarization decreased progressively over time, until an identical clamp step 2 minutes after membrane rupture did not elicit any outward current, as shown in Fig 4B. Similar results were obtained in six cells studied with Cs+ -containing pipettes and were never observed in cells studied with standard K+ -containing electrodes. This finding is in marked contrast to our observations in rabbit atrial myocytes, in which a substantial and apparently unaltered I\textsubscript{nat} is elicited by depolarizations positive to the Cl\textsuperscript{-} equilibrium potential following the replacement of K+ by Cs+ in both the pipette solution and the superfusate.\textsuperscript{12}

**Sensitivity of I\textsubscript{nat} and I\textsubscript{bol} to Block by 4AP**

The most important obstacle in discerning the properties of I\textsubscript{nat} is its separation from I\textsubscript{bol}. Like the latter current, I\textsubscript{nat} is carried by K+ ions, is blocked by 4AP, and is relatively insensitive to the actions of other channel-blocking drugs that we tested. The much greater sensitivity of I\textsubscript{nat} to blockade by 4AP compared with I\textsubscript{bol} indicated the possibility of using 4AP as a tool to separate the currents. Fig 5 shows the results of concentration-response analysis of the effects of 4AP on I\textsubscript{nat} and I\textsubscript{bol}. Currents were elicited both by 200-millisecond depolarizations (1 Hz) from -80 mV to +30 mV and by a train of ten 200-millisecond pulses (from -80 to +30 mV) followed by a single 2-second test pulse. The latter protocol was used to ensure full inactivation of I\textsubscript{bol} by the end of the test pulse; the former was used to ensure steady-state effects at a frequency of 1 Hz. Similar results were obtained with either protocol. After baseline measurements had been obtained, 4AP (10\textsuperscript{-6} to 10\textsuperscript{-2} mol/L) was superfused for 5 minutes, and the measurements were repeated. Fig 5A shows recordings from a representative cell. 4AP (50 \textmu mol/L) reduced I\textsubscript{nat} by approximately 50%, without affecting I\textsubscript{bol}. When the 4AP concentration was increased to 2 mmol/L, I\textsubscript{nat} was completely suppressed, and I\textsubscript{bol} was reduced by approximately 50%. Fig 5B shows mean concentration-response data for 4AP inhibition of I\textsubscript{nat} and I\textsubscript{bol} in seven cells. The symbols represent experimental data, and the solid lines are the best-fit curves according to the equation provided in Fig 5B. The dashed vertical lines indicate IC\textsubscript{50} values for I\textsubscript{nat} and I\textsubscript{bol}. Appreciable effects of 4AP on I\textsubscript{nat} occurred at concentrations as low as 10
higher than that for \( I_{\text{us}} \), and full suppression of \( I_{\text{us}} \) was not achieved until a 4AP concentration of 10 mmol/L. Calculated values of the Hill coefficient were 1.45 for \( I_{\text{us}} \) and 1.95 for \( I_{\text{ol}} \).

**Separation of \( I_{\text{us}} \) From \( I_{\text{ol}} \)**

Since 50 \( \mu \)mol/L 4AP blocks \( I_{\text{us}} \) by over 50% without affecting \( I_{\text{ol}} \), the 50 \( \mu \)mol/L 4AP–sensitive component should consist solely of \( I_{\text{us}} \). Fig 6 shows typical current recordings at 25°C (top) and 36°C (in a different cell, bottom) on depolarization for 100 milliseconds from −50 mV to various test potentials before (left) and after (middle) exposure to 50 \( \mu \)mol/L 4AP. The subtracted current (right) at 25°C reveals a time-dependent current with an activation time constant ranging in the example shown from 13.4 milliseconds at +10 mV to 2.5 milliseconds at +50 mV. At 36°C, the subtracted current appears instantaneous, with small initial fluctuations due to intrinsic variation in early, very large peak \( I_{\text{ol}} \) currents. Similar results were obtained in five other cells, suggesting that \( I_{\text{us}} \) is a very rapidly activating current of the delayed rectifier type.

The use of subtracted currents to analyze \( I_{\text{us}} \) presents a number of difficulties, including the possibilities of voltage- and time-dependent block of \( I_{\text{us}} \) by 4AP, which could distort the results obtained, and contamination by leakage currents. Therefore, we attempted to exploit the voltage-dependent inactivation properties of \( I_{\text{ol}} \) to suppress \( I_{\text{ol}} \) under the conditions necessary to record \( I_{\text{us}} \).

Fig 7A shows the effect of 1000-millisecond conditioning pulses to various voltages in 10 cells on the amplitude of \( I_{\text{us}} \), elicited by a subsequent test pulse to +40 mV. At a conditioning potential of −20 mV, \( I_{\text{ol}} \) was 91.7 ± 0.1% inactivated, and full inactivation occurred at +10 mV. \( I_{\text{us}} \), on the other hand, was not significantly altered by depolarizing prepulses. Fig 7B shows the effect of a 200-millisecond prepulse from an HP of −60 to +40 mV on the amplitude of \( I_{\text{ol}} \) and \( I_{\text{us}} \) during an identical test pulse delivered between 1 and 36 milliseconds later (n=5). Although \( I_{\text{us}} \) was not significantly affected by the prepulse, \( I_{\text{ol}} \) of the test pulse at all coupling intervals was <10% of the value during the prepulse. The data shown in Fig 7A and 7B, recorded at room temperature, indicate that \( I_{\text{ol}} \) is over 90% inactivated at a potential of −20 mV and is fully inactivated by pulses positive to +10 mV and that there is <10% recovery of \( I_{\text{ol}} \) at −60 mV up to 36 milliseconds after a depolarization to +40 mV. Therefore, we estimated that \( I_{\text{ol}} \) should be over 95% inactivated when a test pulse from an HP of −20 mV is applied 10 milliseconds after a 200-millisecond prepulse to +40 mV. We were, in fact, unable to detect any transient outward current over the activation voltage range of \( I_{\text{ol}} \) for test pulses applied under the conditions just described.

Fig 7C shows currents recorded at 25°C when a 40-millisecond test pulse from −20 mV to various potentials is applied 10 milliseconds after a 200-millisecond conditioning pulse to +40 mV. The current activates rapidly, with an activation time constant averaging 2.1 ± 0.2 milliseconds (n=14 cells) at +50 mV. The rate of activation was voltage dependent, with the activation time constant increasing to 18.2 ± 1.8 milliseconds at −10 mV. Results of the same pulse protocol in the same cell at 36°C are shown in Fig 7D. Current amplitudes were not substantially altered by the higher temperature, but activation was
greatly accelerated and could not be separated from the capacitance decay. Exposure to 50 μmol/L 4AP reduced the currents recorded at 25°C by approximately 50% without appreciatively altering their kinetics (Fig 7E), and 2 mmol/L 4AP completely suppressed the time-dependent current (Fig 7F).

**Characterization of \( \text{I}_{\text{su}} \): Current-Voltage Relation and Activation Curve**

To characterize the current-voltage (I-V) relation and steady-state activation properties of \( \text{I}_{\text{su}} \), we used two different protocols. The first is illustrated in Fig 8A and consists of a 1000-millisecond prepulse from an HP...
of −50 to +50 mV to inactivate I_{i01}, followed 20 milliseconds later by a 100-millisecond test pulse to a variety of test potentials, followed by a 100-millisecond clamp to −10 mV (to evaluate tail currents). Experiments were conducted at room temperature, and I_{i01} was measured from the maximum current level following activation. The second protocol, illustrated in Fig 8B, consisted of 2000-millisecond depolarizations from an HP of −60 mV to various test potentials between −40 and +50 mV, with I_{i01} measured from the sustained current as indicated in Fig 1.

The I-V relation obtained in six cells studied with the protocol illustrated in Fig 8A is shown in Fig 8C. The current is evident at potentials positive to −30 mV and appears to show outward rectification. Measurement of I_{i01} in a separate group of cells with the
Activation and Deactivation Kinetics

The steady-state activation curve of $I_{\text{ss}}$ was constructed from data obtained with the protocol in Fig 8A by plotting $I_{\text{ss}}$ tail current (normalized to the maximum current) as a function of test potential (Fig 8E). These data were well described by a single Boltzmann distribution: $I(V) = 1/[1 + \exp((V-V_{1/2})/k)]$, where $I(V)$ is the normalized current as a function of the test potential $V$, $V_{1/2}$ is the voltage at which 50% of the channels are activated, and $k$ is a slope factor. The best fit ($r=.998$) to the experimental data was obtained with $V_{1/2} = -4.3$ mV and $k = 8.0$ mV.

**Activation**

The activation kinetics of $I_{\text{ss}}$ were determined from the type of data shown in Figs 7C and 8A. Fig 8F shows the activation time constants obtained from nine cells by exponential curve fitting of the activation time course as a function of test potential. The activation time constant is voltage dependent, decreasing from $17.7 \pm 2.8$ milliseconds at $-10$ mV to $1.9 \pm 0.2$ milliseconds at $+50$ mV. The kinetics of $I_{\text{ss}}$ tail currents were determined by repolarizing from a conditioning potential of $+60$ mV to a variety of test potentials in six cells. Outward tail currents were very small negative to $-30$ mV, and at potentials positive to $-30$ mV, there is steady-state activation (Fig 8E), making it impossible to study deactivation kinetics independent of activation changes. At $-20$ mV, at which there is $<20\%$ $I_{\text{ss}}$ activation, the time constant of the $I_{\text{ss}}$ tail current averaged $13 \pm 3$ milliseconds.

**Inactivation Properties**

$I_{\text{ss}}$ demonstrated no significant voltage-dependent inactivation during 1000-millisecond conditioning pre-
pulses to between $-60$ and $+40$ mV. However, this does not necessarily exclude slow inactivation that fails to develop perceptibly during a 1000-millisecond conditioning pulse. Therefore, we evaluated $I_{\text{na}}$ amplitude in eight cells held at $-80$, $-50$, and $-20$ mV and pulsed to $+40$ mV at a frequency of 0.2 Hz. $I_{\text{na}}$ averaged 509±72 pA at an HP of $-80$ mV, 468±68 pA at $-50$ mV, and 411±59 pA at $-20$ mV. Holding at $-50$ mV reduced $I_{\text{na}}$ by 7±5% ($P=\text{NS}$) relative to values at $-80$ mV, whereas holding at $-20$ mV reduced $I_{\text{na}}$ by 16±8% ($P<.05$) relative to values at $-80$ mV. Thus, $I_{\text{na}}$ showed slight but statistically significant voltage-dependent inactivation over the range from $-80$ to $-20$ mV.

Time-dependent inactivation was seen in 33 (46%) of 72 cells during strong depolarizations (positive to $+40$ mV). In 13 of 27 cells studied with 2-second depolarizing pulses that showed inactivation, $I_{\text{na}}$ was reduced by an average of 19±2% ($P<.001$) over 2000 milliseconds at $+60$ mV. The time course of inactivation was very slow, and steady state was not achieved by the end of the pulse.

**K⁺ Selectivity of $I_{\text{na}}$ and Rectification Properties**

Fig 9A shows recordings from an experiment studying the reversal potential of $I_{\text{na}}$ based on tail current measurements. In this experiment, $I_{\text{na}}$ tails reversed between $-60$ and $-80$ mV in the presence of 5.4 mmol/L K* in the superfusate. At this [K*], the mean $I_{\text{na}}$ reversal potential in seven cells was $-75\pm3$ mV, which compares with a value of $-83$ mV calculated from the Nernst equation for a temperature of 25°C and an assumed [K⁺] of 130 mmol/L. Increased superfusate K⁺ concentration shifted the reversal potential, as illustrated in Fig 10B. The relation between mean $I_{\text{na}}$ reversal potentials and log [K⁺] was linear, with a correlation coefficient of 0.999 and a slope of 54 mV per decade. Slopes were also calculated from data in each of seven individual experiments and averaged 55.3±2.9 mV.

Rectification properties were studied by analyzing the fully activated I-V relation with the protocol illustrated in Fig 9C. Tail currents were corrected for incomplete deactivation as previously described by dividing the time-dependent (peak minus steady-state) tail current by (1−a—a), where $a−P$ and $a−S$ are the activation variable at the test potential and $+50$ mV, respectively. The results show substantial outward rectification at potentials positive to $-10$ mV.

**Envelope of Tails Test**

Because of the rapid activation of $I_{\text{na}}$ at 25°C, it was difficult to obtain sufficient information for an accurate envelope of tails analysis. Therefore, we studied $I_{\text{na}}$ at 16°C (using prepulses to inactivate $I_{\text{ol}}$ according to the protocol illustrated in Fig 10A). The properties of $I_{\text{na}}$ were similar to those observed at 25°C (Fig 8), except that current kinetics were slowed (eg, the activation time constant at 16°C averaged 46.7±4.1 milliseconds at $+10$ mV versus 7.1±0.8 milliseconds [P<.01] at 25°C and the same potential). The scaled tail currents were superimposed on the activating current tracing (Fig 10A and 10B), indicating that the envelope test was satisfied. Fig 10C shows mean ratios of tail current ($I_{\text{ta}}$) to step current ($I_{\text{sto}}$) as a function of pulse duration in four cells. There is no significant time dependence of the ratio, consistent with the presence of only one current

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**Fig 9.** A, Tail currents were obtained at various potentials with the protocol shown, after a 400-millisecond conditioning pulse from $-50$ to $+50$ mV to inactivate the 4-aminopyridine-sensitive transient outward current ($I_{\text{ol}}$) (at room temperature). Results at 20-mV test voltage increments are shown. Sustained outward tail current ($I_{\text{ta}}$) reversed between $-60$ and $-80$ mV in this cell. B, Graph shows dependence of $I_{\text{ta}}$ reversal potential (RP) on logarithm of [K⁺]. Symbols are mean values of the RP from seven cells (standard errors are within the symbols for means), and the line is the best linear fit, with a slope of 54 mV per decade. C, Graph shows fully activated current-voltage relation, as obtained from measurements of tail currents with the protocol shown in the inset (400-millisecond pulse to $+50$ mV to activate $I_{\text{ta}}$ followed by repolarization to various test potentials [TPs]; 400-millisecond prepulse as in A to inactivate $I_{\text{ol}}$). Values plotted are amplitudes of deactivating tail current, adjusted for incomplete deactivation as described in the text.
component for $I_{\text{so}}$. Tail current deactivation was found to be well fit by a single exponential relation, and the deactivation time constant was unrelated to the duration of the activating pulse. For example, time constants of tail deactivation at $-20 \text{ mV}$ averaged $78 \pm 11$, $77 \pm 11$, $77 \pm 12$, $79 \pm 12$, and $78 \pm 12$ milliseconds, respectively, for 10-, 20-, 50-, 100-, and 300-millisecond activating pulses to $+10 \text{ mV}$.

**Observations in Cells Lacking $I_{\text{so}}$**

In the course of these experiments, we observed 10 cells (out of a total of approximately 100) lacking any current resembling $I_{\text{so}}$ on depolarization from $-80 \text{ mV}$ to potentials as positive as $+60 \text{ mV}$. These cells appeared otherwise normal. Their capacitance ($78 \pm 8 \text{ pF}$ before and $64 \pm 5 \text{ pF}$ after series resistance and capacitance compensation), capacitive time constant ($431 \pm 48$ microseconds before and $135 \pm 11$ microseconds after compensation), series resistance ($5.5 \pm 20 \text{ MΩ}$ before and $2.1 \pm 0.7 \text{ MΩ}$ after compensation), and input resistance ($1.6 \pm 0.6 \text{ GΩ}$) were not significantly different from those determined in other cells. They averaged $92 \pm 6 \text{ μm}$ in length and $9.0 \pm 0.4 \text{ μm}$ in width, similar to other cells.

In these cells, depolarizing pulses at $25°C$ revealed a current with properties of $I_{\text{su}}$, as shown in Fig 11A and 11B. The I-V relation and activation kinetics at $25°C$ of $I_{\text{su}}$ in such cells could be characterized without the use of pharmacologic or voltage protocols otherwise required to separate $I_{\text{su}}$ from $I_{\text{so}}$ and are shown in Fig 11C and 11D. The results are similar to results obtained in cells possessing $I_{\text{so}}$ with the use of depolarizing pre-pulses to inactivate the latter current.

**Effects of Dendrotoxin on $I_{\text{su}}$**

A variety of voltage-gated $K^+$ channels have been cloned, several of which resemble $I_{\text{su}}$. Differences in pharmacologic response characterize the various clones in the Kv1 family and may be useful in comparing native currents with specific clones. Pharmacologic probes of interest include 4AP, TEA, and $Ba^{2+}$. The responses of $I_{\text{su}}$ to 4AP, TEA, and $Ba^{2+}$ were defined in experiments addressing the role of other $K^+$ currents as described above. To complete the pharmacologic characterization of $I_{\text{su}}$, we evaluated its response to 1, 10, and 100 nmol/L dendrotoxin in six cells. Dendrotoxin did not significantly alter $I_{\text{su}}$ at any concentration. For example, $I_{\text{su}}$ averaged $149 \pm 29 \text{ pA}$ at $+10 \text{ mV}$ and $444 \pm 51 \text{ pA}$ at $+50 \text{ mV}$ before dendrotoxin superfusion and $176 \pm 39 \text{ pA}$ at $+10 \text{ mV}$ and $444 \pm 80 \text{ pA}$ at $+50 \text{ mV}$ after exposure to $100 \text{ nmol/L}$ dendrotoxin ($n=6$).

**Effects of Selective Blockade of $I_{\text{su}}$ on the Action Potential**

To evaluate the possible role of $I_{\text{su}}$ in mediating repolarization, the effects of 50 μmol/L 4AP were analyzed. At this concentration, 4AP reduced $I_{\text{su}}$ by 50% without affecting $I_{\text{so}}$. Experiments were carried out at $36°C$ in normal Tyrode’s solution free of channel blockers (to allow for as normal action potentials as possible). Outward currents were evaluated by depolarizing the membrane to various test potentials from an
HP positive to −50 mV to inactivate the Na⁺ current, and action potentials were recorded in the current-clamp mode. After baseline recording of action potentials and outward currents had been obtained under control conditions, cells were superfused with Tyrode’s solution containing 4AP at a concentration of 50 μmol/L, and the same measurements were repeated.

The effects of 4AP are illustrated in Fig 12, in which action potentials (A) and current recordings (B) elicited under control conditions and during exposure to 4AP are shown. The action potential is substantially prolonged by 4AP, without any change in overall morphology. Under control conditions, there is a prominent Iᵦᵦ, followed by a slowly developing rectifier current. Outward current was reduced by 4AP at all times during the pulse by just over 200 pA, with no effect on time-dependent current components per se. The overall effect of 50 μmol/L 4AP on action potential duration in 10 myocytes is shown in the Table. The drug significantly increased action potential duration at various levels of repolarization, with no significant difference in percent change at each level.

Discussion

We have shown that the depolarization-induced sustained current remaining after Iᵦᵦ inactivation in human atrial myocytes is a K⁺ current that can be distinguished from Iᵦᵦ on the basis of differences in voltage-dependent inactivation and sensitivity to block by 4AP. Iᵦᵦ differs from classic Iᵦ in terms of its sensitivity to 4AP, insensitivity to TEA, and extremely rapid activation kinetics. The kinetic properties of Iᵦᵦ are similar to those of a variety of rapidly activating and slowly inactivating K⁺ channels encoded by cDNAs from rat[30,31] and human[13–17,29] heart tissue, suggesting that Iᵦᵦ may be a physiological manifestation of this type of channel identified by DNA cloning.

Comparison Between Iᵦᵦ and Similar Currents Carried by Cloned Channels

Tamkun et al13 described two K⁺ channel clones from human heart, one of which (HK2) is much more abundant in human atrium than ventricle. HK2 was transfected into mouse L cells by Snyders et al,14,16 and the expressed current was found to be highly K⁺ selective (55 mV per decade), rapidly activating (the time constant decreased from 10 milliseconds to approximately 2 milliseconds between 0 and +60 mV), and outwardly rectifying. Slow, partial inactivation was seen after strong depolarization, and the single-channel conductance was 15 picosiemens.14 The current is very sensitive to blockade by 4AP (IC₅₀ at +50 mV, 180 μmol/L) and insensitive to inhibition by TEA (10 mmol/L) or dendrotoxin (100 nmol/L).16

Philipson et al15 cloned cDNA (hPCN1) encoding for an islet cell K⁺ channel from human pancreatic tissue, with significant homology and functional properties similar to HK2. On expression of this cDNA in Xenopus oocytes, the resulting K⁺ currents activated rapidly, with time constants in the range of 20 milliseconds at −10 mV and 1 millisecond at +60 mV. Steady-state inactivation was observed at HPs more positive than −50 mV, and the current was fully inactivated at potentials positive to −10 mV. The current was insensitive to TEA but highly sensitive to 4AP, with 30% to 38% block at 50 μmol/L and 54% to 62% block with 100 μmol/L 4AP.

More recently, Fedida et al17 cloned a cDNA (fHK) from a fetal human heart library. K⁺ channels encoded by fHK carry a K⁺ current with many similarities to the currents described above. There is substantial sequence homology between fHK and hPCN1 and slightly less homology with HK2. The properties of currents resulting from expression of fHK in a human epithelial kidney cell line include rapid activation (time constants of 11.8 milliseconds at 0 mV and 1.6 milliseconds at +60 mV), K⁺ selectivity, and slow inactivation at positive potentials (approximately 40% after 10 seconds at +50 mV).

The voltage dependence, pharmacologic response (to TEA and 4AP), and kinetic properties of current carried by fHK strongly resemble the corresponding characteristics of Iᵦᵦ.17

A rapidly activating and slowly inactivating delayed rectifier current is carried by channels encoded by a
cDNA (designated RAK) cloned from adult rat atrial tissue, as reported by Paulmichl et al.31 This current also activates rapidly, although somewhat more slowly than HK2 (time constant ranging from 58 milliseconds at -20 mV to 6.4 milliseconds at +60 mV). It is insensitive to TEA and is blocked by 4AP with an IC50 of 600 μmol/L.

Cloned K+ channels are now generally described in terms of a common nomenclature proposed by Chandy33 in 1991. The activation and inactivation properties of Irau most resemble those of three members of the Kv1 family: Kv1.1, Kv1.2, and Kv1.5.29 However, Kv1.1 is sensitive to TEA, dendrotoxin, and Ba2+, with IC50s of 2 mmol/L, 12 nmol/L, and 0.8 nmol/L, respectively, and Kv1.2 is exquisitely sensitive to dendrotoxin (IC50, 0.1 nmol/L).29 Only Kv1.5 has a pharmacologic profile similar to that of Irau, including sensitivity to 4AP (IC50 in the order of 100 μmol/L) and insensitivity to TEA and dendrotoxin (200 nmol/L).29 The pharmacologic properties of Irau therefore identify it with the Kv1.5 group of K+ channel clones, including the clones shown to be present in human heart: HK2,13-16 hPCN1,32 and fHK.17

Relation of Irau to Previously Described Native Currents

In 1988, Yue and Marban34 described a novel K+ channel based on single-channel recordings from guinea pig ventricular myocytes, which they designated IKp. This channel was observed in approximately 10% of patches, activated rapidly (time constant, <10 milliseconds), and was highly selective for K+. Depolarization increased open probability, which was 50% of maximum at approximately -10 mV, and no detectable inactivation was noted. Single-channel conductance was 14 picoisemiers, a value close to that noted for fHK17 and HK2.30 Recently, Backx and Marban35 have reported the macroscopic current counterpart of IKp in guinea pig ventricular myocytes. It differs from Irau in that IKp is strongly inhibited by 1 mmol/L Ba2+ and has a relatively linear I-V relation.

Boyle and Nerbome36 have described a rapidly activating delayed rectifier current in rat atrial myocytes. The activation time constant of this current ranged from 5.3 to 1.4 milliseconds over the range of -10 to +50 mV. Activation was voltage dependent, with 50% activation at -1.5 mV, similar to Irau. The rat atrial K+ current was sensitive to 4AP, with an IC50 of approximately 1 mmol/L,36 which is similar to the 4AP sensitivity of the rat atrial delayed rectifier cDNA cloned by Paulmichl et al31 and about one order of magnitude larger than the IC50 for 4AP block of Irau, fHK,17 HK2,16 and hPCN1.32 Finally, the rat atrial K+ current showed considerable voltage-dependent inactivation and was 50% inactivated at approximately -40 mV.36 Rat atrial delayed rectifier currents corresponding to both the cloned31 and native36 channels are somewhat less sensitive to 4AP than the native human current (Irau) and similar channels encoded by human cDNA clones.13-16 The rat atrial channel also appears to manifest stronger voltage-dependent inactivation.

Jeck and Boyden37 have recently observed a very rapidly activating outward current that shows little inactivation in 23% of neonatal puppy cells. The ionic nature of this current was not studied in detail, but it was found to change little or not at all on exposure to 2 mmol/L 4AP. The precise nature of this current and its relation to cloned and other native channels remains to be elucidated.

Relation to Classic Delayed Rectifier Currents and Potential Significance

Delayed rectifier currents in the heart were first described by Noble and Tsien10 in 1969. Since that time, delayed rectifier K+ currents have been shown to exist and play a potential role in action potential repolarization of a variety of cardiac cell types and species.38-45 Recently, it has been suggested that IK in some species consists of two components, IKr (or "rapid IK") and IKs (or "slow IK"), which can be distinguished on the basis of different kinetics, voltage dependence, and response to pharmacologic agents.46,47 A cDNA cloned from neonatal rat heart has been found to encode channels with properties comparable to IKr.48

Classic delayed rectifier currents are present in human atrial myocytes11 and appear to manifest characteristics comparable to those attributed to IKr and IKs in the guinea pig.46,47 Irau, along with the similar cloned and native current systems discussed above, belongs to a new category of delayed rectifier current, which differs from classic IK in activating much more rapidly and, in the case of the

![Diagram](https://example.com/diagram.png)

**Fig. 12.** Tracings showing the effects of 4-aminopyridine (4AP, 50 μmol/L) on the action potential of a single atrial myocyte. Experiments were conducted in normal Tyrode’s solution, and only cells with a resting potential more negative than -50 mV were used. A, Action potentials were recorded in current-clamp mode by clamping the membrane potential to -80 mV and then delivering 3-millisecond rectangular pulses at 1 Hz. Action potentials under control conditions and in the presence of 4AP are superimposed. 4AP prolongs action potential duration without substantially changing the morphology. B, Currents in the same cell were elicited by a 350-millisecond pulse to +30 mV from a holding potential of -40 mV. 4AP reduces total depolarization-induced outward current by approximately 50%, without altering transient outward and delayed rectifier current components.

<table>
<thead>
<tr>
<th>Effect of 4-Aminopyridine (50 μmol/L) on Action Potential Duration in 10 Human Atrial Myocytes</th>
<th>Control</th>
<th>4AP (50 μmol/L)</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>APD20, ms</td>
<td>6 ±1</td>
<td>9 ±2*</td>
<td>49±11</td>
</tr>
<tr>
<td>APD90, ms</td>
<td>52 ±7</td>
<td>90 ±13†</td>
<td>73±8</td>
</tr>
<tr>
<td>APD90, ms</td>
<td>207 ±18</td>
<td>342±32†</td>
<td>66±11</td>
</tr>
</tbody>
</table>

4AP indicates 4-aminopyridine. APD20, APD90, and APD90 action potential duration at 20%, 50%, and 90% repolarization, respectively.

*P < .05 and †P < .01 vs control.
Kv1.5 clones and corresponding native currents, differing in pharmacologic sensitivity (with markedly greater sensitivity to blockade by 4AP and resistance to TEA). It may be appropriate to term these currents “ultrarapid” delayed rectifiers (I_{uhr}), to distinguish them from the slower delayed rectifiers I_K and I_K,C. Because of their rapidity of activation and limited slow inactivation, these recently described delayed rectifier currents have the capacity to contribute significantly to action potential repolarization. Results of exposure to 50 μmol/L 4AP (Fig 12) are consistent with a role for I_{uhr} in repolarizing the human atrial action potential. A deeper understanding of the properties of I_{uhr} may allow for a fuller appreciation of the mechanisms of repolarization of human atrial cells, and the development of safe and specific I_{uhr} blockers could allow for new approaches to the pharmacologic therapy of atrial arrhythmias.

**Potential Limitations**

The most important limitation of these studies is the difficulty of separating I_{uhr} from other currents, particularly I_{loli}. We have achieved this goal in three ways: (1) by studying current selectively blocked by very low (50 μmol/L) concentrations of 4AP, (2) by using conditioning pulses to inactivate I_{loli}, and (3) by studying cells lacking I_{loli}. All of these methods have potential limitations. Blockers like 4AP are notorious for voltage- and frequency-dependent effects, which could distort the subtracted currents. For this reason, we used the 4AP-sensitive current only for qualitative comparison with results obtained by other methods. One can never be sure that conditioning pulses fully remove a contaminating current, and the short interval between the conditioning and test pulse leaves significant I_{loli} activated at the onset of the test pulse, so that an instantaneous outward component is present at the onset of depolarization (compare Fig 7C, with conditioning pulse method, with Fig 6C, top, using 4AP-sensitive current, to study I_{loli}). Finally, cells lacking I_{loli} may be abnormal or unrepresentative.

On the other hand, the results obtained using all three methods to define I_{uhr} are in close agreement. Fig 13A shows the I-V relation for I_{uhr} as established with four different sets of protocols in four separate sets of cells: (1) studies at 25°C, with prepulses to remove I_{loli} (same voltage protocol as in Fig 8A, n=6), (2) studies at 25°C, without prepulses, with I_{uhr} measured from the current at the end of a 2-second pulse (as in Fig 8B, n=4), (3) studies at 36°C, without prepulses, and I_{uhr} measured from the current at 2 seconds of a depolarizing pulse (as in Fig 8B, n=8), and (4) studies at 25°C, in cells lacking I_{loli} (as in Fig 11, n=10). The results are very similar for all protocols in all sets of cells, making it unlikely that they are an artifact of the method used to study I_{uhr}. Similarly, activation kinetics at 25°C (Fig 13B) were not significantly different when assessed with the use of a prepulse to inactivate I_{loli} (as in Fig 8A) or simple voltage steps from the same HP in cells lacking I_{loli} (Fig 11). While recognizing the imperfections of the approaches required to define the properties of I_{uhr} in human atrial myocytes, it must be acknowledged that these approaches are the only ones currently available to isolate and describe the native current. Cloned channels may allow one to study in a relatively pure way the properties of a given current but still leave unanswered the role of that specific current in native tissues.

We tested the relative potency of 4AP in blocking I_{uhr} and I_{loli} at a frequency of 1 Hz. In preliminary studies, we have found that 4AP block of I_{uhr} in human atrium is not rate dependent, whereas 4AP block of I_{loli} shows typical reverse use dependence. Thus, our estimate of the IC_{50} for 4AP block of I_{uhr} is rate independent, but the value obtained for 4AP block of I_{loli} applies, strictly speaking, only to a frequency of 1 Hz. This does not limit the validity of the experiments in which we used 50 μmol/L 4AP to selectively block I_{uhr}, since the latter were all performed at a frequency of 1 Hz. On the other hand, the relative affinity of 4AP for I_{loli} versus I_{loli} needs to be assessed in light of the state-dependent block of

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**Fig 13.** A, Graph shows current-voltage relation for sustained outward current (I_{uhr}) obtained with four different protocols: ○, studies at 25°C, with prepulses to remove the 4-aminopyridine–sensitive transient outward current (I_{loli}) (as in Fig 8A, n=6); ●, studies at 25°C, without prepulses, with I_{uhr} measured from the current at the end of a 2-second pulse (as in Fig 8B, n=4); ▼, studies at 25°C, in cells lacking I_{loli} (as in Fig 11, n=10); and ▼, studies at 36°C, without prepulses and with I_{uhr} measured from the current at 2 seconds of a depolarizing pulse (as in Fig 8B, n=8). B, Graph shows activation time constant (τ) for I_{uhr} at 25°C, as measured with a prepulse to inactivate I_{loli} (Fig 8A) or in cells lacking I_{loli} (Fig 11). TP indicates test potential.
**Ultrarapid Delayed Rectifier in Human Atrium**

I_{to1} by 4AP. The K_d of 4AP for the rested state of I_{to1} is likely to be closer to the K_d for I_{to1} than the relative IC_{50} obtained during pulsing at 1 Hz. Available estimates in the literature suggest that even the resting state I_{to1} affinity for 4AP is probably less than that of I_{to1}.

We used Co^{2+} to block Ca^{2+} current and I_{N}. Since divalent cations can have complex effects on the activation and deactivation of potassium channels and conductive pathways, our results must be interpreted in that light. The use of 2 mmol/L Co^{2+} may have shifted the activation curve in the positive direction, similar to the effect of La^{3+} on cloned Kv1.5. This may be relevant when comparing the position of the activation curve of the human atrial current with cloned Kv1.5 channels that are studied in the absence of divalent cations needed in the present experiments to block Ca^{2+} channels.

**Conclusions**

We have defined the properties of the current underlying the sustained outward current elicited by depolarization of human atrial myocytes. The properties of this current suggest that it is the native counterpart to Kv1.5 channels cloned from human cardiac tissue. Because of its extremely rapid activation and relative lack of inactivation at physiological temperatures, I_{to1} can contribute to repolarization of the human atrial action potential and to the determination of action potential duration. Consideration of this current may lead to new insights into the physiological and pharmacologic determinants of human atrial repolarization.

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