Ultrarapid delayed rectifier current inactivation in human atrial myocytes: properties and consequences

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Feng, jianlin, Donghui Xu, Zhiguo Wang, and Stanley Nattel. Ultrarapid delayed rectifier current inactivation in human atrial myocytes: properties and consequences. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1717–H1725, 1998.—The ultrarapid delayed rectifier current ($I_{K,ur}$) plays a significant role in human atrial repolarization and is generally believed to show little rate dependence because of slow and partial inactivation. This study was designed to evaluate in detail the properties and consequences of $I_{K,ur}$ inactivation in isolated human atrial myocytes. $I_{K,ur}$ inactivated with a biexponential time course and a half-inactivation voltage of $-7.5 \pm 0.6 \text{mV (mean} \pm \text{SE})$, with complete inactivation during 50-s pulses to voltages positive to $+10 \text{mV (37°C)}$. Recovery from inactivation proceeded slowly, with time constants of $0.42 \pm 0.06$ and $7.9 \pm 0.9 \text{s at } -80 \text{mV (37°C)}$. Substantial frequency dependence was observed at $37^\circ C$ over a clinically relevant range of frequencies. Inactivation was faster and occurred at more positive voltages at $37^\circ C$ compared with room temperature. The voltage and time dependencies of $\text{Kv1.5}$ inactivation were studied in Xenopus oocytes to avoid overlapping currents and strongly resembled those of $I_{K,ur}$ in native myocytes. We conclude that, while $I_{K,ur}$ inactivation is slow, it is extensive, and slow recovery from inactivation confers important frequency dependence with significant consequences for understanding the role of $I_{K,ur}$ in human atrial repolarization.

A variety of studies have indicated the presence in human atrial myocytes of a rapidly activating, highly 4-aminopyridine (4-AP)-sensitive $K^+$ current with slow and partial inactivation (1, 10, 21). This current has been variously referred to as $I_{K,ur}$, for “ultrarapid delayed rectifier $K^+$ current” (21), or $I_{so}$, for “sustained outward current” (1, 11). We refer to the macroscopic current as $I_{K,ur}$ in the present manuscript. The voltage- and time-dependent properties of $I_{K,ur}$, along with its sensitivity to small 4-AP concentrations (1, 10, 21), identify it with currents resulting from the expression of the $\text{Kv1.5}$ $K^+$ channel subunit (7, 19). The role of $\text{Kv1.5}$ channel subunits in carrying $I_{K,ur}$ is further supported by the specific downregulation of $I_{K,ur}$ in cultured human atrial myocytes exposed to antisense oligodeoxynucleotides directed to the NH$_2$-terminal coding sequence of $\text{Kv1.5}$ (8).

One element of the physiology of $I_{K,ur}$ that remains poorly understood is its inactivation properties. $\text{Kv1.5}$ currents show significant, albeit slow, inactivation (17, 19). $I_{K,ur}$ inactivation is considered to be slow and partial (1, 10, 11, 21); however, the longest depolarizing pulses used to characterize $I_{K,ur}$ inactivation have been 4 s in duration, and steady-state inactivation did not appear to be achieved (1). There is reason to believe that $I_{K,ur}$ inactivation may be physiologically relevant, because the end-pulse current during 80-ms depolarizing pulses (which contains a significant contribution of $I_{K,ur}$; see Refs. 1, 10, 11, 21) shows a statistically significant decrease at rapid pulsing frequencies (9). We therefore set out to evaluate in detail the inactivation properties of $I_{K,ur}$, with the use of 50-s depolarizing pulses that allow for a much more accurate assessment of inactivation than was previously possible with shorter-duration pulses. In particular, we sought to clarify the voltage and time dependence of the development of $I_{K,ur}$ inactivation at room temperature and body temperature ($37^\circ C$), the time dependence of current recovery from inactivation at potentials comparable to the normal atrial resting potential, $-80 \text{mV (23)}$, and the frequency dependence of $I_{K,ur}$ at a holding potential of $-80 \text{mV}$ and physiological temperature. $I_{K,ur}$ was isolated from other currents, particularly the transient outward current ($I_o$), with the use of previously described protocols that rely on the slower inactivation kinetics and greater 4-AP sensitivity of $I_{K,ur}$ compared with $I_o$ (21). For additional comparison, we measured the inactivation properties that we observed for $I_{K,ur}$ with those of currents carried by $\text{Kv1.5}$ subunits during 50-s depolarizing pulses in Xenopus oocytes.

METHODS

Cell isolation. Specimens of human right atrial appendage were obtained from the hearts of 30 patients (61 ± 2 yr old, range 42–75 yr) undergoing aortocoronary bypass surgery. The procedure for obtaining the tissue was approved by the Ethics Committee of the Montreal Heart Institute. Samples were immersed in nominally $\text{Ca}^{2+}$-free Tyrode solution (100% $\text{O}_2$, $37^\circ C$) of the following composition (in mM): $136.0 \text{NaCl, 5.4 KCl, 1.0 MgCl}_2, 0.33 \text{Na}_2\text{HPO}_4, 10$ dextrose, and 10 HEPES (Sigma), pH adjusted to 7.4 with NaOH. The myocardial specimens were chopped with scissors into cubic chunks and placed in a 25-ml flask containing 10 ml of the $\text{Ca}^{2+}$-free Tyrode solution. The tissue was gently agitated by continuous bubbling with 100% $\text{O}_2$ and stirring with a magnetic bar. After an initial 5 min in this solution, the chunks were reincubated in a similar solution containing 200 U/ml collagenase (CLS II; Worthington Biochemical) and 4 U/ml protease (type XXIV; Sigma). The first supernatant was removed after 45 min and discarded. The chunks were then reincubated in a
fresh enzyme-containing solution. Microscopic examination of the medium was performed every 15 min to determine the number and quality of the isolated cells. When the yield appeared to be maximal, the chunks were suspended in a storage solution of the following composition (in mM): 20 KCl, 10 K2HPO4, 10 glucose, 70 glutamic acid, 10 β-hydroxybutyric acid, 10 taurine, 10 EGTA, and 0.1% albumin, pH adjusted to 7.4 with KOH, and 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 were allowed for cell adherence to the bottom of the chamber, and then the cells were superfused at 3 ml/min with a solution containing (in mM) 136.0 NaCl, 5.4 KCl, 0.8 MgCl2, 1.0 CaCl2, 0.33 NaH2PO4, 10 HEPES, and 5.5 glucose, pH adjusted to 7.4 with NaOH. Experiments were conducted at room temperature (23–25°C) or at 37°C (with the use of a Peltier-effect device). All studies were performed within 12 h of the completion of cell isolation.

Whole cell patch-clamp methods. The whole cell patch-clamp technique was employed to record ionic currents in the voltage-clamp mode. Borosilicate glass electrodes (1.0-mm OD) were used, with tip resistances of 1.5–3 MΩ when filled with the pipette solution. 110 potassium aspartate, 20 KCl, 10 MgCl2, 10 HEPES, 5 EGTA, 5 MgATP, and 5 Na2-glycerophosphate (pH adjusted to 7.4 with KOH) and connected to a patch-clamp amplifier (Axopatch 200A; Axon Instruments). Command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon). Recordings were low-pass filtered at 5 kHz and stored on the hard disk of an IBM compatible computer.

Offset voltages generated when the pipette was inserted in Tyrode solution (2–8 mV) were zeroed before formation of the membrane-pipette seal. Mean seal resistance averaged 10.9 ± 1.8 GΩ (n = 35). Several minutes after seal formation, the membrane was ruptured by gentle suction to establish the whole cell configuration for voltage clamping. The series resistance (R_s) 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 a 5-mV hyperpolarization from a holding potential of −60 mV, divided by the voltage drop). Before R_s compensation, the capacitive time constant was 548 ± 34 μs (cell capacitance: 79 ± 3.8 pF; n = 35). After R_s compensation, the time constant was reduced to 145 ± 12 μs. The initial R_s was calculated to be 6.8 ± 0.2 MΩ, and R_c was reduced to 2.0 ± 0.1 MΩ after compensation. Currents recorded during this study rarely exceeded 2 nA, and the voltage drop across R_s did not exceed 5 mV. Cells with significant leak currents were rejected, and leakage compensation algorithms were not used.

To minimize possible contamination from delayed rectifier (I_k) inward rectifier (I_kr), and acetylcholine-dependent (I_ac) currents, the following chemicals were used in the extracellular solution for I_kr recording: tetraethylammonium chloride (10 mM, to inhibit I_kr; Sigma), atropine (100 mM, to inhibit I_ac; Sigma), and CdCl2 [200 μM, to block the Ca2+ current (I_Ca; Sigma)]. The sodium current (I_Na) was suppressed by isomolar replacement with choline chloride (Sigma) for NaCl in the bath solution. In some experiments, I_KC, the suppressed with the use of 0.5 mM BaCl2, which does not affect I_kr (21).

Functional expression of Kv1.5 in Xenopus oocytes. The Kv1.5 cDNA was subcloned into pSP64 (Promega) for coocyte expression. cRNA for injection into oocytes was prepared as previously described (22) with the mMESSAGE mMACHINE kit (Ambion) using SP6 polymerase after linearization of the plasmid with EcoRI. The samples were dissolved in 0.1 M KCl, stored at −80°C, and diluted immediately before injection. Stage V-VI Xenopus oocytes were injected with 46 nl of cRNA (~300 ng).

Whole cell two-microelectrode voltage-clamp recording. Whole cell macroscopic currents were recorded with conventional two-electrode techniques as previously described (6). Microelectrodes were filled with 3 M KCl and had resistances in the range of 0.8 MΩ. The bath solution contained (in mM) 100 NaCl, 5 KCl, 0.3 CaCl2, 2 MgCl2, and 10 HEPES. The pH was adjusted to 7.4 with Tris base. Signals were acquired with a Geneclamp amplifier, digitized at 10 kHz, and low-pass filtered at 3 kHz. Experiments were conducted at room temperature (20–22°C).

Data analysis. Group data are presented as the means ± SE. Nonlinear curve fitting was performed with the Clampfit routine in pCLAMP 6 (Clampfit algorithm). Ionic current data, which are displayed along with fitted curves in Figs. 1–7, are represented with the use of the data reduction algorithm in pCLAMP 6, so that the relation between fitted curves and data is clear; if all data points were shown, fitted curves would be obscured by data points. Statistical comparisons among groups were performed with analysis of variance (ANOVA). If significant effects were indicated by ANOVA, a t-test with Bonferroni correction or a Dunnett’s test was used to evaluate the significance of differences between individual means. A two-tailed P < 0.05 was taken to indicate statistical significance.

RESULTS

Time-dependent inactivation of I_kur. When a 100-ms pulse was applied to +40 mV, 5 ms after a prepulse to +40 mV to inactivate I_to, a rapidly activating and slowly inactivating current was observed at 37°C, with a small tail current during an 80-ms repolarization to −20 mV (Fig. 1A). These findings are typical of I_kur (21). Figure 1B shows a recording obtained with a 50-s pulse to +40 mV after a similar prepulse. During the prolonged pulse, considerable inactivation is observed. When the data shown in Fig. 1B were fitted by nonlinear curve-fitting methods, the best fit was provided by a biexponential equation. The curve fit to the data in Fig. 1B is shown in Fig. 1C, with time constants of 1.0 and 6.8 s. Note that extensive data reduction was used to provide the data points in Fig. 1C, otherwise, the fitting would be totally obscured by overlying data points; however, curve fitting was performed with the complete set of data shown in Fig. 1B. A similar approach is taken to illustrate curve fits to inactivation data throughout this paper (e.g., see Figs. 3A and 7A).

To evaluate further the possible nature of the slowly but extensively inactivating component, we studied its response to the K+ channel blocker 4-AP. Figure 2A shows the response to 4-AP of current elicited by a 50-s pulse to +40 mV, after a 100-ms prepulse to inactivate I_to. A large inactivating component was seen before 4-AP. Exposure to 10 μM 4-AP substantially depressed the inactivating current. At 250 μM, 4-AP fully suppressed the inactivating current, leaving only a time-independent component. Note that 4-AP had no effect on current at the end of the 50-s pulse (i.e., there was no sustained component to 4-AP-sensitive current). Fig-
ure 2B shows the concentration dependence of 4-AP inhibition of the inactivating current. The latter was measured on the basis of the difference between peak and end-pulse current during a 50-s pulse to +40 mV, with a 100-ms prepulse used to eliminate I\textsubscript{to}. The concentration-response curve at 37°C (mean ± SE of 6 cells) provided an EC\textsubscript{50} for current inhibition of 10.2 ± 1.2 µM, and virtually complete inhibition of current was seen at 100 µM. Results at room temperature are shown in Fig. 2B (mean ± SE of 6 cells) and indicate an EC\textsubscript{50} of 21.6 ± 2.3 µM, significantly higher (P < 0.01) than at 37°C. The 4-AP sensitivity of the inactivating current is compatible with that previously reported for I\textsubscript{K,ur} (1, 13, 21), with reported EC\textsubscript{50} values in the range of 6–50 µM at room temperature and full inhibition at 250–500 µM. The properties of the slowly inactivating current therefore identify it with I\textsubscript{K,ur}. Note that there was no significant 4-AP-sensitive sustained component at 37°C after 50 s at +40 mV. Mean current at 37°C in inhibited by 500 µM 4-AP at the end of a 50-s pulse averaged 4 ± 1 pA, compared with 819 ± 97 pA for peak 4-AP-sensitive current after a 100-ms prepulse to inactivate I\textsubscript{to}, and 821 ± 97 pA for inactivating current during a 50-s pulse after a 100-ms prepulse to remove I\textsubscript{to} (n = 6). These results show that I\textsubscript{K,ur} inactivates completely during 50-s pulses and that the size of the inactivating current during a 50-s pulse (after a prepulse to inactivate I\textsubscript{to}) is an accurate reflection of I\textsubscript{K,ur} amplitude as measured by 4-AP-sensitive current. Because a 50-s pulse was sufficient to achieve complete inactivation of I\textsubscript{K,ur} at 37°C and near-complete inactivation at room temperature, 50-s pulses were used to study I\textsubscript{K,ur} inactivation properties. At least 1 min was allowed at the holding potential between pulse protocols to ensure full recovery from inactivation.

Time and voltage dependence of I\textsubscript{K,ur} inactivation. To analyze the time dependence of I\textsubscript{K,ur} inactivation, we applied 50-s pulses from −50 mV to test potentials between 0 and +50 mV. Experiments were performed at both room temperature and 37°C, with both temperatures studied for each cell, and a 100-ms prepulse to +40 mV was delivered 5 (at 37°C) or 10 (at room temperature) ms before the test pulse to inactivate I\textsubscript{to}. Figure 3A illustrates I\textsubscript{K,ur} inactivation during a 50-s pulse to +40 mV in one cell. Note that inactivation is much faster at 37°C than at room temperature: inactivation was still proceeding (albeit very slowly) at the end of the 50-s pulse at room temperature, whereas it
was completed within ~20 s at 37°C. Data were fitted by biexponential functions of the form \( I = A_1 \exp(-t/\tau_f) + A_2 \exp(-t/\tau_s) \), as illustrated in Fig. 3A, where \( I \) indicates current, \( A_1 \) is the amplitude of the fast component, \( A_2 \) is the amplitude of the slow component, \( t \) is time, \( \tau_f \) is the time constant of the fast component, and \( \tau_s \) is the time constant of the slow component. This analysis provided the mean ± SE time constants for 12 cells per group shown in Fig. 3B. The fast-phase time constants were significantly voltage dependent at both temperatures (\( P < 0.05 \), ANOVA), whereas slow-phase time constants were not voltage dependent. The Time constants were much faster at 37°C; for example, at +40 mV the time constants averaged 1.0 ± 0.1 and 6.8 ± 0.9 s at 37°C compared with 2.3 ± 0.4 and 22.2 ± 1.3 s (\( P < 0.01 \) for each) at room temperature. The relative amplitude (\( A_1/A_2 \)) of fast and slow phases of inactivation were also temperature dependent, as shown in Fig. 3C, with faster inactivation comprising a larger proportion of overall inactivation at higher temperatures. Figure 3D shows the results of experiments designed to assess the development of \( I_{K,ur} \) inactivation at 37°C and +40 mV with the use of conditioning pulses of varied duration, followed 5 ms later by a 100-ms prepulse to inactivate \( I_{K,ur} \) and then 5 ms later by a 100-ms test pulse to +40 mV to elicit \( I_{K,ur} \). This protocol was applied before and after the addition of 500 µM 4-AP, with \( I_{K,ur} \), quantified on the basis of 4-AP-sensitive current during the test pulse. The fast and slow time constants averaged 1.3 ± 0.3 and 6.3 ± 0.8 s, and \( A_1 \) and \( A_2 \) averaged 0.65 ± 0.07 and 0.35 ± 0.05, respectively.

To evaluate the voltage dependence of \( I_{K,ur} \), inactivation, we used 50-s prepulses from the holding potential of −80 mV to various voltages, followed by 240-ms test pulses to +40 mV (protocol delivered every 60 s). Because \( I_{K,ur} \) inactivates fully within 100 ms at 37°C (21), the current at the end of the test pulse consists of \( I_{K,ur} \), nonselective cation current (5), and a small leak component. We separated \( I_{K,ur} \) from the other currents on the basis of its sensitivity to 4-AP, by performing experiments in the absence and presence of 500 µM 4-AP and taking the 4-AP-sensitive end-pulse component as a reflection of \( I_{K,ur} \). Figure 4A shows currents recorded during the 240-ms test pulse from a representative cell at 37°C. Figure 4B shows results from the same cell with the same protocol, after the addition of 500 µM 4-AP to the bath solution. The 4-AP-sensitive current from the same cell is shown in Fig. 4C. Mean data for six cells studied at 37°C are shown in Fig. 4D. Total current amplitude (measured at the end of the pulse) decreases as prepulse voltage becomes more positive, with a steady-state value attained at about +10 mV.
The 4-AP-resistant component shows no voltage-dependent change, whereas the 4-AP-sensitive component shows voltage-dependent inactivation that is complete at +10 mV. Results were fitted by Boltzmann functions as shown. Similar experiments were conducted at room temperature, giving the mean data from six cells shown in Fig. 4E. The results at room temperature are qualitatively similar to those at 37°C. Figure 4F shows the normalized inactivation curves for 4-AP-sensitive current at room temperature and 37°C. The half-inactivation voltage averaged −23.1 ± 2.6 mV at room temperature and −7.5 ± 0.6 mV at 37°C (P < 0.001).

Time-dependent recovery from inactivation and frequency dependence of current. A two-pulse protocol was used to study time-dependent recovery of \( I_{K_{ur}} \) at 37°C, as illustrated in Fig. 5A. A pair of 50-s pulses (first pulse designated \( P_1 \) and second pulse designated \( P_2 \)) from −80 to +40 mV were applied with varying coupling intervals, with the \( P_2 \) pulse preceded by a 100-ms prepulse (ending 5 ms before \( P_2 \)) to inactivate \( I_{K_{ur}} \). As shown in Fig. 5A, there was very little time-dependent current at short \( P_1-P_2 \) intervals, with a progressive recovery in current as the \( P_1-P_2 \) interval increased. Mean ± SE data from six cells are shown in Fig. 5B, along with the best-fit biexponential function. Recovery was complete within ~20 s and was always a biexponential function of the \( P_1-P_2 \) interval. The fast and slow recovery time constants averaged 419 ± 59 ms and 7.2 ± 0.9 s, and the amplitude of the fast and slow components averaged 0.61 ± 0.05 and 0.43 ± 0.05, respectively.

The data provided above indicate that, although \( I_{K_{ur}} \) inactivates slowly, it is capable of inactivating fully, and recovery from inactivation is relatively slow. These observations would suggest the possibility of significant frequency dependence. We therefore studied the frequency dependence of \( I_{K_{ur}} \) with the use of a train of 100 pulses (from a holding potential of −80 to +40 mV) of 100- or 200-ms duration, followed by a single 100-ms prepulse to inactivate \( I_{K_{ur}} \), and then 5 ms later a test pulse (140 ms) to +40 mV, followed by repolarization for 60 ms to −20 mV to record \( I_{K_{ur}} \) tail current. The protocol was applied before and after 500 µM 4-AP, and the frequency dependence of \( I_{K_{ur}} \) was determined in the following two ways: 1) based on the tail current, which with the protocol used represents only \( I_{K_{ur}} \), and 2) 4-AP-sensitive step current. Similar results were obtained with both analyses. Figure 5C shows the 4-AP-sensitive current from a typical experiment. Increases in frequency caused progressive reductions in \( I_{K_{ur}} \) amplitude. Mean results from six experiments with 200-ms pulses and six experiments with 100-ms pulses are shown in Fig. 5D and indicate that \( I_{K_{ur}} \) demonstrates highly significant frequency dependence (P < 0.001 for each pulse duration) over a clinically relevant range of frequencies (0.5–4 Hz).
The final set of experiments in native myocytes was performed to evaluate possible changes in the reversal potential of $I_{Kur}$ during depolarization to assess the possibility that apparent current inactivation may be due to accumulation of $K^+$ in a restricted extracellular space and a positive shift in the reversal potential. A double-pulse protocol was used to measure the reversal potential of current at 37°C after a 100-ms (Fig. 6A) and a 5-s (Fig. 6B) pulse to $+40$ mV. In five cells, the reversal potential averaged $-75.1 \pm 4.1$ mV for the 100-ms pulse and $70.8 \pm 6.2$ mV for the 5-s pulse ($P$ not significant). This small and statistically nonsignificant change in reversal potential can account for only a 5% decrease in $I_{Kur}$, in contrast to the 84% decrease after 5 s at $+40$ mV seen at 37°C in the experiments illustrated in Fig. 3A.

Inactivation properties of Kv1.5 current expressed in Xenopus oocytes. We applied a variety of approaches (including the use of prepulse protocols and the pharmacological probe 4-AP) to isolate $I_{Kur}$; however, it is impossible in native systems that express a variety of currents to be sure that the currents studied are free from contamination by other currents and are undistorted by the manipulations used to suppress other currents. We therefore expressed Kv1.5 channels in Xenopus oocytes and assessed the voltage and time dependence of Kv1.5 inactivation at room temperature during 50-s depolarizing pulses as applied to study $I_{Kur}$.

Fig. 5. Time-dependent recovery and frequency dependence of $I_{Kur}$. A: currents recorded at 37°C during 50-s test pulses (P2) from one cell, after a 50-s conditioning pulse (P1) to $+40$ mV with P1–P2 intervals of 50 s (largest current) and 20, 6, 6, 0.2, and 0.1 s, respectively (progressively decreasing currents). Test pulse was preceded by a 100-ms prepulse to inactivate $I_{to}$. B: recovery of $I_{Kur}$ based on amplitude of inactivating current during 50-s test pulses recorded as illustrated in A. Results at each P1–P2 interval are normalized to current at a P1–P2 interval of 50 s. Results are means ± SE from 6 cells. C: currents recorded at 37°C with the pulse protocol shown, delivered 100 ms after a train of 100 pulses at the frequencies (F) indicated. Currents are shown following a 100-ms prepulse to inactivate $I_{to}$, which was followed 5 ms later by a 140-ms test pulse to record $I_{Kur}$ and a 60-ms repolarization to 20 mV to record $I_{Kur}$ tail currents. The largest current was recorded at 0.1 Hz, and currents decreased progressively as frequency increased to 4 Hz. D: mean ± SE tail currents and 4-AP-sensitive step currents elicited with the protocol shown in C by test pulses after trains of 100- or 200-ms pulses at the frequencies indicated. Results are from 6 cells at each data point.

Fig. 6. Reversal of tail currents recorded from one cell at 37°C with the use of a 100-ms (A) and a 5-s (B) activating pulse. There was no significant change in the reversal potential, indicating that the substantial $I_{Kur}$ decrease that occurred during the 5-s pulse could not be attributed to extracellular $K^+$ accumulation. Similar results were obtained in a total of 5 cells.
Figure 7A shows the time course of Kv1.5 current inactivation during a 50-s pulse from 80 to +40 mV. The data were best fit by a biexponential relation, with time constants of 1.32 and 16.77 s in the example shown. Figure 7B shows mean time constants for Kv1.5 inactivation as measured at room temperature in 12 oocytes, which are of the same order as the I_{K,ur} inactivation time constants at room temperature shown in Fig. 3B. Figure 7C shows recordings from a representative experiment to study the voltage dependence of Kv1.5 inactivation. A 300-ms test pulse to +40 mV was preceded by 50-s conditioning pulses to voltages between −100 and +20 mV. In Fig. 7, only the currents for conditioning pulse voltages of −100, −80, and −60 to 0 mV are shown for the sake of clarity. Figure 7D shows mean data from five oocytes. The data were well fitted by a Boltzmann relation, as shown. The best-fit Boltzmann relation to data from each oocyte had a half-inactivation voltage that averaged 26.1 ± 3.1 mV, of the same order as values obtained for I_{K,ur} at room temperature, as shown in Fig. 4F, which was 23.1 ± 2.6 mV.

**DISCUSSION**

In the present study, we have shown that I_{K,ur} undergoes complete, albeit slow, voltage-dependent inactivation at positive potentials. The voltage and time dependencies of I_{K,ur} inactivation are quite similar to those of Kv1.5 channels expressed in Xenopus oocytes. Recovery of I_{K,ur} from inactivation at diastolic potentials is also very slow, so that I_{K,ur} density is quite sensitive to alterations in frequency over the physiological heart rate range in humans.

Comparison with previous studies of I_{K,ur} and Kv1.5 inactivation. In the first studies of I_{K,ur}, the current was found to have slow and partial inactivation (20% inactivation over 2 s at room temperature; see Ref. 21). Firek and Giles (10) noted a contribution of a slowly inactivating component to the pedestal current in human atrial cells, with a time constant of 1.7 s at 33°C. The pedestal component showed voltage-dependent inactivation when the latter was studied with the use of prepulses, with inactivation increasing as prepulse duration was increased from 400 to 2,500 ms, to a maximum of 50% (10). Amos et al. (1) studied outward current inactivation upon depolarization of human atrial myocytes at room temperature and noted a small slowly inactivating component with a time constant of 1.4 s during 2-s pulses at room temperature. This slowly inactivating component had many of the features of I_{K,ur} (voltage dependence, 4-AP sensitivity, etc.) and had a half-inactivation voltage of −9 ± 1 mV under their experimental conditions.

Studies of Kv1.5 inactivation have also been somewhat limited. Philipson et al. (17) obtained an inactivation time constant for Kv1.5 channels expressed in Xenopus oocytes in the range of 2 s with the use of 1-s pulses at room temperature. The half-inactivation voltage was −26 mV. Snyders et al. (19) expressed Kv1.5 channels in mouse-derived L cells and studied their biophysical properties at room temperature in greater detail. Inactivation was found to be slow and partial, averaging 20 ± 2% after 250 ms at +60 mV and 69 ± 3% during a 5-s pulse to +50 mV. In three cells subjected to 30-s depolarizing pulses, inactivation averaged 86 ± 1%. During 5-s pulses, inactivation developed as a biexponential function with time constants of the order of 200–300 ms and 2–3 s and weak voltage dependence. The recovery from inactivation after 5-s pulses was monoexponential with a time constant of 1.65 ± 0.11 s, and the half-inactivation voltage was −25 ± 4 mV.

The present study is consistent with previous observations in the literature but expands upon them sub-
stantially by studying in detail the time course of development and removal of inactivation in native myocytes with the use of pulses sufficiently long to achieve steady state at 37°C. There are no systematic studies in the literature of $I_{K,ur}$ or Kv1.5 current inactivation with the use of depolarizing pulses of $>5$ s in duration. With slow-phase inactivation time constants in the range of 22 s at room temperature (based on our observations), a 5-s pulse is clearly insufficient to characterize adequately the properties of inactivation. Contrary to most previous observations using shorter pulses, we found that $I_{K,ur}$ and Kv1.5 currents inactivate extensively; in fact, inactivation is complete if depolarization to positive voltages is maintained for sufficient periods of time. Because of relatively slow recovery from inactivation at diastolic potentials, $I_{K,ur}$ shows significant frequency dependence at physiological temperatures.

Novel aspects and potential importance. Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice, and the therapeutic options presently available are suboptimal (16). The frequency-dependent behavior of human atrial repolarization is an important determinant of the occurrence of atrial reentrant arrhythmias and, in particular, AF (2, 3) and may be a target for antiarrhythmic drug action (23). It is therefore very important that a better understanding be obtained of the basic ionic mechanisms underlying the response of the human atrial action potential to changes in heart rate. Because of its slow and partial inactivation, $I_{K,ur}$ has been considered a current that is relatively frequency independent under physiological conditions. The present report shows that this is far from the case. Although $I_{K,ur}$ inactivation develops slowly, it is potentially quite extensive, and recovery is slow at diastolic potentials. Consequently, the degree of inactivation at the onset of an action potential will depend on the relative times the cell spends at voltages positive to $-50$ mV (at which inactivation begins at 37°C) versus voltages negative to $-50$ mV. During rapid arrhythmias, such as AF, atrial myocytes may remain at voltages positive to $-50$ mV for most of the cycle, and substantial $I_{K,ur}$ inactivation might then be expected. Even with pulse durations as short as 100 ms, we found that $I_{K,ur}$ showed substantial rate-dependent behavior at 37°C over the frequency range between 0.1 and 4 Hz (Fig. 5D). Consideration of the kinetics and magnitude of $I_{K,ur}$ inactivation may thus be important in understanding the rate dependence of human atrial repolarization.

As activation rate increases, action potential duration tends to decrease, largely because of $I_{Ca}$ inactivation (4, 15, 24). This rate-dependent acceleration in repolarization decreases the refractory period and therefore the minimum path length over which reentry can occur (the wavelength) and thereby promotes the occurrence of reentrant arrhythmias (18). The rate-dependent decrease in $I_{K,ur}$ may tend to offset the decrease in $I_{Ca}$ resulting from tachycardia and thereby result in a longer action potential during tachycardia than if $I_{K,ur}$ were rate independent. Thus the rate-dependent properties of $I_{K,ur}$ may serve a protective role against reentrant arrhythmia. Theoretically, a similar role may be suggested for $I_{K,ur}$ downregulation, which has been demonstrated in patients with AF (20).

There is considerable evidence that $I_{K,ur}$ is present in human atrium but not human ventricle (1, 8, 12, 14). This observation makes $I_{K,ur}$ a potentially attractive target for the development of ion channel-selective antiarrhythmic drugs with reduced ventricular proarrhythmic potential. The rate-dependent behavior of $I_{K,ur}$ is important to consider for the therapeutic application of such compounds. If $I_{K,ur}$ is decreased substantially at rapid activation rates (e.g., during AF), $I_{K,ur}$ blockers may be much more effective in preventing AF, by virtue of the large amplitude of $I_{K,ur}$ during slower sinus rates, than in terminating the arrhythmia.

Several groups have identified a kinetically slower component of $I_{to}$ in human atrial myocytes and have suggested that this component is related to Kv1.5 channels (1, 10, 11). Our observations provide direct support for this conjecture. The slowly inactivating component in human atrium has been analyzed as a monoexponential function (1, 10, 11). Our results suggest that $I_{K,ur}$ inactivation is, in fact, biexponential, as noted for Kv1.5 currents in our study and previously published findings (19). The observation of slow, monoexponential decay of outward current in human atrial cells is probably due to the relatively short pulse durations used ($<4$ s at room temperature (1), $<2.6$ s at 33°C (10)), which would be insufficient to detect with any precision the slow phase of $I_{K,ur}$ inactivation with time constants ranging from 22 s (at room temperature) to 7 s (at 37°C). The substantial temperature sensitivity of both the relative magnitude and the rate of fast and slow $I_{K,ur}$ inactivation that we observed is important to consider when comparing various studies in the literature and when analyzing the potential physiological consequences of $I_{K,ur}$ inactivation.

Potential limitations. $K^+$ accumulation in extracellular fluids may reduce $K^+$ currents during prolonged depolarizations, giving the impression of inactivation in the absence of true voltage-dependent inactivation. To address this possibility, we measured the reversal potentials of tail currents after short (100-ms) and long (5-s) depolarizing pulses (Fig. 6). If $K^+$ accumulation is contributing importantly to current changes during the depolarizing pulse, significant decreases in reversal potential should be seen during the longer pulse. We found no statistically significant change at 37°C in the reversal potential with a 5-s pulse compared with a 100-ms pulse. On the basis of the actual reversal potentials measured, $K^+$ accumulation would account for a 5% decrease in $I_{K,ur}$ over the course of a 5-s pulse, rather than the 84% decrease observed experimentally.

The potential role of contaminating currents is always a concern in studies of native cells expressing multiple channel types. Experimental conditions were designed to minimize potential contamination by $I_{Na}$, $I_{Ca}$, $I_{K,ur}$, and $I_{K1}$. The most important potential overlapping current remaining was $I_{to}$. We isolated $I_{K,ur}$ with the use of prepulses to inactivate $I_{to}$ and with the use of
4-AP-sensitive current, as previously described (21). Even with these methods, however, potential questions remain with respect to the completeness of the suppression of overlapping currents and the degree to which measures used to isolate $I_{K\text{ur}}$ may have distorted it. We therefore studied the inactivation properties of Kv1.5 channels expressed in Xenopus oocytes and compared them with properties noted for $I_{K\text{ur}}$. The close similarity in the extent, kinetics, and voltage dependence of inactivation between Kv1.5 and $I_{K\text{ur}}$ provides strong support for the physiological validity of our observations regarding $I_{K\text{ur}}$.

We have demonstrated that $I_{K\text{ur}}$ undergoes extensive, albeit slow, inactivation and that slow recovery from inactivation confers substantial rate-dependent properties on $I_{K\text{ur}}$ over the physiologically relevant frequency range. The kinetics of $I_{K\text{ur}}$ inactivation were determined in detail at both room temperature and 37°C, and a close similarity was noted between the inactivation properties of $I_{K\text{ur}}$ and Kv1.5 current, supporting the notion that Kv1.5 channels carry a slowly inactivating outward $K^+$ current during depolarization of human atrial myocytes. Our findings point to a potentially important role of the rate-dependent properties of $I_{K\text{ur}}$ in contributing to rate-dependent behaviors of the human atrium, which are known to be associated with vulnerability to atrial reentrant arrhythmias. Furthermore, our observations need to be considered in the development of novel antiarrhythmic agents that target $I_{K\text{ur}}$ for the treatment of AF.

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