Two Functionally Distinct
4-Aminopyridine-sensitive Outward K⁺
Currents in Rat Atrial Myocytes

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ABSTRACT In the experiments here, the detailed kinetic properties of the
Ca²⁺-independent, depolarization-activated outward currents (I_out) in enzymatically
dispersed adult rat atrial myocytes were studied. Although there is only slight
attenuation of peak I_out during brief (100 ms) voltage steps, substantial decay is
evident during long (10 s) depolarizations. The analyses here reveal that current
inactivation is best described by the sum of two exponential components, which we
have termed I_fast and I_slow to denote the fast and slow components, respectively, of I_out
decay. At all test potentials, I_fast inactivates ~20-fold more rapidly than I_slow. Neither
the decay time constants nor the fraction of I_out remaining at the end of 10-s
depolarizations varies over the potential range of 0 to +50 mV, indicating that the
rates of inactivation and recovery from inactivation are voltage independent. I_fast
recovers from inactivation completely, independent of the recovery of I_slow and I_fast
recovers ~20 times faster than I_slow. The pharmacological properties of I_fast and I_slow
are similar: both components are sensitive to 4-aminopyridine (1–5 mM) and both
are relatively resistant to externally applied tetraethylammonium (50 mM). Taken
together, these findings suggest that I_fast and I_slow correspond to two functionally
distinct K⁺ currents with similar voltage-dependent properties and pharmacologic
sensitivities, but with markedly different rates of inactivation and recovery from
inactivation. From the experimental data, several gating models were developed in
which voltage-independent inactivation is coupled either to channel opening or to
the activation of the individual channel subunits. Experimental testing of predictions
of these models suggests that voltage-independent inactivation is coupled to
activation, and that inactivation of only a single subunit is required to result in
functional inactivation of the channels. This model closely approximates the
properties of I_fast and I_slow, as well as the composite outward currents, measured in
adult rat atrial myocytes.

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INTRODUCTION

In the mammalian myocardium, various types of depolarization-activated outward K$^+$ currents have been distinguished based on differences in time- and voltage-dependent properties and pharmacological sensitivities (Cohen, Dayner, Gintant, and Kline, 1986; Carmeliet, Biermans, Callawaert, and Vereecke, 1987). Transient outward currents, for example, are characterized by rapid activation and inactivation kinetics and sensitivity to millimolar concentrations of 4-aminopyridine (4-AP) (Kenyon and Gibbons, 1979; Coraboeuf and Carmeliet, 1982; Kukushkin, Gainullin, and Sosunov, 1983; Giles and Van Ginneken, 1985; Nakayama and Irisawa, 1985; Escande, Coulombe, Faivre, Deroubiax, and Coraboeuf, 1987; Kenyon and Sutko, 1987; Tseng, Robinson, and Hoffman, 1987; Benndorf and Nilius, 1988; Giles and Imaizumi, 1988; Apkon and Nerbonne, 1991). In Purkinje fibers and some cardiac myocytes, Ca$^{2+}$-dependent transient outward currents have also been described; these currents are blocked selectively by agents that inhibit voltage-dependent Ca$^{2+}$ influx or intracellular Ca$^{2+}$ release, and they are 4-AP resistant (Coraboeuf and Carmeliet, 1982; Escande et al., 1987; Kenyon and Sutko, 1987; Tseng et al., 1987; Giles and Imaizumi, 1988). Delayed outward K$^+$ currents in mammalian cardiac preparations, although somewhat less well characterized, have been distinguished (from transient currents) by slower activation and inactivation kinetics (McDonald and Trautwein, 1978; Hume and Uehara, 1985; Tseng et al., 1987; Giles and Imaizumi, 1988) and greater sensitivity to extracellular tetraethylammonium (Apkon and Nerbonne, 1991).

Recently, we reported the presence of a novel type of Ca$^{2+}$-independent, depolarization-activated outward K$^+$ current ($I_{\text{out}}$) in adult rat atrial myocytes with properties distinct from those of either the transient or delayed K$^+$ currents described in other cardiac preparations (Boyle and Nerbonne, 1991). Although $I_{\text{out}}$ in these cells activates rapidly and is blocked by 4-AP, it is distinct from other myocardial transient K$^+$ currents in that peak $I_{\text{out}}$ decays only slightly (15.7 ± 10.0%; mean ± SD) during brief (100 ms) depolarizations. The time- and voltage-dependent properties and the pharmacological sensitivity of atrial $I_{\text{out}}$ are also distinct from those of delayed outward K$^+$ currents described in other cardiac preparations. Interestingly, the properties of the atrial currents closely resemble those of several K$^+$ channels cloned from rat brain and, more recently, rat heart, cDNA libraries, and expressed in Xenopus oocytes (Stühmer, Stocker, Sakmann, Seeburg, Baumann, Grupe, and Pongs, 1988; Christie, Adelman, Douglass, and North, 1989; Stühmer, Ruppersberg, Schröter, Sakmann, Stocker, Giese, Baumann, and Pongs, 1989; Grupe, Schröter, Ruppersberg, Stocker, Drewes, Beckh, and Pongs, 1990; Koren, Liman, Logothetis, Nadal-Ginard, and Hess, 1990; Swanson, Marshall, Smith, Williams, Boyle, Folander, Luneau, Antanavage, Oliva, Buhrow, Bennett, Stein, and Kaczmarek, 1990; Tseng-Crank, Tseng, Schwartz, and Tanouye, 1990; Paulmichl, Nasmith, Hellmiss, Reed, Boyle, Nerbonne, Peralta, and Clapham, 1991; Roberds and Tamkun, 1991).

Although there is little attenuation of peak atrial $I_{\text{out}}$ during brief depolarizations, substantial decay is evident during prolonged (10 s) depolarizations. Inactivation of atrial $I_{\text{out}}$ is best described by the sum of two exponential components, which we have termed $I_{K_f}$ and $I_{K_i}$ to denote the fast and the slow components, respectively, of $I_{\text{out}}$. 
decay. The analyses here also reveal that $I_{Kf}$ and $I_{Ks}$ recover from inactivation independently and over substantially different time courses, indicating that $I_{Kf}$ and $I_{Ks}$ reflect currents through two functionally distinct populations of depolarization-activated K$^+$ channels. The other time- and voltage-dependent properties and the pharmacological sensitivities of $I_{Kf}$ and $I_{Ks}$, however, are indistinguishable. Several gating models were developed from the experimental data which were capable of generating currents with time- and voltage-dependent properties similar to those of $I_{Kf}$ and $I_{Ks}$, as well as those of the composite whole-cell current, $I_{tot}$. Predictions of these models were then tested experimentally, and one was found to approximate very closely all of the properties of the depolarization-activated K$^+$ currents in adult rat atrial myocytes.

**METHODS**

**Preparation of Isolated Myocytes**

Single atrial myocytes were isolated from adult rat heart using a procedure developed in this laboratory from procedures described previously by others (Powell and Twist, 1976; Wittenberg and Robinson, 1981). In brief, hearts were rapidly removed from anesthetized (halothane/O$_2$) adult Long Evans rats (200–250 g) and retrogradely perfused (at 37°C) through the aorta with Ca$^{2+}$-free Krebs buffer (50 ml) followed by perfusion with buffer containing either 0.10–0.2% type II collagenase (Worthington Biochemical Corp., Freehold, NJ) or 0.01–0.02% type IA collagenase (Sigma Chemical Co., St. Louis, MO) and 10 μM added Ca$^{2+}$. During perfusion, the hearts were kept immersed in the Krebs-enzyme solution, which was filtered (5 μm) and recirculated until the hearts were digested (20–40 min). After perfusion, the atrial appendages were removed, cut into small pieces, incubated in fresh Krebs-enzyme buffer for an additional 5–20 min, and mechanically dispersed with a fire-polished Pasteur pipette. The tissue was then filtered through gauze to remove undigested fragments and washed twice in enzyme-free Krebs containing 150 μM Ca$^{2+}$. The filtrate was enriched for rod-shaped cells by low speed centrifugation (300–500 rpm; 10 min). Dissociated cells were resuspended in serum-free Medium-199 (Irvine Scientific, Santa Ana, CA) supplemented with antibiotics (penicillin/streptomycin) and plated on glass coverslips coated with laminin (Collaborative Research, Inc., Bedford, MA). Rod-shaped, Ca$^{2+}$-tolerant myocytes adhered preferentially to the laminin substrate and damaged, rounded-up cells were removed by replacing the medium 5–10 min and again 30–60 min after plating.

Dissociated myocytes were maintained at 37°C in a humidified air/CO$_2$ (95:5) environment and many myocytes remained rod-shaped for 24–96 h in vitro. Because we wished to study a homogeneous population of cells, all experiments were performed on rod-shaped atrial myocytes within 48 h of isolation.

**Electrophysiological Recordings**

The whole-cell variation of the patch-clamp technique (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981) was used to record depolarization-activated K$^+$ currents in isolated adult rat atrial myocytes. All recordings were performed at room temperature (21–23°C). The voltage-clamp circuit was provided by an Axopatch-1B (0.5 GΩ feedback resistor; Axon Instruments, Inc., Foster City, CA) whole-cell/patch-clamp amplifier. Recording pipettes were fabricated from borosilicate glass, coated with Sylgard (Dow Corning Corp., Midland, MI), and fire-polished to produce electrodes with tip diameters of 1.0–2.0 μm and resistances of 2–4 MΩ when filled with recording solution (see below). After formation of a high resistance giga seal
In the whole-cell configuration, series resistances, estimated from the decay of the uncompensated capacitative transients, were found to equal 1.5–2 times the pipette resistance. Because series resistance was routinely compensated by >80% and peak outward current amplitudes were generally <5 nA, voltage errors due to uncompensated series resistance were small (<8 mV) and were not corrected. In all experiments, series resistance was checked at regular intervals to ensure that no variations occurred over time; if measurable increases developed, the experiment was discontinued and the data were not subsequently analyzed. Only cells with input resistances ≥0.4 GΩ were studied (range: 0.4–1.5 GΩ).

To measure Ca²⁺-independent, depolarization-activated outward K⁺ currents, bath solutions routinely contained (mM): 136 NaCl, 4 KCl, 1 CaCl₂, 2 MgCl₂, 10 HEPES, 10 glucose, 5 CoCl₂, and 0.02 tetrodotoxin (TTX) at pH 7.2–7.4, 300–310 mosmol. In some experiments, designed to explore the effects of high extracellular divalent cation concentrations and Co²⁺ on the properties of the atrial K⁺ currents, the Co²⁺ was replaced by 1 mM Cd²⁺ and 1 μM nifedipine was added to ensure the complete block of voltage-gated Ca²⁺ currents. These experiments (n = 8) revealed that the measured properties of the currents in these cells were largely unaffected, although there was a small shift (~3 mV) in the V₁/₂ for current activation as compared with the currents recorded in the presence of 5 mM Co²⁺. All of the current waveforms displayed and analyzed here were obtained under the routine recording conditions, i.e., with 5 mM Co²⁺ in the bath. Although the bath solution contained 20 μM TTX, complete block of voltage-dependent Na⁺ currents (I₉Na) was not always provided due to the presence of a TTX-resistant component of I₉Na in these cells (Reuter, 1984). In addition, the amplitude of the TTX-resistant component of I₉Na varied considerably among cells.

Recording pipettes routinely contained (mM): 135 KCl, 10 EGTA, 10 HEPES, 5 glucose with 3 Mg-ATP, and 0.5 Tris-GTP (pH 7.2, 300–310 mosmol). To determine if this high intracellular Cl⁻ concentration influenced the measured properties of these cells in general, or the outward K⁺ currents in particular, experiments were also completed using K-aspartate in place of the KCl in the recording pipettes. These experiments (n = 6) revealed that there are no measurable effects on the time- or voltage-dependent properties of the measured K⁺ currents in these cells as a function of the concentration of Cl⁻ or aspartate⁻ in the recording pipettes. All of the current waveforms displayed and analyzed here were obtained under the routine recording conditions, i.e., with 135 mM KCl in the recording pipettes.

Outward K⁺ currents were routinely measured during depolarizations to membrane potentials between −40 and +50 mV from a holding potential (HP) of −60 mV. This HP was chosen to minimize contamination of the outward current waveforms by the TTX-resistant component of I₉Na (see above). For studies of voltage-dependent inactivation, however, the range of HPs was −110–0 mV. Experimental parameters such as HPs, test potentials, and the timing of voltage steps were controlled with an AST-286 or Northgate 386 equipped with a Tecmar Labmaster (Scientific Solutions Inc., Solon, OH) analog/digital interface. Data acquisition was performed using pCLAMP (v4.1; Axon Instruments Inc.). Sampling frequencies ranged from 4 to 10 kHz and current signals were filtered at 3–5 kHz (corner frequency) before digitization and storage. All of the current records displayed (and all of the currents analyzed here) are raw records and no corrections have been made for linear "leakage" currents which are negligible in cells with input resistances of ≥0.4 GΩ. On average, atrial cells are 15 x 100 μm, yielding an estimated surface area of 5 x 10⁻⁵ cm², assuming the cells are cylindrical (Boyle and Nerbonne, 1991). Integration of the capacitative transients evoked during small voltage steps from a HP of ~70 mV provided a mean (±SD) membrane capacitance of 58.0 ± 16.5 pF (n = 13) for these cells.

4-AP (Sigma Chemical Co.) and tetraethylammonium (TEA; Sigma Chemical Co.) were applied to isolated myocytes during electrophysiological recordings by pressure ejection from
small (2–3 mm) puffer pipettes placed within ~20 mm of the cell surface. At least two puffer pipettes were used; one contained only bath solution and served as a control. 4-AP was dissolved in bath solution immediately before use. The TEA-containing solution was prepared by equimolar substitution (50 mM) of TEACl (Sigma Chemical Co.) for NaCl in the standard bath solution.

Data Analyses

Data were compiled and analyzed using pCLAMP, Lotus 1-2-3 (Lotus Corporation, Cambridge, MA), and NFIT (Island Products, Galveston, TX). The kinetics of outward current decay were analyzed using Clampfit (pClamp) as described in the text and the time constants and magnitudes of the current components were determined from the fitting procedure. In several cases, the experimental data and the results provided by the current simulations (see below) were analyzed using NFIT with functions presented in the text. Additional components were included in the fitting equations only if they substantially improved the quality of the fits as judged by the correlation coefficient (R) and by eye. Examples of fits are displayed in the figures together with the data points. All averaged and normalized data are presented as mean ± either the standard deviation of the mean (±SD) or the standard error of the mean (±SEM), as indicated. Statistical significance was examined using the analysis of variance (ANOVA).

Model Simulations

For modeling the atrial outward currents, a diagram was constructed of the kinetic states of the channels, and differential equations were written describing the time-dependent changes in the probability of channels existing in each of the proposed states. Simulations were accomplished using TUTSIM (Tutsim Products, Palo Alto, CA), with the instantaneous probabilities over time obtained by numerical integration at an interval of 100 ms. The modeled currents were generated at the same rate by multiplying the open state probability by the driving force, $I_{m} - V_{rev}$, where $I_{m}$ is the membrane potential and $V_{rev}$ is the mean measured reversal potential of $-75.5$ mV (Boyle and Nerbonne, 1991) of the currents (see text). Voltage-dependent activation (α) and deactivation (β) rate constants were derived from the analyses of the voltage dependences and activation rates of the measured currents (see text). Voltage-independent inactivation (κ) and recovery (λ) rate constants were derived from the inactivation and recovery data. In testing the models, the absolute values of $κ$ and $λ$ were adjusted until the inactivation and recovery kinetics of the simulated currents, as well as the amount of the noninactivating current, closely resembled those observed in the electrophysiological experiments.

RESULTS

Time- and Voltage-dependent Activation and Inactivation

Representative Ca$^{2+}$-independent depolarization-activated outward K$^{+}$ current ($I_{out}$) waveforms evoked in an isolated adult rat atrial myocyte during depolarizations to potentials between $-40$ and $+50$ mV from a HP of $-60$ mV are displayed in Fig. 1. All of the experiments here were completed with inward Ca$^{2+}$ current blocked by the Co$^{2+}$ in the bath. To date, we have not explored the effects of changing the intracellular or extracellular Ca$^{2+}$ concentration on the measured properties of these currents, and we have not determined if there are any Ca$^{2+}$-dependent K$^{+}$ currents in these cells. As previously reported (Boyle and Nerbonne, 1991), on membrane depolarization, $I_{out}$ activates rapidly after a delay and the time course of activation is well described by a fourth power exponential. In addition, current amplitudes as well
Figure 1. Activation and inactivation of depolarization-activated outward K+ currents ($I_{out}$) in an isolated adult rat atrial myocyte. Depolarizations from a HP of -60 mV to potentials between -40 and +50 mV were applied in 10-mV increments. Co2+ (5 mM) and TTX (20 µM) were present in the bath to block voltage-gated inward Ca2+ and Na+ currents, respectively. (A) 100-ms steps presented at 5-s intervals. Current amplitudes and activation rates increase with increasing depolarization and there is little or no attenuation of peak $I_{out}$ during the steps. The largest and most rapidly activating current was evoked at +50 mV. (B) 10-s steps presented at 60-s intervals in the same cell. Substantial inactivation of peak $I_{out}$ develops over several seconds. Calibration bars: 1 nA and 10 ms (A); 1 nA and 900 ms (B).

As activation rates increase with increasing depolarization (Fig. 1 A), although during brief (100 ms) depolarizations (Fig. 1 A) $I_{out}$ decays only slightly, substantial inactivation of peak $I_{out}$ develops over several seconds (Fig. 1 B).

As illustrated in Fig. 2 A, decay of $I_{out}$ during 10-s depolarizations is well described

(C) Mean ($\pm$ SEM; n = 5) values for the fast (●) and slow (○) inactivation time constants plotted as a function of test potential. (D) Mean ($\pm$ SEM; n = 5) normalized current–voltage relations for peak $I_{out}$ (□), $I_{Na}$ (●), $I_{Ks}$ (○), and $I_{Na}$ (▲); all of the currents were normalized to their respective values at +50 mV. Calibration bars: 1 nA and 900 ms (A, B).

Figure 2. Kinetics of outward current inactivation. (A) The outward currents displayed in Fig. 1 B are replotted here (as points) with double exponential fits (solid lines) to the decay phases of the currents superimposed. (Inset) Single exponential fit to the decay phase of the current evoked at +50 mV. (B) $I_{out}$ waveforms recorded at test potentials between 0 and +50 mV (from A) are superimposed after each was scaled to the peak $I_{out}$ recorded at +50 mV.

FIGURE 1. Activation and inactivation of depolarization-activated outward K+ currents ($I_{out}$) in an isolated adult rat atrial myocyte. Depolarizations from a HP of -60 mV to potentials between -40 and +50 mV were applied in 10-mV increments. Co2+ (5 mM) and TTX (20 µM) were present in the bath to block voltage-gated inward Ca2+ and Na+ currents, respectively. (A) 100-ms steps presented at 5-s intervals. Current amplitudes and activation rates increase with increasing depolarization and there is little or no attenuation of peak $I_{out}$ during the steps. The largest and most rapidly activating current was evoked at +50 mV. (B) 10-s steps presented at 60-s intervals in the same cell. Substantial inactivation of peak $I_{out}$ develops over several seconds. Calibration bars: 1 nA and 10 ms (A); 1 nA and 900 ms (B).
by a double exponential function of the form:

$$I_{out}(t) = I_{Kf} \cdot \left[ \exp \left(-\frac{t}{\tau_f}\right) \right] + I_{Ks} \cdot \left[ \exp \left(-\frac{t}{\tau_s}\right) \right] + I_s$$

where $I_{Kf}$ and $I_{Ks}$ are the rapidly and slowly inactivating fractions of the total outward current; $\tau_f$ and $\tau_s$ are the inactivation time constants of the rapidly and slowly decaying components; and $I_s$ is the steady-state current (see also below for further clarification of these terms). The fits were not as good when a single exponential was used (see Fig. 2 A, inset).

The values for $\tau_f$ and $\tau_s$ display no measurable voltage dependence over the potential range of 0 to $+50$ mV (Fig. 2 C). In addition, the magnitudes of $I_{Kf}$, $I_{Ks}$, and $I_s$ increase in parallel with that of peak $I_{out}$ over this same potential range. This is demonstrated by scaling and superimposing the currents recorded at various potentials in individual cells (Fig. 2 B), or by analysis of the normalized current–voltage relations for peak $I_{out}$, $I_{Kf}$, $I_{Ks}$, and $I_s$ in several cells (Fig. 2 D). These results suggest that the voltage dependences of activation of $I_{Kf}$ and $I_{Ks}$ are indistinguishable (see also below). In addition, assuming that $I_s$ reflects incomplete inactivation of $I_{Kf}$ and $I_{Ks}$, these results suggest that the rate constants governing inactivation and recovery from inactivation of $I_{Kf}$ and $I_{Ks}$ are voltage independent. It should be noted that the assumption that $I_s$ represents the noninactivating fractions of $I_{Kf}$ and $I_{Ks}$ is made throughout the remainder of this paper. Although difficult to prove experimentally, this assumption is consistent with all of the experimental data (as discussed below). Thus, although the terms $I_{Kf}$ and $I_{Ks}$ in Eq. 1 (above) and elsewhere in the text refer to the amplitudes of the inactivating fractions of the currents, the actual amplitudes of the two current components include the noninactivating fraction of each, represented by $I_s$.

Because the values for $\tau_f$ and $\tau_s$, as well as the relative magnitudes of $I_{Kf}$ and $I_{Ks}$, are unaffected by voltage over the potential range 0 to $+50$ mV, long depolarizations to a single test potential of $+50$ mV were presented to characterize $I_{out}$ inactivation in 38 additional atrial cells (Table I). In all but 2 of these 38 cells, two exponentials were required to describe the time course of $I_{out}$ decay, and in all cells examined there was an $I_s$ component, i.e., an outward current remaining at the end of the 10-s test pulses. There was some cell to cell variability in the values of $\tau_f$ and $\tau_s$ (Table I), although in most cells the ratio of $\tau_s$ to $\tau_f$ was $\geq 10$, with a mean ($\pm$SD) value for $\tau_s/\tau_f$ of $23.0 \pm 13.6$ ($n = 36$). Variability in the relative contribution of the two current components was observed in these cells.
components to the peak $I_{\text{out}}$ was also evident, although generally $I_{K_s}$ was larger than $I_{K_f}$ (Table I). As would be expected, the relatively small amount of inactivation of peak $I_{\text{out}}$ observed during brief (100 ms) depolarizations of $15.7 \pm 10\%$ (mean $\pm$ SD) correlates with the amount of $I_{K_f}$ inactivation predicted by the fits of peak $I_{\text{out}}$ decay during long depolarizations ($R = 0.95$). Thus, the variability in peak $I_{\text{out}}$ inactivation during brief depolarizations (Boyle and Nerbonne, 1991) appears to result from cell to cell variability in the relative contribution of $I_{K_f}$ to the peak $I_{\text{out}}$, as well as the precise value of $\tau$. 

Recovery from Inactivation

The time courses of recovery from inactivation of peak $I_{\text{out}}$, $I_{K_f}$ and $I_{K_s}$ were examined in paired pulse experiments in which two successive 10-s depolarizing pulses to $+50$ mV from a HP of $-60$ mV were applied at interpulse intervals ranging from 10 ms to 60 s. The decay phase of $I_{\text{out}}$ evoked during the first pulse was fitted according to Eq. 1 and the time constants derived from that fit were then used to fit $I_{\text{out}}$ decay during the second pulse of the pair. For each pair of pulses, the magnitudes of peak $I_{\text{out}}$, $I_{K_f}$ and $I_{K_s}$ derived from the fits were compared to determine the amount of recovery for a given interpulse interval; the cell was held at $-60$ mV for 60 s between pulse pairs to allow complete recovery of peak $I_{\text{out}}$. A sequence of four paired pulses (to the same cell) in which the interpulse interval was varied from 3 to 52 s is shown in Fig. 3. The data and the fits for each pulse pair are displayed, and the magnitudes and percent changes in peak $I_{\text{out}}$, $I_{K_f}$ and $I_{K_s}$ are given in the insets of the figure. Although the various parameters (e.g., $I_{K_f}$, $\tau_f$, etc.) were left free when each of the control current waveforms was fitted, there were no significant differences in the values obtained from these fits (see insets) even though the time required to complete these experiments was rather long and the total time between recording the control currents for each paired pulse experiment was several minutes. These data demonstrate that the properties of the currents do not vary substantially over time.

For the cell in Fig. 3, peak $I_{\text{out}}$ recovered to $\sim 80\%$ of the control value and $I_{K_f}$ recovered completely when the interpulse interval was 3 s (Fig. 3A). The remaining $\sim 20\%$ of the peak $I_{\text{out}}$ recovered over a much longer time course, and complete recovery was only evident when the interpulse interval was increased to 52 s (Fig. 3, B–D). The slow phase of peak $I_{\text{out}}$ recovery was due to the slow recovery of $I_{K_s}$ (Fig. 3, insets). Thus, $I_{K_f}$ recovers from steady-state inactivation completely independent of the recovery of $I_{K_s}$.

Paired pulse experiments, such as that illustrated in Fig. 3, required long periods of stable recording conditions. As such, increased series resistances or "leak" currents often resulted in premature termination of these experiments before sufficient data over a wide range of interpulse intervals could be collected. Nevertheless, in a total of 20 atrial cells, long enough interpulse intervals were tested to demonstrate the complete recovery of $I_{K_f}$ "independent" of the recovery of $I_{K_s}$ (similar to the results presented in Fig. 3). Although the complete recovery of $I_{K_s}$ (and peak $I_{\text{out}}$) was not demonstrated in all of these cells, sufficient data were obtained over a range of interpulse intervals in six cells to demonstrate the complete recovery of both $I_{K_f}$ and $I_{K_s}$ (Fig. 4). In all six of these cells, the complete recovery of $I_{K_f}$ (Fig. 4A) occurred independent of the complete recovery of $I_{K_s}$ (Fig. 4B). These experiments confirmed
FIGURE 3. Recovery of \( I_{\text{out}} \) from inactivation. Pairs of 10-s depolarizing pulses to +50 mV from a HP of -60 mV were applied at interpulse intervals of (A) 3 s, (B) 7 s, (C) 20 s, and (D) 52 s; 60 s was allowed between each pair of pulses to allow complete recovery of peak \( I_{\text{out}} \). The decay phases of the currents (plotted as points) were fitted (solid lines) with double exponential functions. In each case, the fast and slow time constants determined from the fit of the current recorded during the first pulse of each pair (1) was used to fit the second current waveform (2) recorded. (Insets) The amplitudes of peak \( I_{\text{out}} \), \( I_{Kf} \), and \( I_{Ks} \) derived from the fits for the first (1) and second (2) records of each pulse pair are listed. The percent change (\( \Delta \)) in each component was calculated as \( \frac{(I_2 - I_1)}{I_1} \times 100 \). Calibration bars: 800 pA and 1 s (A–D).

FIGURE 4. Time course of recovery from inactivation of (A) \( I_{Kf} \) and (B) \( I_{Ks} \) during paired pulse experiments similar to those illustrated in Fig. 3. The time courses of recovery of \( I_{Kf} \) and \( I_{Ks} \) were determined from double exponential fits to the decay phases of \( I_{\text{out}} \) recorded during pairs of 10-s depolarizing pulses to +50 mV (as in Fig. 3). Data obtained from six atrial cells are presented; each cell is indicated by a different symbol. (A) In general, \( I_{Kf} \) recovered rapidly and completely after interpulse intervals that left \( I_{Ks} \) substantially inactivated, e.g., at times \( \leq 10 \) s. (B) In all six cells, however, \( I_{Ks} \) recovered (to \( \geq 90\% \) of control) after long interpulse intervals.
that the long interpulse intervals required for complete recovery of peak \( I_{\text{out}} \) were clearly the result of the slow recovery of \( I_{Ks} \). In addition, comparison of the time course of recovery suggests that \( I_{Kf} \) recovers from steady-state inactivation ~20 times faster than \( I_{Ks} \).

The finding that \( I_{Kf} \) recovers from inactivation independent of the recovery of \( I_{Ks} \) is most simply interpreted as suggesting that \( I_{Kf} \) and \( I_{Ks} \) are functionally independent currents. This concept can be viewed intuitively by considering that the complete recovery of \( I_{Kf} \) from inactivation requires the recovery of all of the \( I_{Kf} \) channels that opened during the depolarization. This, in turn, implies full recovery of the channels in each and every kinetic state connected to the open and/or inactivated state(s) of \( I_{Kf} \) channels. Thus, if \( I_{Kf} \) and \( I_{Ks} \) share kinetic states such that some channels initially inactivating via the \( I_{Kf} \) pathway end up in the \( I_{Ks} \) pathway, the net effect will be that any \( I_{Ks} \) channels that remain inactivated would decrease the number of channels available to open via \( I_{Kf} \) in a subsequent depolarization, thereby reducing the magnitude of the \( I_{Kf} \) relaxation. In other words, the complete recovery of \( I_{Kf} \) would depend on the recovery of \( I_{Ks} \). Therefore, because \( I_{Kf} \) recovers fully while \( I_{Ks} \) remains substantially inactivated, \( I_{Kf} \) and \( I_{Ks} \) cannot share any kinetic states. \( I_{Kf} \) and \( I_{Ks} \) must, therefore, be functionally independent currents.

To test this hypothesis directly and to examine the molecular mechanisms underlying the gating of these channels, we constructed models of channel gating incorporating the general features of voltage-dependent activation through multiple closed states and voltage-independent inactivation. The models used were similar to those proposed by others (Clark, Giles, and Imaizumi, 1988; Koren et al., 1990; Zagotta and Aldrich, 1990).

**Gating Models of Atrial \( I_{\text{out}} \)**

As noted above, we interpreted the finding that \( I_{Kf} \) and \( I_{Ks} \) recover from inactivation independently as suggesting that these two current components reflect the presence of functionally distinct \( K^+ \) channels or gating pathways. To test this hypothesis directly, we began the modeling by considering gating models incorporating two inactivated states of the same channel, which could be populated either sequentially or in parallel from the open state, to account for the two inactivation and recovery time constants for peak atrial \( I_{\text{out}} \).

Models of atrial \( I_{\text{out}} \) were based on the kinetic and voltage-dependent properties of the measured currents. Because the kinetics of \( I_{\text{out}} \) activation appeared not to be dependent on the relative amplitudes of \( I_{Kf} \) and \( I_{Ks} \), we assumed in this initial modeling that the time and voltage dependences of \( I_{Kf} \) and \( I_{Ks} \) activation were the same (see below). For simplicity, we also assumed that both components had the same reversal potential (of approximately ~75 mV) of the peak \( I_{\text{out}} \) reported previously (Boyle and Nerbonne, 1991; see also below). Atrial \( I_{\text{out}} \) activates with a delay and the time course of activation is best described by a fourth power exponential of the form: \( I_{\text{out}}(t) = I_{\text{max}} [1-\exp(-t/\tau_n)]^4 \) (Fig. 5A). Therefore, the Hodgkin and Huxley (1952) \( n^4 \) model was used in which channel opening requires activation of four independent subunits (or gating particles) governed by voltage-dependent forward (\( \alpha \)) and reverse (\( \beta \)) activation rate constants. This results in four closed states (C) which must be traversed before opening (O) according to the
following kinetic scheme:

\[
C_1 \xlongequal{4a} C_2 \xlongequal{3a} C_3 \xlongequal{2a} C_4 \xlongequal{a} O
\] (A)

Values for \( \alpha \) and \( \beta \) were calculated from the activation time constants (\( \tau_a \)) and the voltage-dependent steady-state probabilities of activation (\( n_a \)) of each subunit, where \( \alpha = n_a/\tau_a \) and \( \beta = (1 - n_a)/\tau_a \). Values for \( \tau_a \) at each voltage were determined from the activation time course of \( I_{out} \) described above (Fig. 5A) and values for \( n_a \) were extracted from the normalized conductance–voltage plot (Fig. 6A). Conductance was maximal at +50 mV and the normalized conductance (\( g/g_{max} \)) was taken to be equivalent to \( n_a^4 \) such that \( n_a = \sqrt[4]{g/g_{max}} \). The data are well described by a fourth power Boltzmann function of the form:

\[
g/g_{max} (n_a^4) = 1/[1 + \exp((V_m - V_{1/2})/s)]^4,
\]

with a half-maximal activation value (\( V_{1/2} \)) of -32 mV, corresponding to an \( n_a \) value of 0.5 (Fig. 6A). Lower power Boltzmann functions did not fit the activation data as well as when \( n = 4 \).

The values of \( \alpha \) and \( \beta \) at each voltage were best fitted (Fig. 6, B and C, solid lines) with continuous functions of voltage of the form:

\[
\alpha(s^{-1}) = 10 \cdot \frac{(V_m + 17)}{1 - \exp \left( \frac{(-V_m - 17)}{10} \right)}
\]

\[
\beta(s^{-1}) = 50 \cdot \exp \left( \frac{(-V_m - 15)}{37} \right)
\]

Figure 5. Modeling of outward current activation. (A) \( I_{out} \) waveforms (plotted as points) recorded in a typical atrial cell during the first 40 ms after voltage steps from a HP of -60 mV to potentials between -40 and +50 mV in 10-mV increments are displayed. The activation phases of the currents evoked at -10 to +50 mV are shown fitted (solid lines) to fourth power exponentials of the form \( I_{out}(t) = I_{max} \cdot [1 - \exp(-t/\tau_a)]^4 \) (see text). (B) Mean ln \( \tau_a \) values (\( n = 56 \)) determined from the fits of \( I_{out} \) activation are plotted as a function of test potential. (C) Simulated \( I_{out} \) waveforms during voltage steps identical to those in A (see text). Calibration bars: 400 pA and 4 ms (A).
where $V_m$ is the membrane potential. Since these functions adequately describe the data (Fig. 6), they were used in the simulations rather than the individual values for $\alpha$ and $\beta$ at each voltage. As can be seen in Fig. 5 C, the waveforms of the simulated currents evoked during brief depolarizations to $-40$ to $+50$ mV from a HP of $-60$ mV closely resemble $I_{out}$ measured in a typical atrial cell (Fig. 5 A).

To account for voltage-independent inactivation without substantial inactivation of closed channels at the resting potential, it has been suggested that inactivation must be coupled to activation (DeCoursey, 1990; Koren et al., 1990; Zagotta and Aldrich, 1990). The rate constants controlling inactivation and recovery from inactivation are voltage independent, and inactivation thereby derives only an "apparent" voltage dependence from the voltage-dependent forward ($\alpha$) and backward ($\beta$) rate constants for activation. This coupled gating model is consistent with the observed voltage-independent inactivation of $I_{out}$ (Fig. 2 C) and in all the models tested (see below) the inactivation and recovery rate constants could be adjusted to approximate the time

![Figure 6. Modeling of activation rate constants and steady-state current activation. (A) Mean (±SEM) normalized membrane conductances for peak $I_{out}$ ($n = 56$) during depolarizations to potentials from $-40$ to $+50$ mV (HP $-60$ mV), calculated assuming a reversal potential of $-75$ mV (see text). Note that no error bars are apparent because the symbols themselves reflect the mean ± SEM. The data points are shown fitted (solid line) to a fourth power Boltzmann function with a $V_{1/2}$ of $-32$ mV corresponding to an $n_\infty$ value of 0.5 (see text). It is important to point out that, in spite of the apparent goodness of the fit, the reliability of low $n_\infty$ values is limited due to the fact that this is a fourth power function. (B) The $n_\infty$ values derived from the conductance-voltage plot (in A) and the $\tau_n$ values derived from analyses of the kinetics of $I_{out}$ activation (Fig. 5 B) were used to calculate the voltage-dependent activation rate constants ($\alpha$) where $\alpha = n_\infty/\tau_n$. The calculated values (plotted as points) are shown fitted (solid line) to a continuous Hodgkin-Huxley function of voltage (see text). (C) Voltage-dependent deactivation (closing) rate constants ($\beta$) were calculated from the same data as in B, where $\beta = (1 - n_\infty)/\tau_n$. The calculated values for $\beta$ (plotted as points) were fitted (solid line) to a continuous Hodgkin-Huxley function of voltage (see text).
courses of inactivation at +50 mV and recovery from inactivation at −60 mV observed in atrial cells.

Simulations of the currents evoked during single voltage steps with models incorporating multiple inactivation pathways revealed outward current waveforms that were similar to those observed in the typical atrial cell. However, these models could not account for the experimentally observed complete recovery of \( I_K \) independent of \( I_K \) recovery. Successive depolarizations presented at varying interpulse intervals with these models demonstrated that the complete recovery of \( I_K \) requires the complete recovery of \( I_K \). In fact, simulations with these and other similar models in which the two current components shared any kinetic state(s) along the gating pathway indicated that complete recovery of the shared state is required for the complete recovery of either component. Recovery from inactivation of the simulated currents could be reconciled with the experimental data only when \( I_{out} \) was modeled as two functionally separate currents (\( I_{KF} \) and \( I_{KS} \)) with no shared kinetic states. These results are interpreted as consistent with the interpretation (discussed above) that \( I_{KF} \) and \( I_{KS} \) are functionally independent channel populations.

All subsequent modeling, therefore, was completed with \( I_{KF} \) and \( I_{KS} \) treated as two functionally distinct gating pathways. This required that we determine directly if the voltage dependences of activation of these two current components were indeed identical, as suggested by the data in Fig. 2 and as assumed in the initial modeling (see above). To do this, it was necessary to design voltage-clamp paradigms that would allow the reversal potentials and the kinetics of activation of \( I_{KF} \) and \( I_{KS} \) to be measured independently. This was achieved experimentally by taking advantage of the differing time courses of inactivation and recovery from inactivation of \( I_{KF} \) and \( I_{KS} \). Specifically, to determine the reversal potential of \( I_{KS} \), hyperpolarizing test pulses were presented 1 s after the onset of depolarizations to +50 mV (i.e., at a time when \( I_{KF} \) is inactivated and \( I_{KS} \) remains); the resulting tail currents should largely reflect the deactivation of \( I_{KS} \). To determine the reversal potential of \( I_{KF} \) it was necessary to use a more complicated voltage-clamp paradigm. In this case, cells were first depolarized to +50 mV for 10 s, followed by a 1-s repolarization to −70 mV (i.e., a time sufficient to allow nearly complete recovery of \( I_{KF} \) but little or no recovery of \( I_{KS} \)). A second depolarization to +50 mV was then presented to evoke \( I_{KF} \) and the reversal potential of this component was determined from tail currents evoked on hyperpolarizations presented at the peak of the outward current. Linear interpolation of the tail current amplitudes measured 2.5 ms after the onset of the hyperpolarizations provided mean (±SD) reversal potentials for \( I_{KF} \) and \( I_{KS} \) of −76.2 ± 1.6 and −75.8 ± 2.0 mV, respectively. These values are not significantly different from each other or from the average value of −75.5 ± 1.6 mV for the composite current determined previously (Boyle and Nerbonne, 1991). These values are, however, positive to the calculated value of \( E_K \) under the recording conditions used (−90 mV). Thus, it appears that, although \( K^+ \) selective, \( I_{KF} \) and \( I_{KS} \), like the composite \( I_{out} \) channels (Boyle and Nerbonne, 1991), have finite permeabilities to other ions.

A protocol similar to that used to determine the reversal potential of \( I_{KS} \) (above) was employed to examine \( I_{KS} \) activation, except that after the initial depolarization to +50 mV, cells were repolarized to −70 mV for 5 ms (insufficient time for \( I_{KF} \) to recover) and then depolarized to potentials between −40 and +60 mV to activate \( I_{KS} \). To examine \( I_{KF} \) activation, a protocol similar to that described above was also used,
except that after the 1-s repolarization to -70 mV, cells were depolarized to test potentials of -40 to +60 mV. The kinetics of activation of the evoked currents, which reflect only $I_{Kf}$, were then analyzed. These experiments confirmed that both $I_{Kf}$ and $I_{Kr}$ activate rapidly on membrane depolarization, and that the kinetics of activation of the two components are indistinguishable from each other and from those of the composite $I_{out}$. All subsequent modeling was completed, therefore, with $I_{Kf}$ and $I_{Kr}$ treated as two functionally separate currents with identical kinetics and voltage dependences of activation.

Three basic gating models ultimately emerged that could account for the observed activation, inactivation, and recovery data. These are illustrated below and the rate constants for inactivation ($\kappa$) and recovery from inactivation ($\lambda$) used in the models are provided in the accompanying tables.

\[
\begin{array}{ccc}
C_1 & \overset{4\alpha}{\underset{\beta}{\rightleftharpoons}} & C_2 \\
2\beta & \overset{3\alpha}{\rightleftharpoons} & C_3 \\
3\beta & \overset{2\alpha}{\rightleftharpoons} & C_4 \\
4\beta & \overset{\alpha}{\rightleftharpoons} & O \\
& \overset{\kappa}{\rightleftharpoons} & I \\
\end{array}
\]

(B)

\[
\begin{array}{ccc}
\kappa(s^{-1}) & \lambda(s^{-1}) \\
I_{Kf} & 8 & 1.6 \\
I_{Kr} & 0.4 & 0.08 \\
\end{array}
\]

(C)

\[
\begin{array}{ccc}
C_1 & \overset{4\alpha}{\underset{\beta}{\rightleftharpoons}} & C_2 \\
2\beta & \overset{3\alpha}{\rightleftharpoons} & C_3 \\
3\beta & \overset{2\alpha}{\rightleftharpoons} & C_4 \\
4\beta & \overset{\alpha}{\rightleftharpoons} & O \\
& \overset{\kappa}{\rightleftharpoons} & I \\
\end{array}
\]

\[
\begin{array}{ccc}
\kappa(s^{-1}) & \lambda(s^{-1}) \\
I_{Kf} & 2 & 1.6 \\
I_{Kr} & 0.1 & 0.08 \\
\end{array}
\]

(D)

\[
\begin{array}{ccc}
C_1 & \overset{4\alpha}{\underset{\beta}{\rightleftharpoons}} & C_2 \\
2\beta & \overset{3\alpha}{\rightleftharpoons} & C_3 \\
3\beta & \overset{2\alpha}{\rightleftharpoons} & C_4 \\
4\beta & \overset{\alpha}{\rightleftharpoons} & O \\
& \overset{\kappa}{\rightleftharpoons} & I \\
\end{array}
\]

\[
\begin{array}{ccc}
\kappa(s^{-1}) & \lambda(s^{-1}) \\
I_{Kf} & 2.2 & 3.9 \\
I_{Kr} & 0.11 & 0.195 \\
\end{array}
\]
Model B is analogous to models of K⁺ channel gating proposed by others in which voltage-independent inactivation is coupled to opening of the channel (DeCoursey, 1990; Koren et al., 1990; Zagotta and Aldrich, 1990). Models C and D allow voltage-independent inactivation from closed states by coupling inactivation to activation of the individual subunits. Thus, inactivation does not occur from the deepest closed state (C₁) in models C and D, and voltage-dependent activation develops as the rate of inactivation (κ to 4κ), as well as the number of channels with at least one activated subunit increases along the gating pathway (C₂ → O). Both models C and D are consistent with recent data showing that the inactivation "gate" of Shaker K⁺ channels is an NH₂-terminal cytoplasmic domain of the individual subunits that associates with the activated channel in a voltage-independent manner (Hoshi, Zagotta, and Aldrich, 1990). Model D is analogous to one of the models explored by Zagotta and Aldrich (1990) for gating of Shaker K⁺ channels in which each of four subunits can inactivate independently after activation. Model C is similar except that, in this model, once a single (activated) subunit inactivates, the channel is functionally inactivated and inactivation of other activated subunits cannot occur. This model (C) appears to be more consistent with the observed concentration dependence of NH₂-terminal peptide-induced inactivation of Shaker K⁺ channels, which suggests that there is only a single inactivation gate binding site (Zagotta, Hoshi, and Aldrich, 1990).

To simulate the whole-cell current (I_{out}) with each of these models (B–D), I_{Kf} and I_{Ks} were modeled as separate currents with identical activation (α) and deactivation (β) rate constants (see above), and the composite whole-cell current, I_{out}, was modeled as 40% I_{Kf} and 60% I_{Ks}. The ratios of λ to κ for both I_{Kf} and I_{Ks} in these simulations were adjusted to produce a steady-state current (I_{ss}) equal to ~20% of the peak I_{out}, as was observed experimentally (see Table I). The inactivation (κ) and recovery (λ) rate constants for I_{Kf} and I_{Ks} shown in the tables with each of the models were chosen so that all three gating models produced similar simulated I_{out} waveforms with activation and inactivation kinetics which also closely approximated those observed in typical atrial cells (Fig. 7 A). Because the two current components were modeled as functionally distinct currents with no shared kinetic states along the gating pathway, I_{Kf} and I_{Ks} recover from inactivation independently (Fig. 7 B). In addition, using the rate constants presented in the tables accompanying the models, the time courses of recovery from inactivation of the simulated I_{Kf} and I_{Ks} (Fig. 7 B) were similar to those observed experimentally (Fig. 4).

Inactivation late in the activation pathway for both I_{Kf} and I_{Ks} was required to account for the similar voltage-dependent activation properties of peak I_{out}, I_{Kf}, and I_{Ks}. Models in which inactivation occurred only from states early (e.g., C₁ to C₃) in the activation pathway predicted a voltage-dependent decrease in the extent of peak I_{out} inactivation at more depolarized test potentials and therefore different voltage-dependent activation properties of peak I_{out} compared with those of I_{Kf} and I_{Ks}, neither of which was observed experimentally (Fig. 2 D). Although these findings exclude closed-state inactivation as the only pathway, they do not eliminate the possibility of multiple inactivation pathways that include some closed-state inactivation (such as in Schemes C and D).

Interestingly, as shown in Fig. 7, C and D, the models make quite different
predictions regarding the time and voltage dependences of inactivation at membrane potentials < 0 mV. For example, models B and C predict substantial slowing of inactivation at membrane potentials < 0 mV (Fig. 7 C). This "apparent" voltage dependence of inactivation rate (in models B and C) results from the marked slowing of current activation. With model D, in contrast, there is much less effect of membrane potential on the apparent rate of inactivation as compared with the predictions with models B and C (Fig. 7 C). The results obtained from the simulations of voltage-dependent inactivation with these models are shown fitted in Fig. 7 D.
to Boltzmann functions (solid lines) of the form:

\[ I(V) = \frac{1}{1 + \exp[(V - V_{1/2})/s]} \] (2)

where \( I(V) \) is the normalized current as a function of the HP (V), \( V_{1/2} \) is the voltage at which 50% of the channels are inactivated, and \( s \) is the slope factor. Model B, in which inactivation is coupled to channel opening, predicts a \( V_{1/2} \) for inactivation (for \( I_{Kf} \) and \( I_{Kr} \)) of \(-19 \) mV. Models C and D, in which inactivation is coupled to activation of the individual subunits, however, predict \( V_{1/2} \) values of \(-46 \) and \(-39 \) mV, respectively. These predictions of models B–D regarding the time and voltage dependences of inactivation of \( I_{Kf} \) and \( I_{Kr} \) at membrane potentials \(<0 \) mV were then tested experimentally in atrial cells in an attempt to distinguish among the models.

**Steady-State Inactivation**

The time courses of inactivation of peak \( I_{out} \), \( I_{Kf} \), and \( I_{Kr} \) at membrane potentials negative to \(0 \) mV were examined by application of varying duration prepulses to \(-30 \) mV from a HP \(-90 \) mV before the 10-s depolarizations to \(+50 \) mV (Fig. 8). The decay of peak \( I_{out} \) during depolarization directly from \(-90 \) mV was fitted (Eq. 1) and the values for \( \tau_{f} \) and \( \tau_{s} \) obtained from this fit were then used to fit \( I_{out} \) decay during...
subsequent depolarizations that were preceded by prepulses; depolarizations were presented at 60-s intervals to allow complete recovery of peak $I_{\text{out}}$. In Fig. 8 A, data are shown from one such experiment in which 250-ms to 64-s prepulses to -30 mV were applied. Using the values of $\tau_l$ and $\tau_s$ derived from the fit of the current evoked directly from -90 mV (Fig. 8 A, top trace), the decay phases of $I_{\text{out}}$ evoked from each prepulse potential were well described. In these experiments, the first 100 ms of the +50-mV depolarization was recorded at a high sampling frequency (Fig. 8 A, inset) to allow parallel assessment of the rate of $I_{\text{out}}$ activation (see below).

To determine the kinetics of $I_{\text{out}}$, $I_{\text{Kf}}$, and $I_{\text{Ks}}$ inactivation at -30 mV, the currents were normalized to their respective maximal values measured during the depolarization with no prepulse. The mean ($\pm$ SEM; $n = 4$) normalized current amplitudes are plotted as a function of prepulse duration and fitted to exponential decay functions in Fig. 8, B and C. The time course of peak $I_{\text{out}}$ decay was best fitted ($R = 0.99$) by the sum of two exponentials using Eq. 1 (above) with time constants of 980 ms and 13.1 s (Fig. 8 B). As shown in Fig. 8 C, the inactivation time courses of $I_{\text{Kf}}$ and $I_{\text{Ks}}$ plotted separately were well described ($R = 0.99$) by single exponentials with similar time constants of 1,060 ms and 11.4 s, respectively. These results indicate that $I_{\text{Kf}}$ and $I_{\text{Ks}}$ can be distinguished based on their differing inactivation kinetics at membrane potentials < 0 mV. In addition, both $I_{\text{Kf}}$ and $I_{\text{Ks}}$ inactivate approximately five times more slowly at -30 mV than at +50 mV, consistent with the slowing of inactivation predicted by models B and C (Fig. 7 C). Although the rates of $I_{\text{Kf}}$ and $I_{\text{Kf}}$ inactivation at -30 mV were measured indirectly in these experiments, the validity of the conclusions is supported by the finding that the inactivation time constants for $I_{\text{Kf}}$ and $I_{\text{Ks}}$ derived from the fits were essentially identical to those that described the time course of peak $I_{\text{out}}$ inactivation. In addition, as shown in the inset of Fig. 8 A, there was no measurable change in activation rate when the current amplitude was decreased by the prepulses. Because the short duration prepulses selectively inactivated $I_{\text{Kf}}$, these data (Fig. 8 A, inset) illustrate that $I_{\text{Ks}}$ activates rapidly on membrane depolarization with kinetics of activation that are indistinguishable from those of $I_{\text{out}}$ (see also above). With long duration prepulses, both $I_{\text{Kf}}$ and $I_{\text{Ks}}$ are completely inactivated, and the residual current ($I_{\text{ss}}$) also activates rapidly, consistent with the assumption noted above that $I_{\text{ss}}$ represents the noninactivating portions of $I_{\text{Kf}}$ and $I_{\text{Ks}}$.

The voltage dependences of steady-state inactivation of peak $I_{\text{out}}$, $I_{\text{Kf}}$, and $I_{\text{Ks}}$ were determined at HPs between -90 and 0 mV (Fig. 9). Cells were held at each HP for 64 s to ensure that steady state was reached before the 10-s depolarizations to +50 mV to evoke the outward currents; a 60-s recovery period at a HP of -90 mV was allowed between each pulse sequence. More hyperpolarized HPs (-100 to -120 mV) did not result in measurable increases in peak $I_{\text{out}}$, $I_{\text{Kf}}$, or $I_{\text{Ks}}$. In these experiments, $I_{\text{out}}$, measured during a 10-s depolarization to +50 mV directly from -90 mV was fitted first and the values for $\tau_l$ and $\tau_s$ obtained from this fit were then used to fit $I_{\text{out}}$ decay during subsequent depolarizations from the more depolarized HPs (Fig. 9 A). $I_{\text{out}}$, $I_{\text{Kf}}$, and $I_{\text{Ks}}$ amplitudes were then normalized to the values obtained for the depolarization directly from -90 mV, and the mean ($\pm$ SEM; $n = 3$) normalized current amplitudes are plotted a function of HP in Fig. 9, B and C. The Boltzmann fit to the data for peak $I_{\text{out}}$ (Fig. 9 B, solid line) yielded values of $V_{1/2} = -44$ mV and $s = 10$ mV ($R = 0.99$). The variations in $I_{\text{Kf}}$ and $I_{\text{Ks}}$ amplitudes with HP were similar (Fig.
with the best fits yielding $V_{1/2} = -46 \text{ mV}$ and $s = 8 \text{ mV ($R = 0.99$)}$ for $I_{Kr}$ and $V_{1/2} = -43 \text{ mV}$ and $s = 11 \text{ mV ($R = 0.99$)}$ for $I_{Kf}$. Both the time- and voltage-dependent inactivation properties of $I_{Kr}$ and $I_{Kf}$ at membrane potentials $< 0 \text{ mV}$ thus appeared strikingly similar to those predicted by model C (Fig. 7 D). In particular, model C predicted the slowing of inactivation at membrane potentials $< 0 \text{ mV}$ and a $V_{1/2}$ essentially identical to that observed experimentally (see Discussion).

Pharmacologic Sensitivity of the Currents

The sensitivities of $I_{Kf}$ and $I_{Kr}$ to extracellular 4-AP and TEA were examined during repetitive 10-s depolarizations to $+50 \text{ mV}$ from a HP of $-60 \text{ mV}$ delivered at 60-s intervals. The cells were then exposed to 4-AP or TEA continuously for at least 2 min to allow the level of block to stabilize. The extent of $I_{Kf}$ and $I_{Kr}$ attenuation was determined by comparing the amplitudes of the current components derived from the pre- and posttreatment fits of $I_{out}$ decay; the values for $\tau_i$ and $\tau_e$ determined from the pretreatment fit were used for the posttreatment fits. As shown in Fig. 10, A and B, puffer applications of 1 and 5 mM 4-AP were accompanied by marked attenuation of peak $I_{out}$, $I_{Kf}$, and $I_{Kr}$. As shown in Table II, the percent block (mean ± SD) by 4-AP appeared to be dose dependent over the concentration range of 1 mM ($n = 3$) to 5 mM ($n = 4$), and the effects of 4-AP on $I_{Kf}$ and $I_{Kr}$ were similar.

![Figure 9](image-url)
FIGURE 10. Blockade of $I_{\text{out}}$, $I_{Kt}$, and $I_{Kr}$ by (A, B) 4-AP and (C) TEA. In all three panels, $I_{\text{out}}$ evoked during 10-s depolarizing pulses to +50 mV from a HP of -60 mV was recorded before (1) and during (2) continuous application of 1 mM 4-AP (A), 5 mM 4-AP (B), or 50 mM TEA (C) to the surface of the cell from a puffer pipette (see Methods). The decay phases of $I_{\text{out}}$ are plotted as points and the double exponential fits to the decay phases are plotted as solid lines. From these, the amplitudes of $I_{Kt}$, $I_{Kr}$, and $I_{Kn}$ were calculated (see text). (Insets) The magnitudes of peak $I_{\text{out}}$, $I_{Kt}$, and $I_{Kr}$ determined from the fits are displayed, together with the percent change ($\Delta$) in the currents produced by the drug application, calculated as $[(I_2 - I_1)/I_1] * 100$. Calibration bars: 200 pA and 1 s (A, C); 500 pA and 1 s (B).

TABLE II

Percent Block of Atrial $I_{\text{out}}$ by 4-AP and TEA

|          | 5 mM 4-AP (n = 4) | 1 mM 4-AP (n = 3) | 50 mM TEA (n = 7) |
|----------|-----------------|-----------------|------------------|
| $I_{\text{out}}$ | 82.6 ± 8.2      | 47.6 ± 5.3      | 24.4 ± 11.9*     |
| $I_{Kt}$    | 90.7 ± 7.6      | 66.1 ± 19.5     | 27.8 ± 17.1*     |
| $I_{Kr}$    | 97.7 ± 2.4      | 76.3 ± 7.8      | 40.6 ± 17.7*     |

Mean ± SD.

*P < 0.05 compared with 1 or 5 mM 4-AP.
An example of the effect of 50 mM TEA on the outward currents is shown in Fig. 10 C. Qualitatively similar results were obtained in six other atrial cells (Table II). Although there was some cell to cell variability in the response to TEA, when data from many cells (n = 7) were averaged, the degrees to which \( I_{Kf} \) and \( I_{Ks} \) were affected by TEA were not significantly different (Table II). Thus, these experiments reveal that both \( I_{Kf} \) and \( I_{Ks} \) display similar sensitivities to 4-AP and that both currents are relatively insensitive to externally applied TEA. In addition, we found that the effects of 4-AP and TEA on the noninactivating current \( (I_{sa}) \) are essentially identical to those seen on \( I_{Kf} \) and \( I_{Ks} \) (see Fig. 10), again consistent with the assumption (noted previously) that \( I_{sa} \) represents the noninactivating fractions of \( I_{Kf} \) and \( I_{Ks} \).

**DISCUSSION**

The results presented here reveal the presence of two components, \( I_{Kf} \) and \( I_{Ks} \), of the total Ca\(^{2+}\)-independent, depolarization-activated outward currents \( (I_{out}) \) in adult rat atrial myocytes with distinct rates of inactivation and recovery from inactivation. In particular, \( I_{Kf} \) inactivates and recovers from inactivation ~20 times more rapidly than \( I_{Ks} \). Although previously we noted that peak \( I_{out} \) is stable during repetitive 100-ms depolarizations presented at 5-s intervals (Boyle and Nerbonne, 1991), the experiments here indicate that considerably longer interpulse intervals are needed for complete recovery of peak \( I_{out} \) after long depolarizations. These experiments also reveal that the difference in the time courses of recovery of peak \( I_{out} \) after brief and long depolarizations is due to the distinct inactivation and recovery kinetics of \( I_{Kf} \) and \( I_{Ks} \). During brief steps, only \( I_{Kf} \) inactivates to any extent. Recovery of peak \( I_{out} \), then, parallels the recovery of \( I_{Kf} \) and is complete within a few seconds. In contrast, \( I_{Ks} \) inactivates only during long depolarizations, and the slow recovery of this component results in the long interpulse intervals required for complete recovery of peak \( I_{out} \) after long depolarizations.

Interestingly, other than the differences in the kinetics of inactivation and recovery from inactivation, the two components of \( I_{out} \) could not be distinguished by other established criteria for separating K\(^+\) currents. This primarily reflects the fact that \( I_{Kf} \) and \( I_{Ks} \) display indistinguishable voltage dependences and kinetics of activation and similar sensitivities to 4-AP and TEA. Nevertheless, the observation that \( I_{Kf} \) recovery from inactivation is independent of \( I_{Ks} \) recovery indicates that \( I_{Kf} \) and \( I_{Ks} \) cannot share any kinetic states and therefore are functionally independent currents. \( I_{Kf} \) and \( I_{Ks} \) could reflect alternate pathways for gating of a single type of K\(^+\) channel; however, if they do, interconversion between the two gating pathways must be too slow to be evident during the time course of our experiments.

From a physiological standpoint, the rapid activation of \( I_{Kf} \) and \( I_{Ks} \) suggests that both components contribute to repolarization of the action potential (AP) in adult rat atrial myocytes. In addition, the relatively slow rates of recovery from inactivation, particularly for \( I_{Ks} \), suggest that the amount of steady-state inactivation may vary considerably as a function of stimulation frequency. Thus, accumulation of inactivation of one or both components may occur at the high heart rates observed under normal physiological conditions in the rat. In this regard, the physiological roles of \( I_{Kf} \) and \( I_{Ks} \) may differ. The relatively rapid inactivation and recovery of \( I_{Kf} \) compared
with $I_{Ks}$ suggest that changes in the steady-state availability of this current after a change in heart rate would occur relatively rapidly. In contrast, the slow inactivation and recovery of $I_{Ks}$ suggest that changes in the availability of this current would occur more slowly. Interestingly, accumulated inactivation of 4-AP-sensitive outward currents due to slow recovery has been shown to be the major factor responsible for frequency-dependent changes in AP configuration in other cardiac preparations (Boyett, 1981a, b; Kukushkin et al., 1983; Mitchell, Powell, Terrar, and Twist, 1984; Escande, Loisance, Planche, and Coraboeuf, 1985; Giles and Van Ginneken, 1985; Giles and Imaizumi, 1988; Litovsky and Antzelevitch, 1988, 1989). Although we have not tested the effect of stimulation frequency directly, the properties of $I_{Kf}$ and $I_{Ks}$ suggest that increasing the heart rate would lead to progressive inactivation of both components, thereby resulting in a higher plateau potential and a longer AP duration. Because AP duration is a primary determinant of the refractory period, progressive lengthening of the AP due to accumulated inactivation would also be expected to increase the refractory period. Thus, the slow recovery of outward currents could serve to protect cells from excessively high stimulation rates that might occur in certain physiological or pathological conditions.

Interestingly, biexponential decay of Ca$^{2+}$-independent, 4-AP-sensitive outward currents such as observed here for atrial $I_{out}$ has been described previously in other mammalian cardiac preparations. For example, two inactivation time constants reportedly best describe the decay of the 4-AP-sensitive currents in Purkinje fibers from sheep (Kenyon and Gibbons, 1979; Coraboeuf and Carmeliet, 1982), calf (Kenyon and Sutko, 1987), and dog (Nakayama and Fozzard, 1988) heart, in isolated cells from the rabbit atrioventricular node (Nakayama and Irisawa, 1985), atria (Clark, Giles, and Imaizumi, 1988; Giles and Imaizumi, 1988), and ventricles (Hiraoka and Kawano, 1989), and in isolated mouse ventricular myocytes (Benndorf, 1988; Benndorf and Nilius, 1988). Biexponential recovery kinetics have been described for some of these currents (Benndorf and Nilius, 1988; Giles and Imaizumi, 1988; Nakayama and Fozzard, 1988; Hiraoka and Kawano, 1989), although analysis of the independent recovery of the two components has not been reported, and the functional basis for the components has not been determined. Two components of inactivation have been distinguished in the ensemble averages of the currents through a single type (as defined by single channel conductance) of transient K$^+$ channel in rabbit AV nodal cells with time constants closely resembling those derived from analysis of the decay of the whole-cell current (Nakayama and Irisawa, 1985). Similar results have been reported in rabbit atrial cells (Clark et al., 1988) and for cloned K$^+$ channels expressed in Xenopus oocytes (Koren et al., 1990), suggesting that multiple components of inactivation may arise from a homogenous population of K$^+$ channels. In contrast, the two components of inactivation of the 4-AP-sensitive transient K$^+$ current in mouse ventricular cells appear to reflect distinct channel populations (Benndorf, 1988).

In many respects, $I_{Kf}$ in rat atrial cells resembles the transient outward current ($I_{to}$) in rat ventricular myocytes (Aptkon and Nerbbonne, 1991). The time and voltage dependences of activation and the pharmacological sensitivities of the two currents are similar. In addition, the inactivation and recovery kinetics of atrial $I_{Kf}$ and ventricular $I_{to}$ are both relatively fast monoexponential processes, albeit ~10 times
faster for ventricular $I_{Ko}$. In contrast, the properties of the slowly inactivating rat atrial $I_{Ks}$ and rat ventricular $I_K$ differ markedly. $I_K$ is sensitive to externally applied TEA (Apkon and Nerbonne, 1991) and, in contrast to rat atrial $I_{Ks}$, rat ventricular $I_K$ is more slowly activating, undergoes voltage-dependent inactivation at more hyperpolarized potentials, and is resistant to 4-AP. Interestingly, the properties of atrial $I_{Ks}$ are remarkably similar to those of several $K^+$ channels cloned from rat brain and heart cDNA libraries and expressed in *Xenopus* oocytes (Stühmer et al., 1988, 1989; Christie et al., 1989; Grupe et al., 1990; Swanson et al., 1990; Tseng-Crank et al., 1990; Paulmichl et al., 1991; Roberds and Tamkun, 1991).

Although the conclusion here that $I_{Kf}$ and $I_{Ks}$ represent functionally distinct population of channels was reached based purely on kinetic arguments, a variety of potential molecular mechanisms might underlie the distinct kinetic properties of $I_{Kf}$ and $I_{Ks}$. It is possible, for example, that $I_{Kf}$ and $I_{Ks}$ reflect the expression of two distinct $K^+$ channel genes. Indeed, several distinct $K^+$ channel cDNAs have been cloned from rat heart cDNA libraries, including $K_1$ (Swanson et al., 1990), RHK1 (Tseng-Crank et al., 1990), RK1 through RK5 (Roberds and Tamkun, 1991), and RAK (Paulmichl et al., 1991). Expression of $K_1$ (Swanson et al., 1990) or RAK (Paulmichl et al., 1991) in *Xenopus* oocytes reveals a rapidly activating, slowly inactivating current with kinetic properties similar (but not identical) to $I_{Ks}$ whereas expression of RHK1 (Tseng-Crank et al., 1990) results in a rapidly activating and inactivating current with electrophysiological and pharmacological properties similar (but not identical) to $I_{Kf}$. Interestingly, RHK1 differs from $K_1$ and RAK mainly in the NH$_2$-terminal portion of the polypeptide, consistent with the suggestion that the NH$_2$-terminal sequence is important in determining inactivation kinetics (Kamb, Tseng-Crank, and Tanouye, 1988; Schwarz, Tempel, Papazian, Jan, and Jan, 1988; Timpe, Jan, and Jan, 1988; Hoshi et al., 1990; Zagotta et al., 1990). Thus, $I_{Kf}$ and $I_{Ks}$ may reflect the expression of channels from the same gene family which differ little in terms of primary structure except possibly at the NH$_2$-terminus. It may also be, however, that coexpression of two alternatively spliced *Shaker* clones (Isacoff, Jan, and Jan, 1990) or two members of the same mammalian $K^+$ channel gene family (Christie, North, Osborne, Douglass, and Adelman, 1990; Ruppersburg, Schröter, Sakmann, Stocker, Sewing, and Pongs, 1990) results in the expression of $K^+$ currents with kinetic properties distinct from either gene product expressed alone. Presumably this reflects the formation of heteromultimeric channels. It is possible, therefore, that $I_{Kf}$ and $I_{Ks}$ are heteromultimeric channels that differ only with respect to subunit composition. $I_{Kf}$ and $I_{Ks}$ could also represent different posttranslational modifications of a single type of $K^+$ channel. Recently, β-adrenergic agonists have been shown to alter the inactivation kinetics of the transient $K^+$ current in canine ventricular myocytes, presumably due to channel phosphorylation (Nakayama and Fozzard, 1988). Thus, $I_{Kf}$ and $I_{Ks}$ could correspond to distinct gating modes of a homogenous channel population that are modulated by the degree of channel phosphorylation. Certainly, other posttranslational modifications are also possible.

The initial purpose of modeling the atrial $K^+$ currents was to provide insight into the kinds of kinetic models that could account for the observed kinetics of inactivation and recovery from inactivation. To this end, the modeling provided a direct means of testing the hypothesis that complete recovery of $I_{Kf}$ independent of $I_{Ks}$
recovery cannot occur if \( I_{Kf} \) and \( I_{Ka} \) share any states in the gating pathway. In addition, a model in which voltage-independent inactivation was coupled to activation of individual subunits produced simulated currents with time- and voltage-dependent properties remarkably similar to those of \( I_{Kf} \) and \( I_{Ka} \), as well as those of the composite whole-cell current, \( I_{out} \). This contrasted with the open channel-coupled model, which could account for many of the properties of \( I_{Kf} \) and \( I_{Ka} \) but predicted a much more depolarized \( V_{1/2} \) for inactivation than was observed experimentally. Interestingly, it was possible to apply basic concepts regarding the structure and function of \( K^+ \) channels and to construct a gating model for \( I_{Ka} \) and \( I_{Kf} \) from the whole-cell data that behaves in a manner analogous to the native currents. This strongly suggests that the model reflects features of the gating mechanism of single (\( I_{Kf} \) and \( I_{Ka} \)) channels. In particular, the subunit activation-coupled model provides for the voltage-independent inactivation observed at depolarized membrane potentials, which has also been observed for a variety of similar \( K^+ \) currents (Kenyon and Gibbons, 1979; Giles and Van Ginneken, 1985; Benndorf and Nilius, 1988; Clark et al., 1988; Hiraoka and Kawano, 1989; DeCoursey, 1990; Koren et al., 1990; Zagotta and Aldrich, 1990). This gating mechanism also allows for inactivation of closed channels which was suggested by the hyperpolarized \( V_{1/2} \) for inactivation. Zagotta and Aldrich (1990) demonstrated that the rate of inactivation of \( Shaker \) A-type \( K^+ \) channels in \textit{Drosophila} is voltage independent, but also concluded that some closed-state inactivation of those channels must occur to account for the voltage-dependent increase in the number of blank single channel records at less depolarized potentials. Although they found that the subunit activation-coupled model, among other things, predicted too much inactivation at hyperpolarized potentials, we did not find this to be the case for rat atrial \( I_{Kf} \) and \( I_{Ka} \). The subunit activation-coupled model predicted \( V_{1/2} \) values very similar to those determined experimentally. This model also predicted that the number of channels available to open is not substantially affected over the range of typical resting membrane potentials observed in atrial myocytes (\(-60 \) to \(-90 \) mV), and this was confirmed experimentally. Thus, we conclude that the subunit activation-coupled model provides a simple model of channel gating that can account for the behavior of the macroscopic depolarization-activated \( K^+ \) currents in adult rat atrial myocytes.

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