

Characterization of Two Distinct Depolarization-activated K^+ Currents in Isolated Adult Rat Ventricular Myocytes

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ABSTRACT Depolarization-activated outward K^+ currents in isolated adult rat ventricular myocytes were characterized using the whole-cell variation of the patch-clamp recording technique. During brief depolarizations to potentials positive to -40 mV, Ca^{2+} -independent outward K^+ currents in these cells rise to a transient peak, followed by a slower decay to an apparent plateau. The analyses completed here reveal that the observed outward current waveforms result from the activation of two kinetically distinct voltage-dependent K^+ currents: one that activates and inactivates rapidly, and one that activates and inactivates slowly, on membrane depolarization. These currents are referred to here as I_{to} (transient outward) and I_K (delayed rectifier), respectively, because their properties are similar (although not identical) to these K^+ current types in other cells. Although the voltage dependences of I_{to} and I_K activation are similar, I_{to} activates ≈ 10 -fold and inactivates ≈ 30 -fold more rapidly than I_K at all test potentials. In the composite current waveforms measured during brief depolarizations, therefore, the peak current predominantly reflects I_{to} , whereas I_K is the primary determinant of the plateau. There are also marked differences in the voltage dependences of steady-state inactivation of these two K^+ currents: I_K undergoes steady-state inactivation at all potentials positive to -120 mV, and is 50% inactivated at -69 mV; I_{to} , in contrast, is insensitive to steady-state inactivation at membrane potentials negative to -50 mV. In addition, I_{to} recovers from steady-state inactivation faster than I_K : at -90 mV, for example, $\approx 70\%$ recovery from the inactivation produced at -20 mV is observed within 20 ms for I_{to} ; I_K recovers ≈ 25 -fold more slowly. The pharmacological properties of I_{to} and I_K are also distinct: 4-aminopyridine preferentially attenuates I_{to} , and tetraethylammonium suppresses predominantly I_K . The voltage- and time-dependent properties of these currents are interpreted here in terms of a model in which I_{to} underlies the initial, rapid repolarization phase of the action potential (AP), and I_K is responsible for the slower phase of AP repolarization back to the resting membrane potential, in adult rat ventricular myocytes.

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INTRODUCTION

In myocardial preparations, a variety of K^+ currents with differing kinetic and voltage-dependent properties have been described (Carmeliet and Vereecke, 1979; DiFrancesco and Noble, 1985; Cohen et al., 1986; Carmeliet et al., 1987) and, as in other excitable cells (Thompson, 1977; Hagiwara, 1983; Latorre et al., 1984), K^+ channels in cardiac cells appear to be more numerous and diverse than other types of ion channels. It seems that the various K^+ currents in cardiac cells function to control resting membrane potentials, action potential (AP) waveforms, refractoriness, and automaticity and, in addition, may be important targets for the actions of the many neurotransmitters, neurohormones, and intracellular second messengers known to modulate cardiac function. Differences in the properties and distributions of K^+ currents may also account for variations in the detailed electrical properties of different myocardial cell types.

Depolarization-activated outward currents are expected to influence the duration and amplitude of the plateau phase of the cardiac AP. Transient outward K^+ currents that might fulfill this function have been described in Purkinje fibers (Deck et al., 1964; Dudel et al., 1967; Fozzard and Hiraoka, 1973; Kenyon and Gibbons, 1977, 1979*a, b*; Siegelbaum and Tsien, 1980; Boyett, 1981*a, b*; Coraboeuf and Carmeliet, 1982; Carmeliet et al., 1987; Kenyon and Sutko, 1987), as well as in ventricular (Kukuskin et al., 1983; Josephson et al., 1984; Ng et al., 1987; Tseng et al., 1987; Giles and Imaizumi, 1988; Litkovsky and Antzelevitch, 1988), atrial (Escande et al., 1987; Clark et al., 1988; Giles and Imaizumi, 1988), nodal (Nakayama and Irisawa, 1985), and crista terminalis (Giles and Van Ginneken, 1985) preparations. In some preparations, Ca^{2+} -dependent and Ca^{2+} -independent transient outward currents have been distinguished (Coraboeuf and Carmeliet, 1982; Lipsius and Gibbons, 1982; Escande et al., 1987; Kenyon and Sutko, 1987; Tseng et al., 1987; Litovsky and Antzelevitch, 1988). In others, however, only a Ca^{2+} -independent transient outward current component has been described (Josephson et al., 1984; Giles and Van Ginneken, 1985; Nakayama and Irisawa, 1985). In spite of the fact that a variety of terms, such as "early outward" (I_{eo}) (Kenyon and Gibbons, 1979*b*; Lipsius and Gibbons, 1982; Josephson et al., 1984), "brief outward" (I_{bo}) (Coraboeuf and Carmeliet, 1982; Escande et al., 1987), "long-lasting outward" (I_{lo}) (Coraboeuf and Carmeliet, 1982; Escande et al., 1987), "transient outward" (I_t , I_{to} , I_{to1} , and I_{to2}) (Boyett, 1981*a, b*; Tseng et al., 1987; Clark et al., 1988; Giles and Imaizumi, 1988; Litovsky and Antzelevitch, 1988), and I_A (Giles and Van Ginneken, 1985) have been used to describe the measured currents, there are similarities in the time- and voltage-dependent properties, as well as the pharmacologic sensitivities, of the transient outward currents in different preparations.

Depolarization-activated outward K^+ currents, which activate slowly on membrane depolarization and are similar to the delayed rectifier (I_K) in other cells (Armstrong, 1975; Thompson, 1977; Latorre et al., 1984), have also been described in myocardial tissues (Noble and Tsien, 1969; Noma and Irisawa, 1976; McDonald and Trautwein, 1978*a, b*; Brown and DiFrancesco, 1980; Kass et al., 1982; Kass, 1984; Gintant et al., 1985; Shibasaki, 1987; Tseng et al., 1987). Although two, kinetically distinct, slowly activating currents have been described in some preparations (Noble and Tsien,

1969; McDonald and Trautwein, 1978*a, b*; Shibasaki, 1987; Tseng et al., 1987), it is not clear if similar currents are present in all myocardial cells, primarily because this possibility has not been explored (Kenyon and Gibbons, 1979*a*; Coraboeuf and Carmeliet, 1982; Kukushkin et al., 1983; Josephson et al., 1984; Nakayama and Irisawa, 1985; Kenyon and Sutko, 1987; Ng et al., 1987). The presence of delayed K⁺ currents is suggested in some preparations, however, by the observation that net outward currents remain after the transient components are blocked (Kenyon and Gibbons, 1979*a*; Kukushkin et al., 1983). In the rabbit sino-atrial node, a Ca²⁺-dependent delayed K⁺ current has been described (Brown and DiFrancesco, 1980), although it is not clear if similar currents are present in other myocardial cells (Eisner and Vaughan-Jones, 1983; Kass, 1984).

A rapidly activating and inactivating, Ca²⁺-independent depolarization-activated outward current, termed *I_{eo}*, has been described previously in isolated adult rat ventricular myocytes (Josephson et al., 1984). A sustained outward current remained at all test potentials (Josephson et al., 1984) and, as a result, it was not clear if *I_{eo}* inactivation was incomplete or, alternatively, if additional depolarization-activated outward current components were present in these cells. Because of our interest in the role of K⁺ currents in regulating membrane excitability and in mediating the responses to neurotransmitters and second messengers in adult rat ventricular myocytes (Apkon and Nerbonne, 1988), we have undertaken a detailed examination of the voltage- and time-dependent properties of depolarization-activated, Ca²⁺-independent K⁺ currents in these cells using the whole-cell patch-clamp recording technique (Hamill et al., 1981). The results reveal that two distinct, depolarization-activated outward K⁺ currents are present in these cells. A preliminary account of some of this work has appeared previously in abstract form (Apkon and Nerbonne, 1986).

METHODS

Cell Preparations

Single ventricular myocytes were isolated from 8–12-wk-old Long Evans rat hearts using a protocol based on previously described procedures (Powell and Twist, 1976; Powell et al., 1980; Wittenberg and Robinson, 1981; Wittenberg et al., 1986). Briefly, hearts were removed and perfused (at 37°C) retrogradely through the aorta with 25 ml of Ca²⁺-free Krebs buffer, followed by 15–30 min with Krebs buffer containing 5 μM Ca²⁺ and 0.04–0.2% type II collagenase (Worthington Biochemical Corp., Freehold, NJ) or 0.01–0.02% type IA collagenase (Sigma Chemical Co., St. Louis, MO). After perfusion, the separated ventricles were chopped into small (≈ 1 mm³) pieces, incubated in fresh enzyme solution for 5–10 min, and mechanically dispersed with a large bore fire-polished pasteur pipette. After repeated washing by a series of centrifugations and resuspensions in enzyme-free Krebs buffer, intact cells were usually separated by low speed (≈ 300 rpm; 10 min) centrifugation. In some cases, however, myocytes were separated from cellular fragments and debris by density centrifugation over Percoll (Wittenberg and Robinson, 1981). After centrifugation (and, if necessary, removal of the residual Percoll), 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., Waltham, MA). Dissociated myocytes were maintained at 37°C in an air/CO₂ (95:5) environment. Rod-shaped, Ca²⁺-tolerant

ventricular myocytes adhered preferentially to the laminin substrate (Piper et al., 1985), and damaged or rounded-up cells were removed by replacing the culture medium 1–3 h after plating; thereafter, the medium was exchanged daily.

Although most isolated ventricular myocytes remained rod-shaped during the first week in vitro, alterations in morphology were evident in some cells over this period: these cells developed rounded centers, spread along the laminin substrate, and in addition, showed spontaneous and rhythmic, contractile activity. Similar observations have been reported by others (Jacobson and Piper, 1986). If maintained for 2–3 wk in vitro, eventually all cells adopt these “dedifferentiated” (Jacobson and Piper, 1986) characteristics. To study a homogeneous population of cells, all of the experiments here were performed on rod-shaped ventricular myocytes within the first 3–5 d after isolation. Neither the waveforms nor the voltage-dependent properties of the currents varied measurably over this time in vitro. The electrophysiological properties of “dedifferentiated” cells have not been examined.

Electrophysiological Recordings

The whole-cell variation of the patch-clamp recording technique (Hamill et al., 1981) was employed to record depolarization-activated K^+ currents and AP waveforms in isolated ventricular myocytes; all recordings were obtained at room temperature (22–24°C). The voltage-clamp/current-clamp circuit was provided by either an Axopatch-1B (Axon Instruments, Inc., Burlingame, CA; $\beta = 1$) or a model 8900 (DAGAN Corp, Minneapolis, MN; 1-G Ω feedback resistor) whole-cell/patch-clamp amplifier. Recording pipettes were fabricated from flint glass and the shanks, up to the tips, were coated with Sylgard (Dow Corning Corp., Midland, MI); the Sylgard coating markedly reduced pipette capacitance and eliminated the slow component of the capacitance of “soft” glass patch pipettes (Corey and Stevens, 1983; Sakmann and Neher, 1983). Pipettes were fire-polished before use to produce electrodes with tip diameters of 1.0–2.0 μm and resistances of 2–4 M Ω when filled with recording solution (see below). After formation of a high-resistance (“giga”) seal between the recording electrode and the myocyte membrane, electrode capacitance was fully compensated electronically before obtaining a whole-cell recording; this allowed the subsequent measurement of cell membrane capacitance (see below) after a whole-cell recording was obtained. Seal resistances were in the range of 5–20 G Ω .

In the whole-cell configuration, series resistances were estimated from the decay of the uncompensated capacitive transients, and were found to equal 1.5–2 times the pipette resistance (Marty and Neher, 1983). Series resistances could be compensated in all cells by $\geq 80\%$. Even for the largest currents of 10 nA, therefore, voltage errors resulting from the uncompensated series resistance were always < 8 mV, and were not corrected. In all voltage-clamp experiments, series resistance compensation was checked at regular intervals to ensure that there were no variations with time; data were discarded if increases in series resistance were evident during the course of an experiment. Cell input resistances were in the range of 1–5 G Ω , and capacitive transients (after series resistance compensation) decayed completely to baseline in all cells within 3.0 ms (mean \pm SD = 2.1 ± 0.6 ; $n = 24$). Whole-cell membrane capacitances, determined from integration of the capacitive transients measured during small (5–10 mV) voltage steps from a holding potential of -70 mV, were in the range of 90–240 pF (mean \pm SD = 140 ± 44 pF; $n = 24$). In experiments conducted using the Axopatch-1B ($n = 10$), it was possible to compensate partially for whole-cell membrane capacitances, although the maximal capacity compensation that could be achieved was 100 pF. On average, therefore, whole-cell membrane capacitances could only be compensated by $\approx 60\%$ ($n = 10$). Nevertheless, this approach did minimize the amplitudes of the capacitive transients, thereby facilitating analyses of the rising phases of the total depolarization-activated outward currents

and determination of the reversal potentials (from tail current measurements) of the peak and plateau outward current components.

In most experiments, depolarization-activated outward K^+ currents were measured during depolarizations to test potentials between -40 and $+60$ mV from a holding potential (HP) of -70 mV. All experimental parameters were controlled with an IBM PC-AT equipped with a Tecmar Labmaster (Scientific Solutions, Cincinnati, OH) analog/digital interface to the electrophysiological equipment. Sampling frequencies ranged from 100 Hz to 20 kHz; current and voltage signals were filtered at 3–5 kHz, digitized, and stored on floppy disks. 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 ≥ 1 G Ω . In all of the current records displayed, however, there are gaps (in the current records) for the first ≈ 1 –2 ms after the onset of the voltage steps. These result from the large capacitive transients which, during voltage steps to evoke outward currents, saturate the recording amplifier. Data acquisition was performed using pCLAMP (Axon Instruments, Inc.), and data were analyzed using pCLAMP, ASYSTANT (Macmillan Software, New York, NY) and NFIT (Island Products, Galveston, TX).

Solutions

To measure Ca^{2+} -independent, depolarization-activated outward K^+ currents, bath solutions routinely contained (in mM): 136 NaCl, 4 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 10 glucose, 5 $CoCl_2$ (to suppress voltage-activated Ca^{2+} currents), and 0.02 tetrodotoxin (TTX, to suppress Na^+ [I_{Na}] currents) at pH 7.2–7.4; 300–310 mosM. Recording pipettes contained (in mM): 135 KCl, 10 EGTA, 10 HEPES, 5 glucose with 3 Mg-ATP, and 0.5 Tris-GTP (pH 7.2; 300–310 mosM). ATP and GTP were included in the pipette solution because preliminary experiments revealed that more stable and reproducible recordings were obtained with these agents present. For AP measurements, similar solutions were employed, except that the TTX and $CoCl_2$ were omitted from the bath, and the bath concentration of $CaCl_2$ was increased to 2.5 mM. APs were evoked by brief depolarizing current pulses, delivered via the recording pipettes.

In spite of the fact that 20 μ M TTX was used in all voltage-clamp experiments, complete block of I_{Na} in adult rat ventricular myocytes was not provided primarily because cardiac Na^+ channels are much less sensitive to TTX (Reuter, 1984) than are the Na^+ channels in other cells. In most experiments, therefore, outward K^+ currents were measured during depolarizations from HPs ≥ -70 , because at these potentials, the TTX-resistant component of I_{Na} was reduced (or eliminated) owing to steady-state inactivation. When outward currents were evoked from HPs negative to -70 mV, the residual I_{Na} was clearly evident in the records. To minimize this current, and to facilitate the measurement of outward currents evoked from hyperpolarized membrane potentials, the voltage-clamp paradigm was altered in some experiments to include brief (15 ms) depolarizations to -20 mV to inactivate I_{Na} before the depolarizations to evoke outward K^+ currents (see Results).

Pharmacological agents were applied to isolated myocytes under electrophysiological investigation by pressure ejection from small (2–3 μ m) "puffer" pipettes placed within ≈ 25 μ m of the cell surface. In all of these experiments, two puffer pipettes were used: one contained the test agent and the other contained only bath solution, and served as a control. To examine the effects of 4-aminopyridine (4-AP) on depolarization-activated outward K^+ currents, 4-AP was dissolved in bath solution at 3–6 mM immediately before use. At these concentrations, the addition of 4-AP did not measurably affect solution pH or osmolarity. In some experiments, tetraethylammonium (TEA)-containing solutions were prepared by equimolar substitution of TEACl for NaCl in the standard bath solution (described above). Because the TEA-sensitive component of the total depolarization-activated outward current was maximally activated on

depolarizations from HPs ≤ -90 mV (see Results), however, these experiments were complicated by the presence of the TTX-insensitive component of I_{Na} (see Reuter, 1984, and above). To minimize this current, the voltage-clamp paradigm was altered (as noted above) to include brief depolarizations to -20 mV before the depolarizations to activate the outward currents. To further minimize the amplitude of the TTX-resistant Na^+ current and to eliminate changes in the driving force on Na^+ resulting from the replacement of 50 mM TEACl for NaCl, the control solution in these experiments contained ≈ 100 mM mannitol (substituted for 50 mM NaCl), and the TEA-containing solution was prepared by replacing the mannitol with 50 mM TEACl. The pH and the osmolarities of these solutions were measured and adjusted (to 7.2–7.4, and 300–310 mosM, respectively) before use.

Data Analyses

In most experiments, the time- and voltage-dependent properties of the peak and plateau components of the total depolarization-activated outward currents evoked during brief depolarizations were determined and compared. Peak currents at each test potential were measured as the difference between the maximal outward current amplitude and the zero current level; plateau currents were measured as the difference between the outward currents amplitudes remaining 100 ms after the onset of depolarizing voltage steps and the zero current level. The waveforms of the “4-AP-sensitive” and “TEA-sensitive” components were determined by subtraction of the currents measured in the presence of 4-AP or TEA from those measured in control bath solutions. Rate constants (or time constants) for activation and inactivation of the total outward currents, the 4-AP-sensitive, and the TEA-sensitive current components were determined as described in the text. In all cases, correlation coefficients (R) were determined to assess the quality of the fits to the experimental data; R values for the analyses presented here were in the range of 0.950 to 0.999.

All averaged and normalized data are presented as mean \pm the standard deviation of the mean (\pm SD). The statistical significance of differences in the calculated mean values was evaluated using the Student's t test, and P values are provided (in parentheses) in the text. In all cases in which the data were fit by defined functions, the functions used are described in the text; some of the fits are displayed in the figures with the data points. In addition, R values were determined to assess the quality of the fits and R values for fits presented here are provided (in parentheses) in the text.

RESULTS

Depolarization-activated Outward Currents

In voltage-clamped isolated adult rat ventricular myocytes, brief depolarizations to potentials positive to 0 mV from a HP of -70 mV evoke outward currents (Fig. 1), which rise rapidly to a peak followed by a slower decay to an apparent plateau. Although the current waveforms displayed in Fig. 1 are similar to those measured using the single microelectrode voltage-clamp technique (Josephson et al., 1984), the whole-cell recording method has the advantage of providing resolution of the rising phases of the evoked currents. Outward currents were measured within the first 1–2 min after establishing the whole-cell configuration, and no measurable changes in current properties were evident over time during recordings lasting 5–60 min. The rates of rise of the outward currents, as well as the peak and the plateau outward current amplitudes (see Methods), increase during depolarizations to more positive

test potentials; the largest and most rapidly activating current in Fig. 1 was evoked at +60 mV.

All depolarization-activated outward current was blocked when the K⁺ in the recording pipettes was replaced with equimolar Cs⁺, suggesting that the measured currents (Fig. 1) are carried primarily or exclusively by K⁺. In addition, it was assumed that the currents were not dependent on the presence of voltage-activated Ca²⁺ influx, because inward Ca²⁺ currents (I_{Ca}) should have been blocked by the Co²⁺

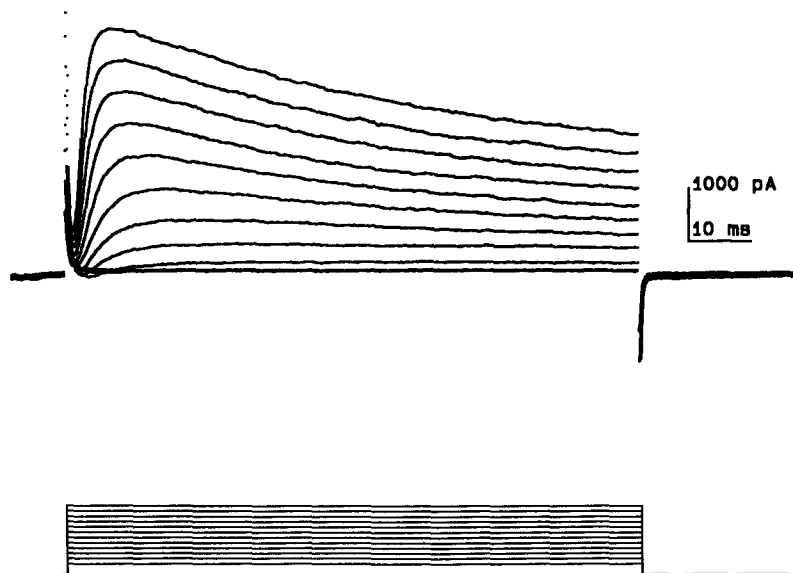


FIGURE 1. Waveforms of depolarization-activated outward K⁺ currents in an isolated adult rat ventricular myocyte. Currents, recorded using the whole-cell patch-clamp technique, were evoked during 100-ms depolarizations to test potentials between -50 and +60 mV from a HP of -70 mV. Depolarizing voltage steps were presented in 10-mV increments at 5-s intervals. The voltage-clamp paradigm in this figure (and in all subsequent figures in which data from single cells are displayed) is drawn below the current records for illustration purposes. Recordings were obtained as described in Methods with Co²⁺ (5 mM) and TTX (20 μ M) in the bath to diminish voltage-gated inward Ca²⁺ and Na⁺ currents, respectively. The apparent gaps in the current records for the first 1-2 ms after the onset of the voltage steps in this figure (and in subsequent figures in which records from single cells are displayed) result from the large capacitive transients which saturate the recording amplifier (see Methods).

in the bath. Blockade of I_{Ca} by 5 mM Co²⁺ was confirmed in separate experiments in which outward currents were suppressed by the replacement of Cs⁺ for the K⁺ in the pipettes. Under these conditions, no inward Ca²⁺ currents (and no outward currents) were measured during depolarizations to potentials between -30 and +60 mV from a HP of -70 mV. Thus, we conclude that the currents characterized here represent exclusively depolarization-activated, Ca²⁺-independent outward currents.

Although outward current waveforms similar to those in Fig. 1 were measured in all isolated adult rat ventricular myocytes, the relative peak to plateau current amplitudes varied markedly among cells. On average, the current remaining 100 ms after the onset of a depolarization to +30 mV from a HP of -70 mV was $54 \pm 15\%$ (mean \pm SD; $n = 19$) of the peak current evoked during the same voltage step; the range was 26–79%. In addition, the relative peak to plateau amplitudes (in the same cell) varied as a function of the HP from which the currents were evoked. In Fig. 2, for example, the plateau current at +40 mV was 54% of the peak when the HP was -90 mV (Fig. 2 *A*), whereas the corresponding ratios for currents evoked from -70 mV (Fig. 2 *B*) and -50 mV (Fig. 2 *C*) were 45% and 37%, respectively. The reductions in the ratios of the plateau to peak current amplitudes result from the fact that the plateau current is more dramatically affected when the HP is depolarized. On changing the HP from -90 to -50 mV, for example, the plateau outward current amplitude evoked at +30 mV was reduced (from 4,700 to 2,550 pA) by 46%, whereas the peak current was attenuated (from 8,500 to 6,700 pA) by only 17%. In Fig. 2 *A*, I_{Na} is also evident during depolarizations from a HP of -90 mV, confirming that I_{Na} in adult rat ventricular myocytes is not completely blocked at 20 μ M TTX (Reuter, 1984). Although seen in recordings from all cells during depolarizations from HPs \leq -70 mV (Fig. 3 *A*), the TTX-resistant component of I_{Na} could be eliminated by brief depolarizations to -20 mV before the depolarizations to evoke the outward currents (Fig. 3 *B*). This procedure, which did not measurably alter the amplitudes of the peak or plateau outward currents, facilitated measurement of the currents evoked from hyperpolarized HPs.

Reversal Potentials of the Depolarization-activated Outward Currents

To examine the K^+ selectivity of the currents, reversal potentials were estimated by measuring current "tails" evoked during hyperpolarizations to potentials between -30 and -140 mV from a depolarized test potential of +20 mV (delivered from a HP of -70 mV to evoke the currents). Because the relative amplitudes of the peak and plateau currents varied as a function of HP (Fig. 2), it seemed likely that the measured waveforms reflected the presence of two (or more) current components. Tail currents were elicited, therefore, during hyperpolarizations presented at the peak of the outward current, i.e., 10 ms after the onset of the depolarizations (Fig. 4), and after the current had reached an apparent plateau level, i.e., 100 ms after the onset of the depolarizations (Fig. 5). Hyperpolarizations to potentials negative to -90 mV in these cells, however, also evoke large inward currents, resulting from the activation of inwardly rectifying K^+ channels (Matsuda et al., 1987). As a result, the instantaneous current amplitudes measured during hyperpolarizations to potentials

FIGURE 2. (*opposite*) The relative amplitudes of the peak and plateau outward K^+ currents vary as a function of HP. Outward currents were evoked as described in the legend of Fig. 1 during membrane depolarizations to potentials between -40 and +40 mV from HPs of (*A*) -90 (*B*) -70, and (*C*) -50 mV; the recordings displayed were obtained from the same cell and the voltage-clamp paradigms are illustrated below each set of current records. At the more depolarized HPs (*B* and *C*), the plateau current amplitudes were reduced to a larger extent than the peak currents at all test potentials. Similar results were obtained in eight cells.

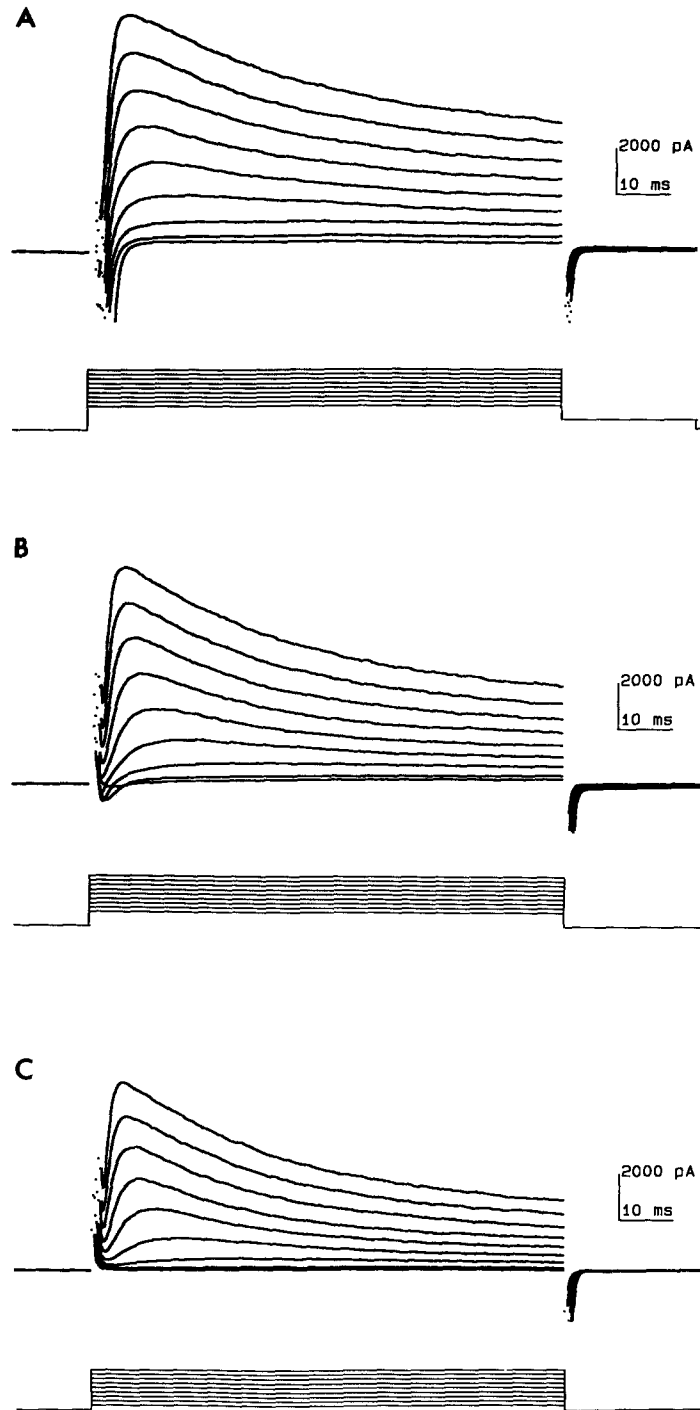


FIGURE 2.

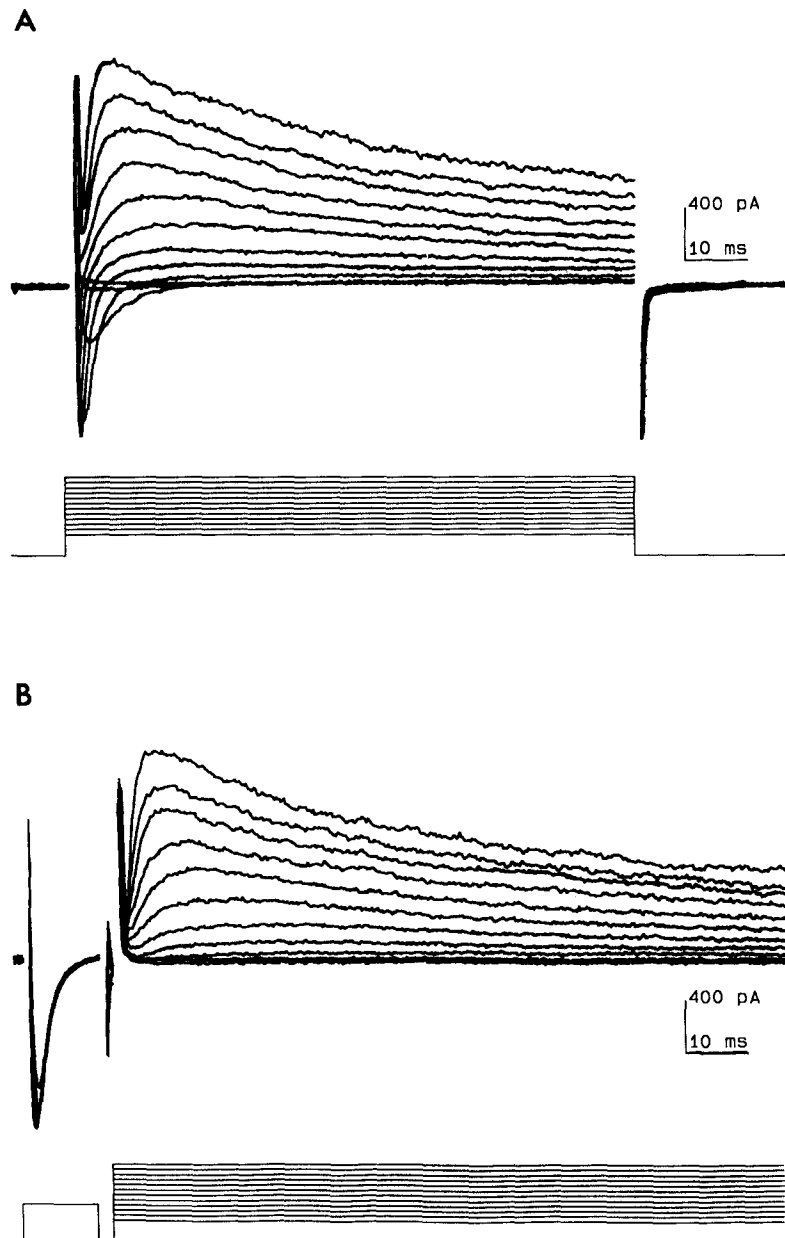


FIGURE 3. The component of I_{Na} persisting in $20 \mu\text{M}$ TTX can be eliminated by brief depolarization. (A) Outward currents were evoked as described in the legend of Fig. 1 during membrane depolarizations directly from a HP of -90 mV . (B) Outward currents were then remeasured during identical voltage steps from a HP of -90 mV preceded by 15-ms depolarizations to -20 mV to inactivate I_{Na} . Both sets of records were obtained from the same cell; the voltage-clamp paradigms are illustrated below the records. The residual I_{Na} was eliminated using the voltage-clamp protocol in B, whereas the amplitudes of the peak and plateau outward currents were not measurably altered (compare A and B). Similar results were obtained in 11 cells.

negative to ≈ -90 mV (Figs. 4 *A* and 5 *A*) reflect currents through these channels, in addition to the tail currents of the depolarization-activated K⁺ channels. To estimate the amplitudes of the currents through the inwardly rectifying K⁺ channels, tail currents were also elicited during voltage steps from -20 mV to potentials between -30 and -140 mV (Figs. 4 *B* and 5 *B*). These records were then subtracted from those recorded following the hyperpolarizations from $+20$ mV (Figs. 4 *A* and 5 *A*). Although depolarization-activated currents were evident during voltage steps to -20 mV (Figs. 4 *B* and 5 *B*) this potential was selected to optimize the amplitudes of the capacitative transients for subtraction, thereby providing better resolution of the tail currents. The subtracted records (Figs. 4 *C* and 5 *C*), therefore, should reflect only the relaxation of the outward K⁺ currents activated on depolarization. In addition, the subtracted records for hyperpolarizations presented 10 (Fig. 4 *C*) and 100 (Fig. 5 *C*) ms after the onset of depolarization should correspond to the tail currents of the peak and plateau outward current components, respectively.

Reversal potentials (mean \pm SD; $n = 8$), estimated by linear interpolation (plateau current) or extrapolation (peak current) of isochronal tail current–voltage plots for tail currents measured 5 ms after the onset of the hyperpolarizations from subtracted records such as those in Fig. 4 *C* and 5 *C*, were -74.5 ± 8.5 and -65.0 ± 7.6 mV for the peak and plateau components, respectively. In separate experiments ($n = 3$), tail currents were measured during hyperpolarizations to potentials between -30 and -140 mV from a depolarized test potential of $+20$ mV in bath solution containing 1 mM Ba²⁺ to block the currents through inwardly rectifying K⁺ channels. The reversal potentials of the peak and plateau components determined in these experiments were -74.2 ± 1.6 and -66.0 ± 2.2 mV, respectively. These values are not significantly different ($P > 0.50$) from those obtained using the subtraction method (Figs. 4 and 5). Because the equilibrium potential for K⁺ (E_K) under the recording conditions employed here was -90 mV, the estimated reversal potentials are consistent with K⁺ being the predominant ion carrying the current through the channels opened on membrane depolarization (see Discussion).

Although we have not examined the K⁺ concentration dependences of the reversal potentials, we have observed an anomalous relationship between the apparent K⁺ conductance and the extracellular concentration of K⁺ (K_0^+). When K_0^+ is decreased from 4 mM, the driving force for current flow at depolarized potentials, and hence the measured outward current amplitudes, should increase because the equilibrium potential for K⁺ becomes more negative. In spite of this predicted behavior, we found that the peak and plateau current amplitudes decreased when K_0^+ was decreased below 4 mM. During depolarizations to $+50$ mV from a HP of -70 mV, for example, outward current amplitudes were decreased $\approx 30\%$ ($n = 3$) when K_0^+ was decreased from 4 to 0 mM. Current amplitudes returned to control levels when K_0^+ was increased to 4 mM. Similar anomalous dependences of K⁺ conductance on K_0^+ have been observed in other excitable cells (Clay, 1986; Matteson and Swenson, 1986; Wagoner and Oxford, 1987), including cardiac myocytes (Schreibmayer et al., 1985; Shibasaki, 1987). It has been suggested that changes in K_0^+ directly alter K⁺ channel gating (Clay, 1986; Matteson and Swenson, 1986) or conductance (Shibasaki, 1987). To date, we have not explored this issue further, and all of the experiments here were conducted at 4 mM K_0^+ .

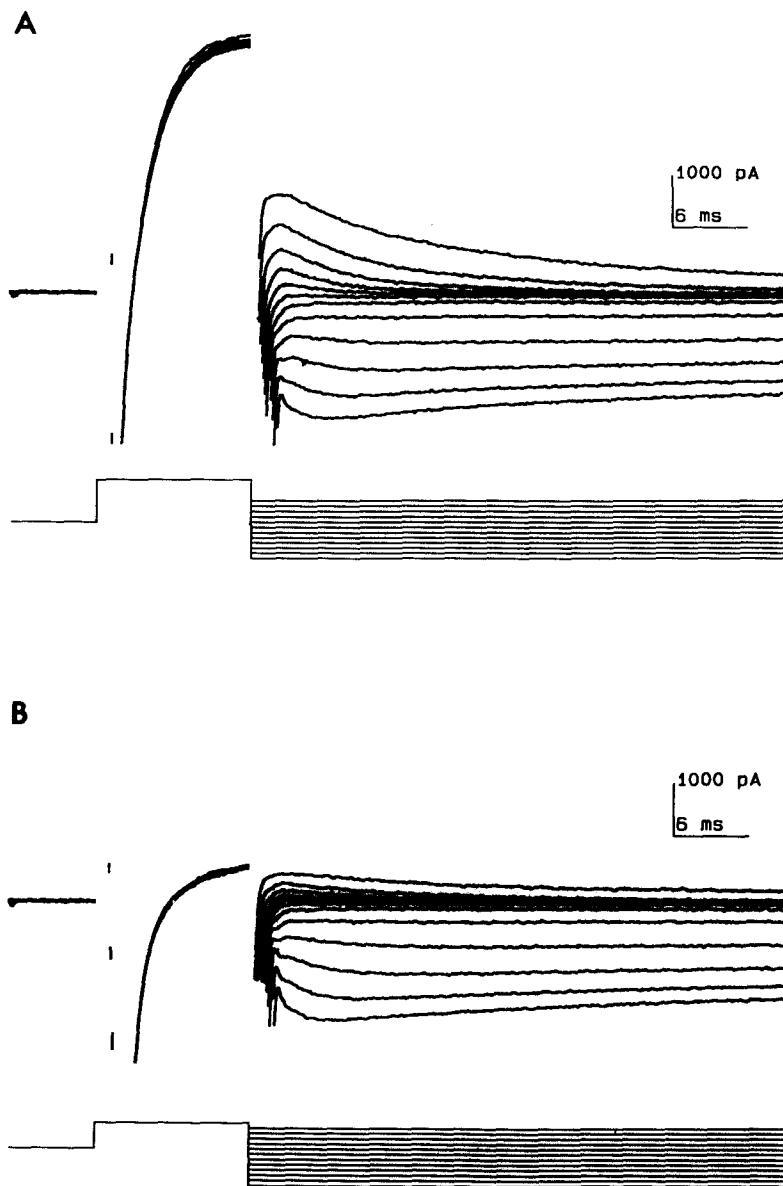


FIGURE 4. Tail currents of the peak component of the total depolarization-activated outward current. (A) After depolarizations to +20 mV from a HP of -70 mV, tail currents were elicited during membrane hyperpolarizations to potentials between -30 and -140 mV. Hyperpolarizing voltage steps were presented 10 ms after the onset of the depolarizations of +20 mV; the voltage-clamp paradigm is illustrated below the records. (B) Because hyperpolarizations to potentials ≤ -90 mV also evoke currents through inwardly rectifying K^+ channels (see text), the amplitudes of these currents were measured separately during voltage steps to -30 to -140 mV from a test potential of -20 mV. (C) The current tails, attributed to the closing of the K^+ channels opened on depolarization, were obtained by subtracting the records in B from those in A. Similar results were obtained in eight cells.

Separation of Outward Currents

The outward K^+ current waveforms (Figs. 1–3) could result from the activation of a single population of K^+ channels or, alternatively, could reflect contributions from multiple K^+ channel types. Owing to the known diversity of K^+ channels (Thompson, 1977; Hagiwara, 1983; Latorre et al., 1984; DiFrancesco and Noble, 1985; Cohen et al., 1986), it seemed likely to us that the observed current waveforms resulted from the integrated activity of more than one type of K^+ channel. The findings that the ratio of the plateau to peak current varied as a function of HP (Fig. 2) and that the estimated reversal potentials of the peak and plateau were distinct (Figs. 4 C and 5 C) also suggested to us the presence of multiple outward current components. By analogy to other cells, we speculated that, if the total currents (Figs. 1–3) reflect activation of more than one type of K^+ channel, it should be possible to distinguish

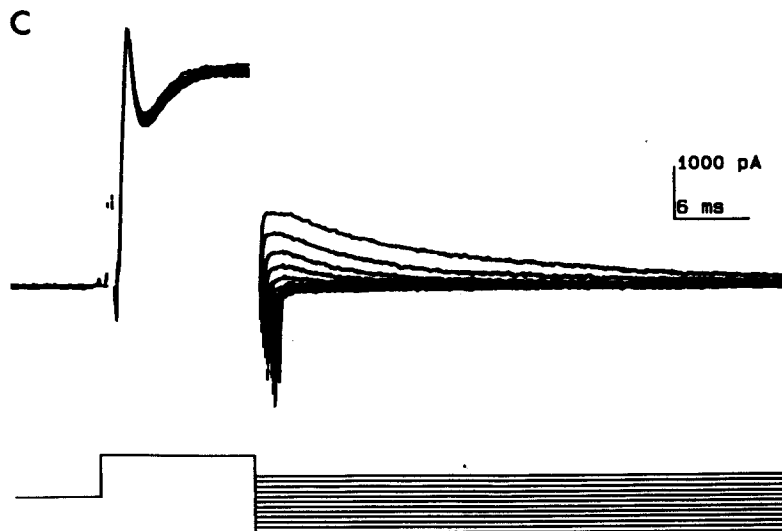


FIGURE 4 (continued).

these by exploiting differences in pharmacologic sensitivities as well, perhaps, as differences in voltage- and time-dependent properties. To test this possibility, we examined the effects of 4-AP and TEA because extracellular application of these agents has been shown to block different types of K^+ channels in other cells. In molluscan neurons, for example, 4-AP blocks preferentially the fast, transient outward K^+ current, I_A , whereas TEA exhibits greater selectivity in blocking the delayed rectifier, I_K (Thompson, 1977). Blockade of Ca^{++} -independent transient outward K^+ currents by extracellular 4-AP has also been described in Purkinje fibers (Kenyon and Gibbons, 1979b; Coraboeuf and Carmeliet, 1982; Lipsius and Gibbons, 1982; Kenyon and Sutko, 1987), ventricular muscle strips (Litovsky and Antzelevitch, 1988) and in isolated ventricular (Josephson et al., 1984; Mitchell et al., 1984; Tseng et al., 1987), crista terminalis (Giles and Van Ginneken, 1985) and atrial (Escande et al., 1987) cells. Although not studied extensively, TEA reportedly blocks outward

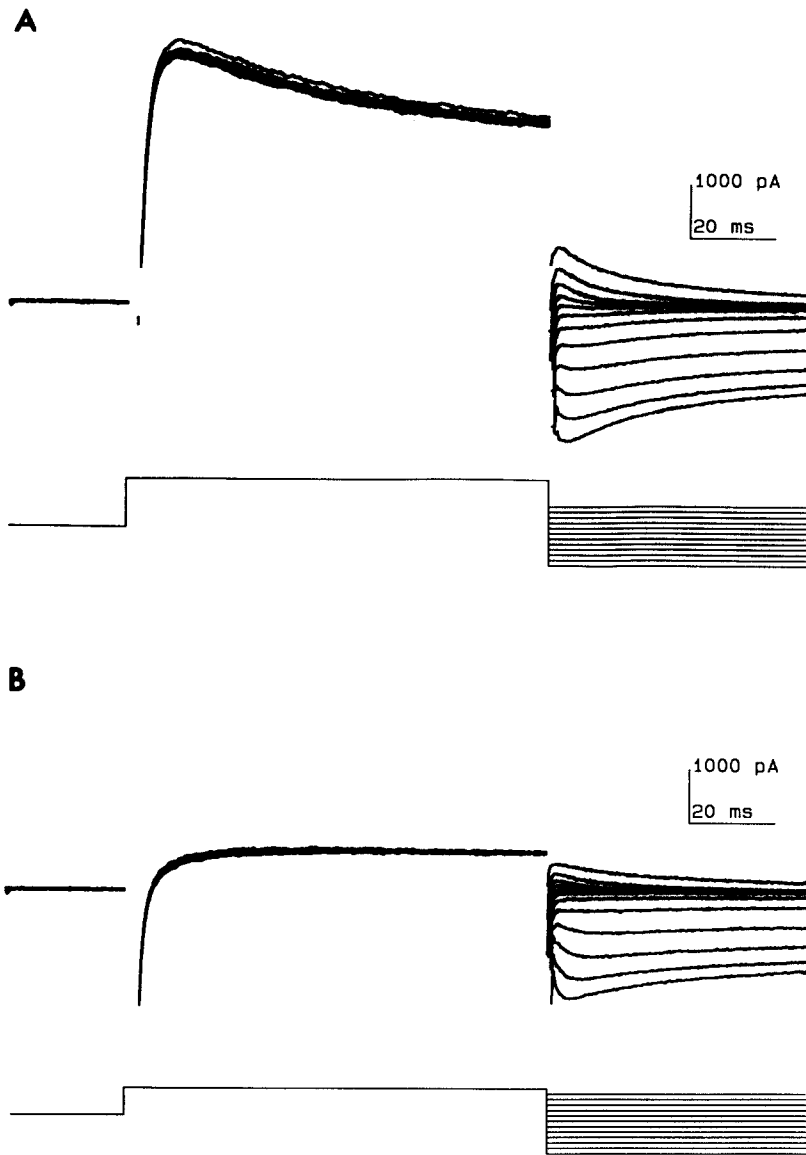


FIGURE 5. Tail currents of the plateau component of the total depolarization-activated outward current. (A) After depolarizations to +20 mV from a HP of -70 mV, tail currents were elicited during membrane hyperpolarizations to potentials between -30 and -140 mV. Hyperpolarizing voltage steps were presented 100 ms after the onset of the depolarizations to +20 mV; the voltage-clamp paradigm is illustrated below the current records. (B) Because hyperpolarizations to potentials ≤ -90 mV also evoke currents through inwardly rectifying K^+ channels (see text), the amplitudes of these currents were measured separately during voltage steps to -30 to -140 mV from a test potential of -20 mV. (C) The current tails, attributed to the closing of the K^+ channels opened on depolarization, were obtained by subtraction of the records in B from those in A. Similar results were obtained in eight cells.

currents in Purkinje fibers (Kenyon and Gibbons, 1979a; Kass et al., 1982) and single K^+ channel currents in ventricular myocytes (Schreibmayer et al., 1985).

To evaluate the effects of 4-AP, outward currents evoked (in the same cell) under control conditions (Fig. 6 A) and in the presence of 4-AP (Fig. 6 B) were compared. During continuous puffer application of a solution containing 3 mM 4-AP, substantial attenuation of the peak current was evident (Fig. 6 B), although little or no suppression of the plateau current was observed. Peak current amplitudes returned to control levels following cessation of the 4-AP application (not shown) and recovery could be accelerated by puffer applications of bath solution (not shown). The 4-AP-sensitive component (Fig. 6 C) of the total depolarization-activated outward current was determined by subtraction of the currents recorded (in the same cell) during depolarizations to potentials between -30 and $+50$ mV from a HP of -70 mV in the presence of 4-AP (Fig. 6 B) from those measured in its absence (Fig. 6 A).

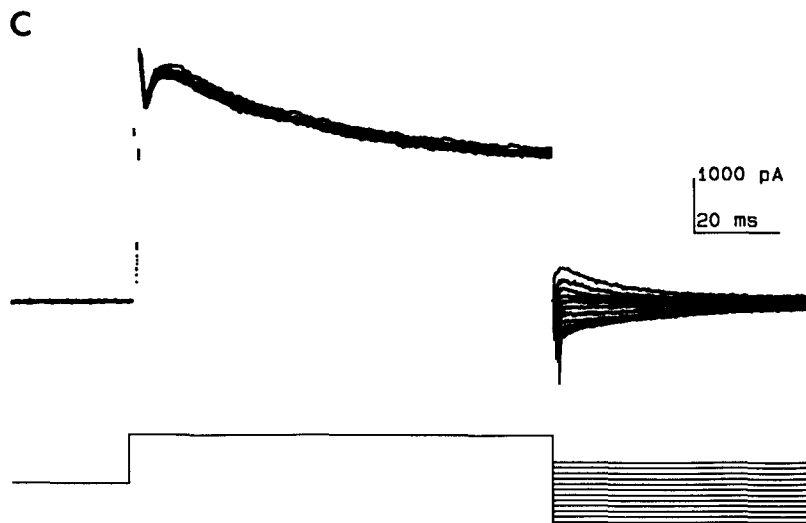


FIGURE 5 (continued).

Examination of the waveforms of the 4-AP-sensitive currents (Fig. 6 C) reveals currents which rise rapidly to a transient peak followed by a slower decline to baseline and the decay of the 4-AP-sensitive currents (Fig. 6 C) is complete, or nearly complete, by the end of 125-ms voltage steps at all test potentials. In addition, the rising phases of the 4-AP-sensitive (Fig. 6 C) and the total depolarization-activated outward currents (Fig. 6 A) are similar (see below).

In preliminary experiments, attempts were made to examine the TEA-sensitive current using a similar paradigm, i.e., currents were recorded before and during puffer applications of a solution in which TEACl (50 mM) was substituted for NaCl (50 mM). To maximize the amplitude of the plateau component (see Fig. 2), depolarizations were evoked from a HP of -90 mV. These experiments, however, were complicated by the fact that inward Na^+ currents were also evoked because I_{Na} is not completely blocked by $20 \mu M$ TTX (Reuter, 1984). In addition, the residual I_{Na}

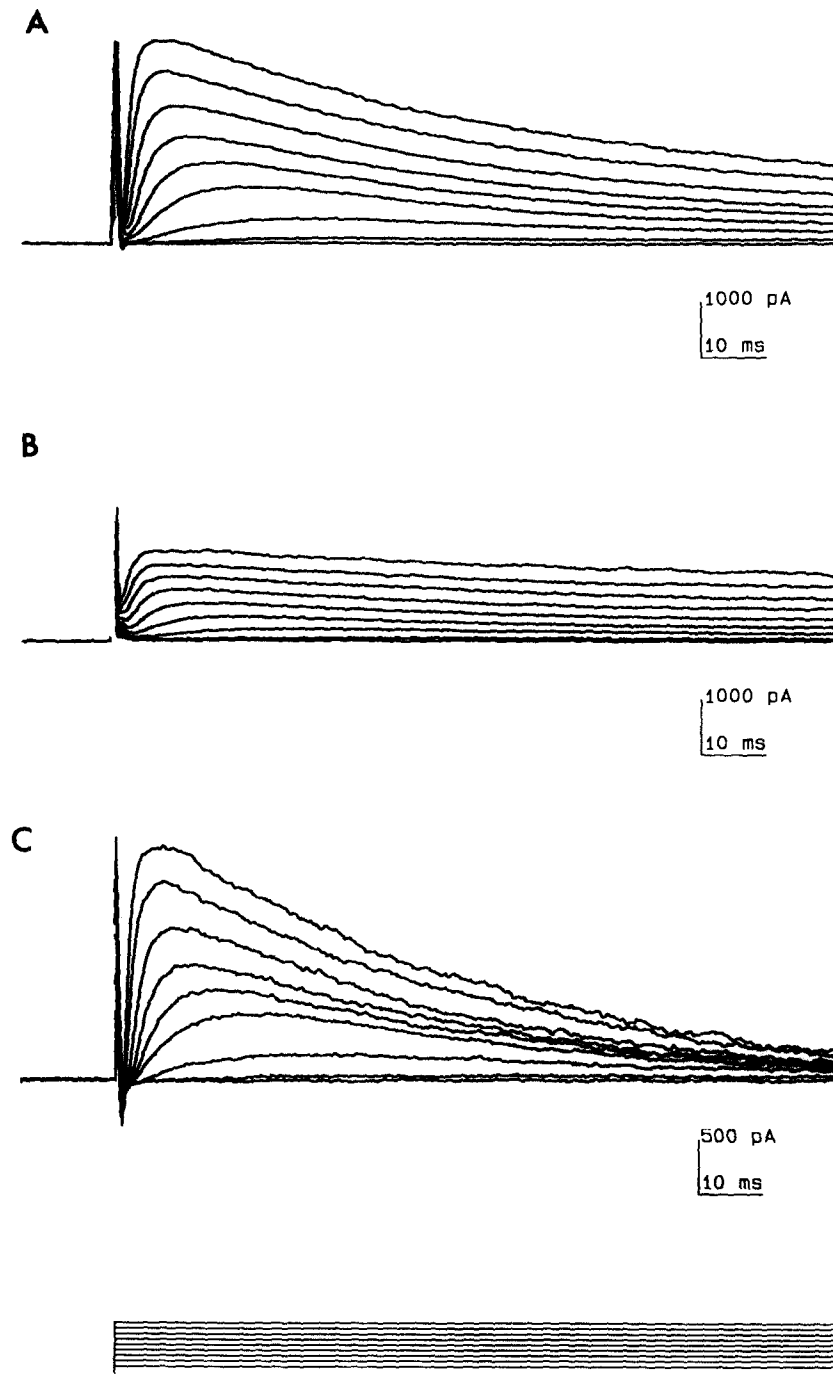


FIGURE 6. 4-AP-sensitive component of the total depolarization-activated outward current. Outward currents were evoked on depolarizations to potentials between -30 and $+50$ mV from a HP of -60 mV as described in the legend of Fig. 1. After recording control currents (*A*), the

was reduced during application of the TEA-containing solution due to a change in the driving force for Na⁺. As a result, subtracted records revealed not only the TEA-sensitive outward currents but, also, TEA-sensitive inward Na⁺ currents. To circumvent this problem, the voltage-clamp paradigm was altered to include brief depolarizations to -20 mV to inactivate the residual I_{Na} ; cells were then repolarized to -90 mV for 2 ms (insufficient time to allow recovery of I_{Na} from steady-state inactivation), and subsequently depolarized to potentials between -30 and +50 mV to evoke outward K⁺ currents (Fig. 3). In addition, to eliminate effects due to changes in the driving force on Na⁺ the control bath solution contained 100 mM mannitol (substituted for 50 mM NaCl), and the TEA-containing solution was prepared by substituting TEACl (50 mM) for the mannitol (see Methods). The TEA-sensitive component (Fig. 7 C) of the total depolarization-activated outward current was obtained by subtraction of the currents evoked in the presence of TEACl (Fig. 7 B) from those recorded in its absence (Fig. 7 A). The TEA-sensitive currents (Fig. 7 C) activate with a delay after the onset of the depolarizations and rise slowly to plateau levels. Intracellular applications of TEA (not shown) via perfusable patch pipettes (Lapointe and Szabo, 1987), in contrast, suppressed both the peak and plateau current amplitudes; intracellular TEA application has similar effects in Purkinje fibers (Kass, 1984).

The simplest interpretation of these results is that there are two K⁺ current components which contribute to the total depolarization-activated outward currents in adult rat ventricular myocytes: (a) a 4-AP-sensitive component which activates and inactivates rapidly on depolarization and (b) a TEA-sensitive component which activates with a delay slowly to a plateau level (during brief depolarizations). We refer to the rapidly activating and inactivating component as I_{to} (transient outward) because this term best describes the properties of the measured current waveforms. Although transient outward K⁺ currents have been studied in a number of myocardial preparations (Cohen et al., 1986), there has been no consensus concerning terminology and various names have been used (Kenyon and Gibbons, 1979b; Boyett, 1981a, b; Coraboeuf and Carmeliet, 1982; Josephson et al., 1984; Giles and Van Ginneken, 1985; Escande et al., 1987; Ng et al., 1987; Tseng et al., 1987; Clark et al., 1988; Giles and Imaizumi, 1988; Litovsky and Antzelevitch, 1988). A rapidly activating outward K⁺ current has been described previously in isolated adult rat ventricular myocytes (Josephson et al., 1984) and referred to as I_{eo} (early outward). Although (as will be demonstrated) the properties of I_{to} described here are similar in some respects to those of I_{eo} , there are also important differences. In particular, the second outward current component was not distinguished in the previous work and, as a result, the fact that the current underlying the peak decays to baseline during brief depolarizations was not evident (Josephson et al., 1984). The term I_{to} , therefore, seems more

cell was exposed continuously to a solution containing 3 mM 4-AP by pressure ejection from a "puffer" pipette and outward currents were remeasured (B). Substantial suppression of the peak current is evident in the presence of 4-AP (B), although there is little or no attenuation of the plateau. The 4-AP-sensitive component (C) was obtained by subtraction of the records in B from those in A; note the change in current scale in C. Similar results were obtained in seven cells.

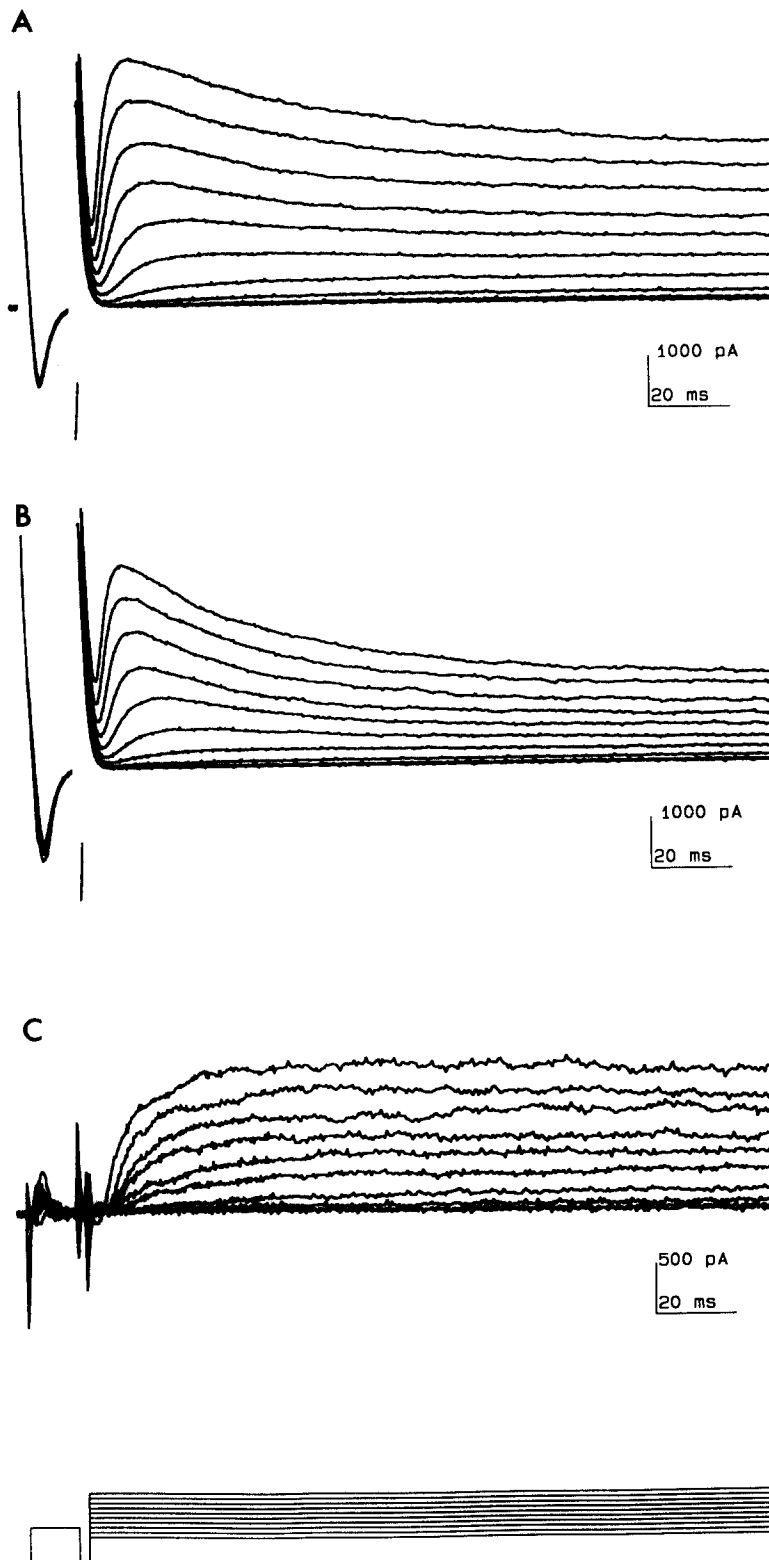


FIGURE 7.

appropriate to describe the outward K^+ current component in adult rat ventricular myocytes which activates rapidly and transiently on membrane depolarization (see Discussion).

The slowly activating component is referred to here as I_K owing to its similarity with delayed (outward rectifier) K^+ currents in other cells (Thompson, 1977; McDonald and Trautwein 1978*a, b*; Kass et al., 1982; Latorre et al., 1984; Gintant et al., 1985; Shibasaki, 1987; Tseng et al., 1987). As will be demonstrated, however, the voltage-dependent properties of I_K in adult rat ventricular myocytes are not identical with those of currents termed I_K in other cells. In the composite outward current waveforms (Figs. 1–3), I_{to} appears to dominate the rapid rise of the currents and is largely inactivated at later times and I_K appears to provide the maintained contribution to the currents remaining 100 ms after the onset of the depolarizations. The peak outward currents, therefore, should reflect primarily I_{to} , whereas the currents remaining at 100 ms should be dominated by I_K . To evaluate the validity of this interpretation, we examined the voltage- and time-dependent properties of outward current activation and inactivation during voltage steps and of steady-state inactivation of the currents, by comparing the properties of the peak currents with those of the plateau currents remaining 100 ms after the onset of depolarizations. The 100-ms time point was selected because, in adult rat ventricular myocytes, APs are brief, and $\approx 90\%$ repolarization is observed within ≈ 40 –150 ms (Powell et al., 1980; Watanabe et al., 1983; Mitchell et al., 1984*a, b*; Schouten and Ter Keurs, 1985; Meier et al., 1986). In addition, we measured outward current waveforms evoked during prolonged depolarizations to examine the kinetics and voltage dependence of I_K inactivation, which is not apparent during brief depolarizations.

Current–Voltage Relations

The voltage-dependence of activation was investigated for currents evoked during depolarizations to potentials between -40 and $+60$ mV from a HP of -70 mV (Fig. 8). Peak and plateau currents in individual cells were measured at each test potential, and subsequently normalized to their respective (peak and plateau) amplitudes measured during depolarizations to $+30$ mV. Data obtained from many cells ($n = 18$) were then averaged and are plotted in Fig. 8*A*. Both components begin to activate at ≈ -30 mV and the normalized current–voltage relations are similar. Membrane conductances, corresponding to the peak and plateau currents, were calculated using the expression $g(V) = i/(V - E)$, where i is the measured current at each test

FIGURE 7. (*opposite*) TEA-sensitive component of the total depolarization-activated outward current. Outward currents were evoked during depolarizations to potentials between -30 and $+50$ mV from a HP of -90 mV; a 15-ms prepulse to -20 mV was delivered in each trial to inactivate the residual I_{Na} before the depolarizations to evoke outward currents (the paradigm is illustrated at the bottom). (*A*) Control currents were recorded in bath solution containing 100 mM mannitol before exposing the cell to a solution containing 50 mM TEACl from a “puffer” pipette (see Methods). (*B*). In the presence of TEA, substantial attenuation of the plateau current is evident at all test potentials. (*C*) The TEA-sensitive component was determined by subtraction of the records in *B* from those in *A*; note the change in the current scale in *C*. Similar results were obtained in five cells.

potential, V ; and E is the calculated reversal potential for K^+ under the recording conditions employed. Normalized average conductances ($n = 18$) were then calculated and are plotted in Fig. 8 *B* as a function of test potential. Both components (Fig. 8 *B*) are voltage dependent, and no apparent saturation in conductance is evident over the potential range -30 to $+60$ mV. Similar results were obtained if the reversal potentials estimated from the analyses of tail currents (Figs. 4 and 5), rather than the theoretical E_K , were used to calculate the conductances, although, in this

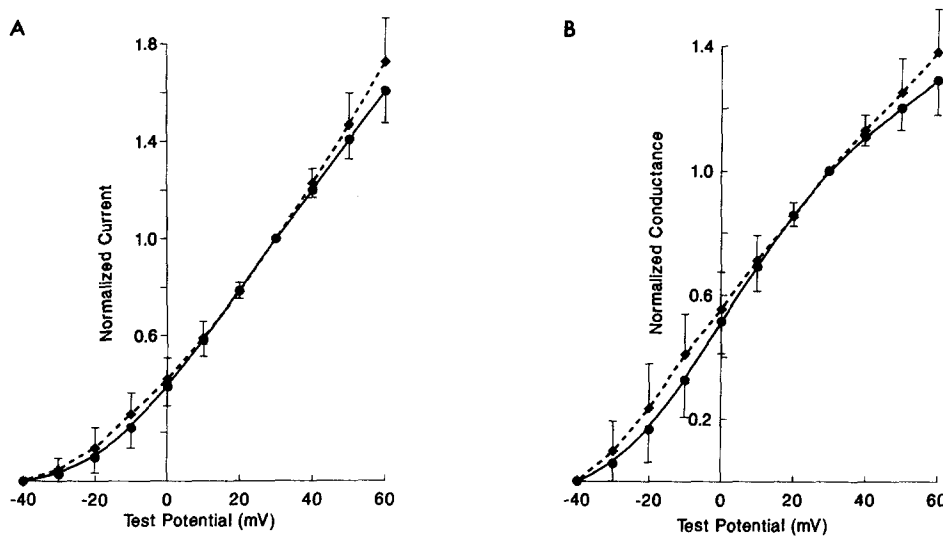


FIGURE 8. (*A*) Normalized current–voltage and (*B*) conductance–voltage relations of the peak and plateau depolarization-activated outward current components. (*A*) Outward currents were evoked during 100-ms depolarizations to potentials between -40 and $+60$ mV from a HP of -70 mV as described in the legend of Fig. 1. For each cell, the amplitudes of the peak and plateau currents were measured at each test potential and normalized to their respective (peak and plateau) amplitudes evoked at $+30$ mV. Mean (\pm SD) normalized values ($n = 18$) for the peak (●) and plateau (◆) current components are plotted. (*B*) Membrane conductances corresponding to the peak and plateau currents were calculated at each test potential in each cell (see text) and normalized to their respective (peak and plateau) values determined at $+30$ mV. Mean (\pm SD) values ($n = 18$) for the peak (●) and plateau (◆) outward currents are plotted.

case, the curves were displaced slightly to the right (i.e., in the depolarizing direction). To explore the possibility that the conductances saturate at more positive potentials, current amplitudes were also measured in some cells ($n = 4$) during depolarizations to potentials between -40 and $+100$ mV. These experiments confirmed that no saturation in conductance was evident up to $+100$ mV; average normalized (to $+30$ mV) conductances for the peak and plateau outward current components at $+100$ mV, for example, were 1.67 and 1.98, respectively ($n = 4$).

Kinetics of Outward Current Activation

Employing the whole-cell recording technique, the waveforms of the total depolarization-activated outward K^+ currents were sufficiently well resolved to permit kinetic analyses of the rising phases of the currents. Activation rate constants were calculated, therefore, from single exponential fits to the rising phases of the currents from records such as those in Fig. 1. Activation rate constants ($n = 14$) increased from 0.12 to 0.96 ms^{-1} between -30 and $+60$ mV (Fig. 9) with no apparent saturation. If the

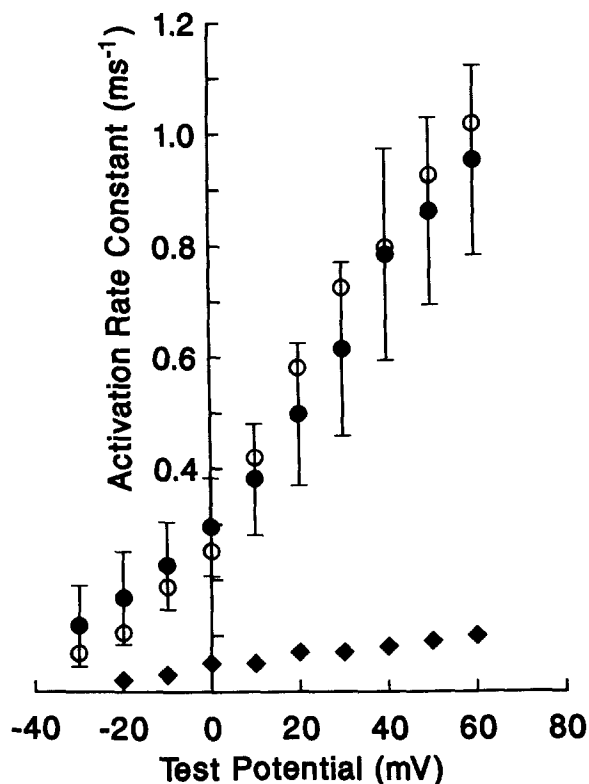


FIGURE 9. Activation rate constants for the depolarization-activated outward currents. Outward currents were evoked during membrane depolarizations to potentials between -30 and $+60$ mV from a HP of -70 mV as described in the legend of Fig. 1. From single exponential fits to the rising phases of the currents (from the onset of the depolarization to the peak), activation rate constants for the total depolarization-activated outward currents were determined; mean (\pm SD) values ($n = 14$) are plotted (\bullet) as a function of test potential. Similar analyses were completed to determine the rate constants of activation of the 4-AP-sensitive outward currents from subtracted records such as those in Fig. 6 C; average values (\circ , $n = 6$) are plotted. Rate constants for the activation of the TEA-sensitive (\blacklozenge)

component of the total outward current were determined from single exponential fits to the rising phases of the currents (after the delay) in records such as those in Fig. 7 C; average values ($n = 5$) are plotted.

rising phases of the currents are dominated by I_{to} (as suggested above) then, activation rate constants determined for the total depolarization-activated outward currents should reflect the rates of activation of I_{to} . Analyses of the rates of activation of the 4-AP-sensitive component of the total outward current from records such as those in Fig. 6 C yielded rate constants indistinguishable from those determined for the total outward currents (Fig. 9). The rates of activation of the currents evoked during depolarizations from a HP of -70 mV, therefore, appear to be determined by the kinetics of I_{to} activation, with no measurable contribution from I_K .

Given the results above, it is evident that contamination from I_{to} precluded simultaneous determination of the rate constants for activation of I_k from the waveforms of the total outward currents: the rising phases of I_k are obscured at all test potentials because I_{to} is the predominant current during the first ≈ 10 ms after the onset of the depolarizations. Reliable measurements of the rates of activation of I_k , therefore, could only be provided from analyses of the TEA-sensitive component of total outward current in subtracted records such as those in Fig. 7 C. The rate constants for activation of the TEA-sensitive currents (I_k) were calculated from single exponential fits to the rising phases of the currents after the delay. Activation rate constants for I_k (Fig. 9) determined in this manner are voltage dependent, increasing from 0.02 to 0.10 ms^{-1} at potentials between -20 and $+60$ mV. I_k , therefore, activates ~ 10 -fold more slowly than I_{to} over the range of test potentials examined.

Kinetics of Outward Current Inactivation

In initial experiments, attempts were made to determine the time constants of I_{to} inactivation by analyzing the decay phases of the total depolarization-activated outward currents evoked during 100-ms voltage steps. At test potentials between 0 and $+60$ mV, the decay phases of the currents were well fit ($R \geq 0.980$) by single exponentials, and the currents remaining at the end of the depolarizations were reflected in these fits as offset terms. At more hyperpolarized test potentials, the data were also best fit by single exponentials, although the quality of the fits was reduced, presumably because the current amplitudes were small. Nevertheless, these analyses revealed that the time constants for decay of the total depolarization-activated outward currents were similar at all test potentials; mean (\pm SD) time constants for the currents evoked at 0 and $+60$ mV were 58.5 ± 18.7 and 52.8 ± 16.9 ms, respectively ($n = 18$). These values are not significantly different ($P > 0.20$), suggesting that the kinetics of I_{to} inactivation are relatively voltage insensitive. It was clear, however, that these analyses were potentially complicated because I_k activates slowly on membrane depolarization (Fig. 7 C) and the rates of I_k activation increase with increasing depolarization (Fig. 9). The decay phases of the currents measured during brief depolarizations, therefore, may be contaminated (and to a variable degree) by I_k activation. If so, then the time constants determined from single exponential fits to the decay phases of the total depolarization-activated outward currents likely do not reflect the "true" kinetics of I_{to} inactivation. To provide more reliable estimates of the kinetics of I_{to} decay, therefore, inactivation time constants were determined from single exponential fits ($R \geq 0.980$) to the decay phases of the 4-AP-sensitive outward currents in subtracted records, such as those in Fig. 6 C. Mean (\pm SD) decay time constants for the currents evoked at 0 and $+60$ mV were 50.7 ± 20.4 and 39.7 ± 10.7 ms, respectively ($n = 7$). The difference in the mean values is not significant ($P > 0.20$), revealing that the rates of inactivation of the 4-AP-sensitive current, i.e., I_{to} , are fast and voltage insensitive. When data from all cells at all test potentials were pooled, the mean (\pm SD) decay time constant was 41.0 ± 14.5 ms.

The above analyses suggest that the decay phases of the total depolarization-activated outward currents elicited during brief (100 ms) depolarizations results from inactivation of I_{to} and that I_k determines the plateau currents. It was not clear from these analyses, however, if I_k is noninactivating, or, alternatively, if inactivation of I_k

was too slow to be revealed during brief depolarizations. To determine if I_K does inactivate, outward currents were also measured during prolonged depolarizations. As is evident in Fig. 10, these experiments revealed that when currents were measured during 500 ms (Fig. 10 B) or 1 s (Fig. 10 C) depolarizations, considerable decay of the "plateau" component was observed. During 5-s depolarizations, the outward currents evoked at all test potentials in this cell decayed to zero (not shown). Similar results were obtained in 9 (of 10) cells examined under identical conditions (see below).

To determine the kinetics of I_K inactivation, functions representing the sum of two exponentials were fit to the decay phases of the total outward currents using the expression: $A_f * [\exp(-t/\tau_f)] + A_s * [\exp(-t/\tau_s)]$, where A_f and A_s are the amplitudes, and τ_f and τ_s are the time constants, of the rapidly decaying and the slowly decaying current components, respectively. At test potentials positive to -10 mV, the data were well fit ($R \geq 0.960$) by this expression and, in all cases, the sum of A_f and A_s accounted for the peak outward current amplitude. Mean (\pm SD) values for τ_s obtained from these analyses were $1,265 \pm 693$ and $1,214 \pm 557$ ms for the currents evoked at 0 and $+60$ mV, respectively ($n = 9$). Although there is considerable variability in the data, the differences in the mean values at different test potentials are not statistically significant ($P > 0.50$), revealing that the rate of I_K (like I_{to}) inactivation is voltage insensitive. When data from all cells at all test potentials were pooled, the mean (\pm SD) decay time constant was $1,238 \pm 566$ ms. On average, therefore, I_K inactivates ≈ 30 -fold more slowly than I_{to} . These analyses provided mean (\pm SD) values for τ_f at 0 and $+60$ mV of 51.9 ± 7.5 and 49.1 ± 8.3 ms, respectively ($n = 9$). The similarity between these values and those obtained from the analyses of the decay phases of the 4-AP-sensitive outward currents (above) is consistent with the previous suggestions that the rapidly decaying component reflects inactivation of I_{to} . As in the analyses of the decay phases of the total outward currents evoked during brief depolarizations, however, the measured values likely are inaccurate owing to the presence of I_K and the kinetics and voltage dependence of I_K activation. The more reliable estimates of the time constants for I_{to} inactivation, therefore, are assumed to be those provided from the analyses of the decay phases of the 4-AP-sensitive currents.

In 1 of the 10 cells examined during prolonged depolarizations, the outward currents did not decay to zero, i.e., a "noninactivating" current component was evident 5 s after the onset of the depolarizations. Kinetic data obtained from this cell were not included in the analyses above. The amplitude of this residual outward current was small, corresponding to $\approx 10\%$ of the peak current amplitude at all test potentials. Nevertheless, these results suggest the presence of a noninactivating outward current component in some ($\leq 10\%$) isolated adult rat ventricular myocytes. Further experiments will be necessary to evaluate the time- and voltage-dependent properties of this current.

Voltage and Time Dependence of Steady-State Inactivation

Some of the experiments described above (see, for example, Fig. 2) revealed that depolarization-activated K^+ current amplitudes in isolated adult rat ventricular myocytes vary as a function of HP. In addition, comparison of the waveforms of the

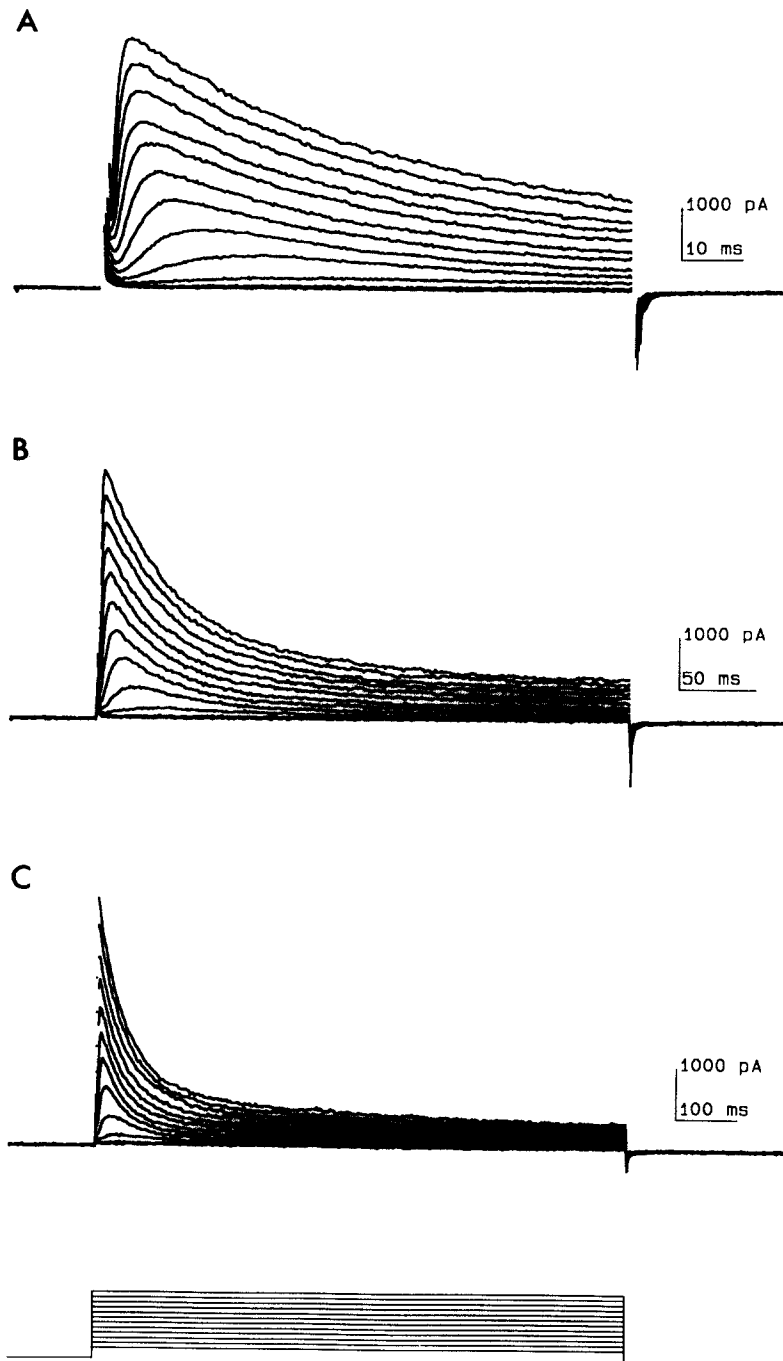


FIGURE 10. Inactivation of the plateau current component is evident during prolonged depolarizations. Outward currents were evoked as described in the legend of Fig. 1 during (A) 100-ms, (B) 500-ms, and (C) 1-s depolarizations to test potentials between -50 and $+60$ mV from a HP of -70 mV. The records displayed were obtained from the same cell and the voltage-clamp paradigm is illustrated at the bottom of the figure. As is evident, substantial attenuation of the "plateau" current is observed during prolonged depolarizations. Similar results were obtained in 9 (of 10) cells (see text).

currents evoked from different HPs suggests that the plateau current is more sensitive to changes in HP than the peak. Because both I_K and I_{to} begin to activate at ≈ -30 mV (Fig. 8A), the reductions in current amplitudes at depolarized HPs must result from steady-state inactivation. To examine the voltage dependence of steady-state inactivation in greater detail, therefore, peak and plateau outward current amplitudes evoked during depolarizations to +30 mV from HPs between -120 to 0 mV were measured. Alternatively, cells were held at conditioning potentials between -120 and 0 mV for 10 s before the depolarizations (from -70 mV) to +30 mV (Fig. 11); the 10-s conditioning pulse was selected because there were no measurable changes in current amplitudes when conditioning pulses longer than 2.5 s were used

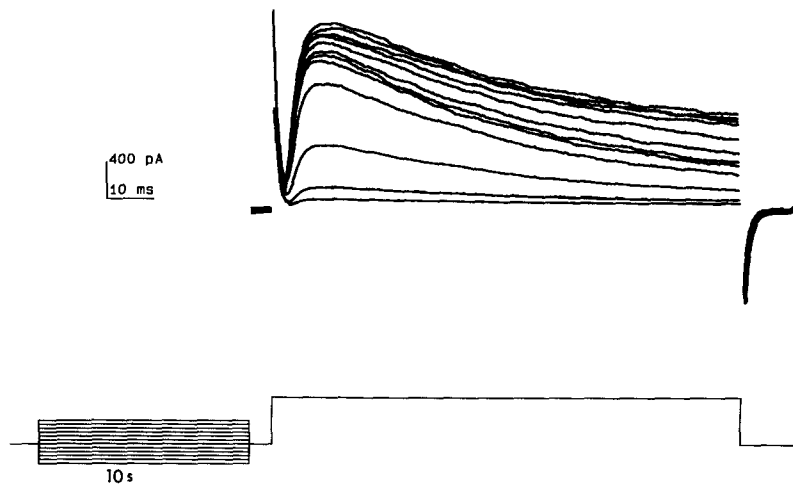


FIGURE 11. Steady-state inactivation of the depolarization-activated outward currents. Outward currents were evoked at 15-s intervals during 100-ms depolarization to +30 mV from a HP of -70 mV. Prior to each depolarization to +30 mV, the cell was held for 10 s at a conditioning potential between -120 and -10 mV; the voltage-clamp paradigm is illustrated below the records. Although the peak and plateau current amplitudes are reduced at conditioning potentials positive to -120 mV, the plateau component is attenuated more than the peak at potentials negative to -40 mV. Similar results were obtained in eight cells.

(see below). The peak and plateau current amplitudes evoked on depolarizations to +30 mV from each conditioning potential were then determined and normalized to their respective (peak and plateau) amplitudes measured during depolarizations from -120 mV (Fig. 12). The normalized amplitude of the peak current varied by $\leq 30\%$ for currents evoked from conditioning potentials between -120 and -50 mV, confirming that the peak current is relatively insensitive to steady-state inactivation over this potential range; 50% inactivation of the peak current is observed at ~ -35 mV (Fig. 12B). Steady-state inactivation of the plateau current, in contrast, is observed at all potentials positive to -120 mV (Fig. 12A).

In the simplest model of steady-state inactivation, channels are removed, in a voltage-dependent manner, from the population of available channels which can be activated on depolarization. The fraction of channels available at any potential, V , may be described by a Boltzmann distribution of the form: $i(V) = A/[1 + \exp [(V - V_{1/2})/k]]$, where i is the normalized current as a function of the conditioning potential, V ; A is an amplitude factor; $V_{1/2}$ is the voltage at which 50% of

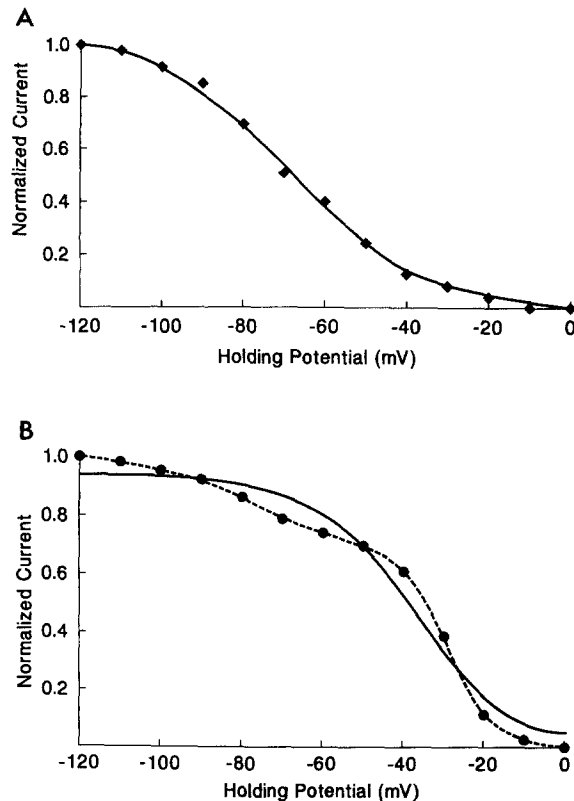


FIGURE 12. Voltage dependence of steady-state inactivation of the (B) peak and (A) plateau components of the total depolarization-activated outward K^+ currents. Outward currents were evoked during depolarization to +30 mV after 10-s conditioning at potentials between -120 and 0 mV (using a voltage-clamp paradigm similar to that illustrated in Fig. 11). The amplitudes of the peak and plateau currents evoked from each conditioning potential were measured in individual cells and normalized to their respective amplitudes evoked from -120 mV. Normalized current amplitudes were then averaged ($n = 8$) and mean values for the (B) peak and (A) plateau current components are plotted as a function of the conditioning potential. Steady-state inactivation data for the plateau component (A) were analyzed assuming a simple Boltzmann relation (see text). The best fit to the data points (*solid line*) was obtained with $V_{1/2} = -69$ mV and $k = 15.3$ mV. Assuming a simple Boltzmann relation, the least-squares fit to the steady-state inactivation data for the peak current (B) yielded $V_{1/2} = -37$ mV and $k = 12.1$ mV (*solid line*). When these data were reanalyzed using the sum of two Boltzmann distributions (see text), the least squares best fit (*dotted line*) yielded: $A_1 = 0.34$; $V_{1/2(1)} = -77$ mV; $k_1 = 13.2$ mV; $A_2 = 0.66$; $V_{1/2(2)} = -29$ mV; and $k_2 = 5.5$ mV. As is evident, a much closer fit to the data points was provided.

simple Boltzmann relation (see text). The best fit to the data points (*solid line*) was obtained with $V_{1/2} = -69$ mV and $k = 15.3$ mV. Assuming a simple Boltzmann relation, the least-squares fit to the steady-state inactivation data for the peak current (B) yielded $V_{1/2} = -37$ mV and $k = 12.1$ mV (*solid line*). When these data were reanalyzed using the sum of two Boltzmann distributions (see text), the least squares best fit (*dotted line*) yielded: $A_1 = 0.34$; $V_{1/2(1)} = -77$ mV; $k_1 = 13.2$ mV; $A_2 = 0.66$; $V_{1/2(2)} = -29$ mV; and $k_2 = 5.5$ mV. As is evident, a much closer fit to the data points was provided.

the channels are inactivated; and k is the Boltzmann factor. Using this expression, least squares best fits (*solid lines*) to the normalized peak ($R = 0.967$; Fig. 12 B) and plateau ($R = 0.999$; Fig. 12 A) current amplitudes as functions of the conditioning potential yielded $V_{1/2}$ values of -37 mV ($k = 12.1$ mV) and -69 mV ($k = 15.3$ mV) for the peak and plateau currents, respectively. This treatment satisfactorily describes the observed steady-state inactivation of the plateau current (Fig. 12 A, *solid line*). For the

peak current, however, there is a substantial discrepancy between the observed steady-state inactivation and that predicted by a single Boltzmann distribution (Fig. 12 B, *solid line*). In fact, the voltage dependence of steady-state inactivation of the peak current (Fig. 12 B) appeared to contain two components: $\approx 30\%$ of the current inactivated at potentials < -50 mV, whereas the remaining 70% inactivated at more positive potentials. The simplest interpretation of this biphasic behavior is that it reflects steady-state inactivation of two channel populations, either two kinetically different states of the same K^+ channel, or two distinct K^+ channel types. The latter interpretation is consistent with the results of the previous experiments which suggested that there are two underlying K^+ current components, i.e., I_{to} and I_k , contributing to the total depolarization-activated outward currents. Since the plateau component (i.e., I_k) undergoes substantial steady-state inactivation at conditioning potentials in the range -50 to -120 mV (Fig. 12 A), we speculated that inactivation of the peak current, although reflecting predominantly steady-state inactivation of I_{to} at potentials positive to -50 mV, contains a component at potentials negative to -50 mV that is due to inactivation of I_k . In spite of the fact that it is slower to activate than I_{to} , I_k will, nevertheless, reach a finite amplitude within the first ≈ 10 ms after the onset of depolarizations to $+30$ mV, and will, therefore, contribute to the peak current during depolarizations evoked from potentials ≤ -50 mV. Assuming a time constant for activation at $+30$ mV of 15 ms (Fig. 9), I_k will reach $\approx 25\%$ of its maximal value 7 ms (the time to peak of the total outward current) after the onset of depolarization. For the average cell, with a plateau to peak current ratio of 0.54, therefore, I_k will contribute $\approx 15\%$ to the peak outward current evoked at $+30$ mV from a HP of -70 mV. Owing to the voltage dependence of steady-state inactivation of I_k (Fig. 12 A), the contribution of I_k to the peak current will be increased during depolarizations from more hyperpolarized potentials.

If both I_{to} and I_k indeed contribute to the peak outward currents elicited by depolarizations following conditioning at potentials < -50 mV, then steady-state inactivation of the peak current as a function of V should be best described by the sum of two Boltzmann distributions (Hagiwara et al., 1975). When the inactivation of the peak current was fit according to the expression: $i(V) = A_1 * \{1/(1 + \exp [(V - V_{1/2(1)})/k_1])\} + A_2 * \{1/(1 + \exp [(V - V_{1/2(2)})/k_2])\}$, the data were well described by this function (Fig. 12 B, *dotted line*). The best fit ($R = 0.999$) parameters were: $A_1 = 0.34$; $V_{1/2(1)} = -77$ mV; $k_1 = 13.2$ mV; $A_2 = 0.66$; $V_{1/2(2)} = -29$ mV; and $k_2 = 5.5$ mV. The $V_{1/2}$ for the first component corresponds closely to the $V_{1/2}$ (-69 mV) determined for I_k (Fig. 12 A), suggesting that the contribution of I_k is responsible for the observed steady-state inactivation of the peak current over the range of -120 to -50 mV. The $V_{1/2}$ (-29 mV) of the second component is interpreted as reflecting the voltage dependence of steady-state inactivation of I_{to} .

In addition to differences in voltage dependence, the rates of recovery from steady-state inactivation for the peak and plateau components are also distinct. For each component, the time course of recovery from the inactivation produced at a HP of -20 mV was evaluated in experiments in which hyperpolarizing prepulses to -90 mV of varying durations were presented prior to depolarizations to $+30$ mV. Peak and plateau current amplitudes evoked during 100-ms depolarizations to $+30$ mV were then measured for each conditioning prepulse duration and normalized to their

respective (peak and plateau) amplitudes measured after a 2.5-s prepulse (Fig. 13); longer conditioning prepulses did not result in further increases in current amplitudes. Examination of the average normalized currents as a function of the prepulse duration (Fig. 13) reveals that the plateau current recovers slowly: $\approx 70\%$ recovery requires several hundred milliseconds. Assuming that recovery followed an exponential time course, the data were analyzed using the expression: $i(t) = 1 - \exp(-t/\tau)$, where i is the normalized current at time, t ; and τ is the time constant. For the plateau current, the data were well fit ($R = 0.996$) by this expression with a time constant of 445 ms. Recovery of the peak current, however, suggests a multiexponential timecourse (Fig. 13), and the data were not well fit using the simple expression described above. Rather, the data for the peak current were best fit ($R = 0.999$) by the sum of two exponential processes using the expression: $i(t) = A_1 * [1 - \exp$

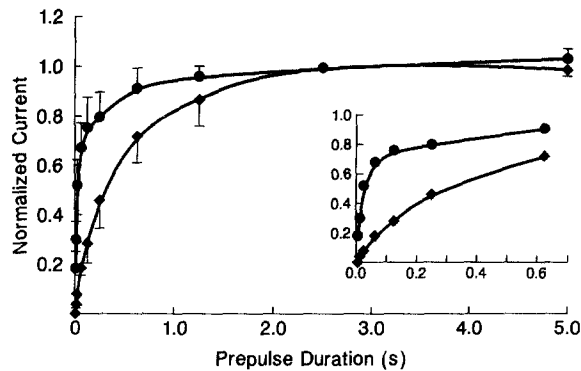


FIGURE 13. Time course of recovery from steady-state inactivation for the depolarization-activated outward currents. Outward currents were evoked during depolarizations to +30 mV after variable length prepulses to -90 mV from a HP of -20 mV. The amplitudes of the peak and plateau currents at +30 mV (after each prepulse) were determined and normalized to their respective (peak

and plateau) amplitudes observed after the 2.5-s prepulse. The normalized data were averaged ($n = 10$) and mean (\pm SD) values for the peak (\bullet) and plateau (\blacklozenge) components are plotted as a function of prepulse duration. The mean normalized current amplitudes measured at short recovery times are replotted on a single expanded time scale in the *inset*. Recovery of the plateau component (\blacklozenge) follows a single exponential time course and the best fit (*solid line*) to the data points yielded a time constant of 445 ms. Recovery of the peak component (\bullet), in contrast, follows a more complex time course, and the data were best fit (*solid line*) by the sum of two exponentials with time constants of 20 and 520 ms (see text).

$(-t/\tau_1)] + A_2 * [1 - \exp(-t/\tau_2)]$, and the best-fit parameters were: $A_1 = 0.68$; $\tau_1 = 20$ ms; $A_2 = 0.32$; and $\tau_2 = 520$ ms. The similarity between the slower of these time constants (520 ms) and the time constant for recovery of I_K (445 ms) is interpreted as revealing the contribution of I_K to the peak outward current during depolarizations from -90 mV. Although I_K is slower to activate than I_{to} , it, nevertheless, does make a contribution to the peak current evoked on membrane depolarizations from -90 mV. The faster time constant (20 ms) is most simply interpreted as reflecting the true rate of recovery from steady-state inactivation for I_{to} .

If the interpretation above is correct, it should be possible to separate I_{to} and I_K based on differences in the time courses of recovery from steady state-inactivation. To examine this possibility directly, a steady-state inactivation protocol similar to that described above (Fig. 11) was employed, except that cells were held at -70 mV for

≈ 75 ms before depolarizing to $+30$ mV to evoke the outward currents. Using this protocol, the plateau current was decreased for currents evoked following conditioning at all potentials positive to -120 mV, whereas the peak current was reduced by $\leq 30\%$ over the entire range of conditioning voltages (Fig. 14). The decrement in the plateau current following conditioning at potentials between -120 and -70 mV is

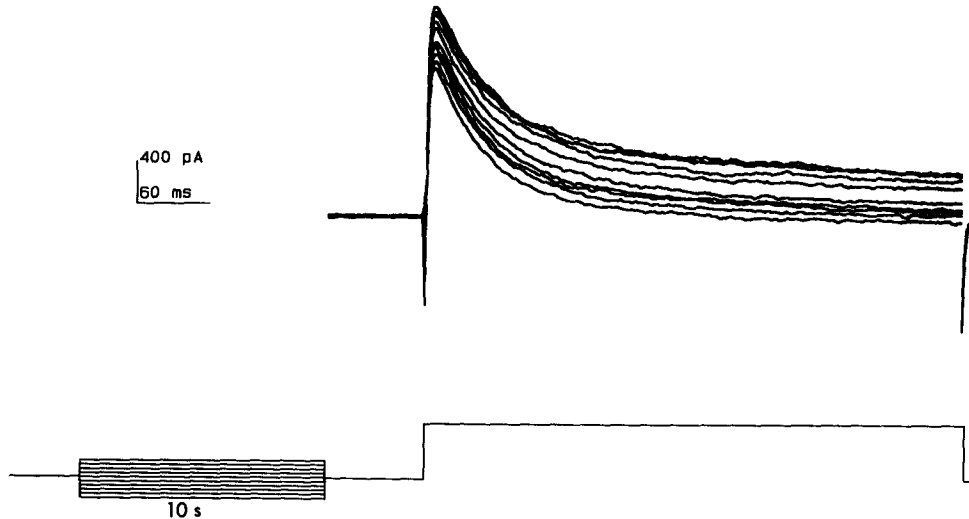


FIGURE 14. The two K^+ current components can be separated on the basis of differing time dependences of recovery from steady-state inactivation. Outward currents were evoked at 15-s intervals using a voltage-clamp paradigm similar to that illustrated in Fig. 11. This cell was held for 10 s at various conditioning potentials between -120 and -30 mV, and subsequently at -70 mV for ≈ 75 ms before depolarizing to $+30$ mV. The plateau current is decreased for currents evoked after conditioning at all potentials positive to -120 mV, whereas the peak current is reduced by $\leq 30\%$ after all conditioning potentials. The decrement in the plateau current is attributed to steady-state inactivation of I_K ; holding the cell at -70 mV for 75 ms does not result in appreciable recovery from the inactivation produced at conditioning potentials positive to -70 mV. Owing to selective attenuation of the plateau current, the waveforms of the evoked currents vary as a function of the conditioning potential. After conditioning at -30 mV, the current evoked from -70 mV is transient, decaying to baseline within ≈ 150 ms, apparently reflecting only the activation of the current component which recovers rapidly from steady-state inactivation, i.e., I_{to} . The ($\leq 30\%$) reductions in the peak current amplitudes as a function of the conditioning potential are attributed to the varying contribution of I_K to the peak (see text). Similar results were obtained in five cells.

attributed to steady-state inactivation of I_K , i.e., I_K is $\approx 50\%$ inactivated -70 mV (Fig. 12A). The finding that the plateau current is further decreased after conditioning at more depolarized potentials in spite of the hyperpolarization to -70 mV is consistent with the finding that recovery of I_K from steady-state inactivation is slow (Fig. 13). Holding the cell at -70 mV for 75 ms, therefore, does not result in appreciable

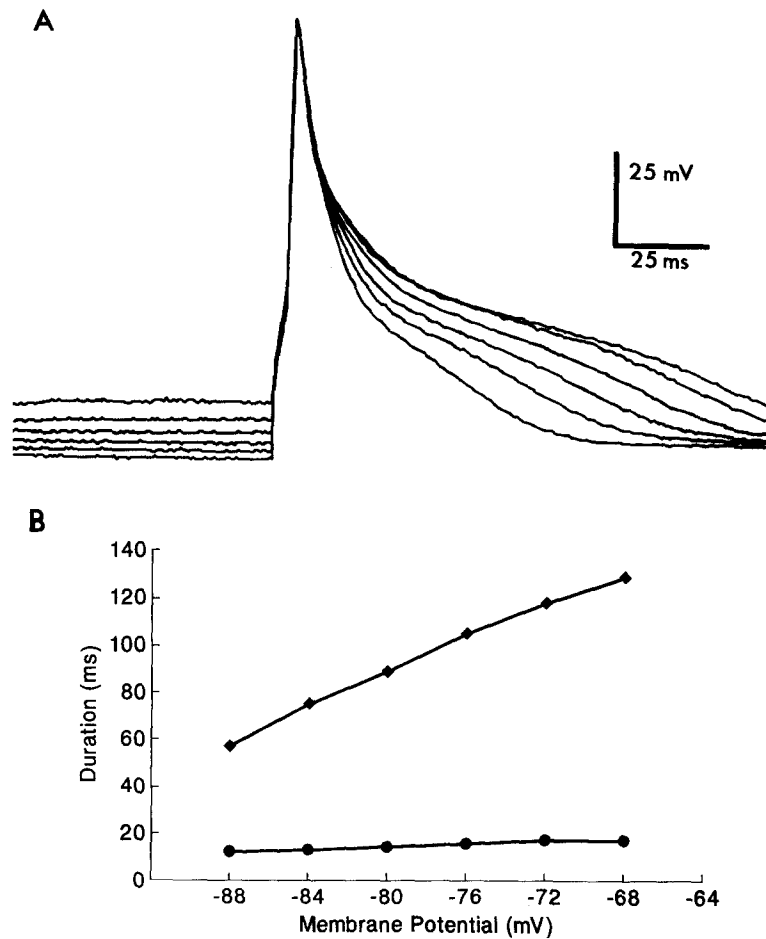


FIGURE 15. AP waveforms vary as a function of membrane potential. (A) Whole-cell APs, recorded under current-clamp in the absence of Co^{2+} and TTX, were evoked by 200-pA depolarizing (10 ms) current pulses delivered at 10-s intervals. The resting membrane potential (i.e., zero current level) of this cell was -80 mV and the membrane potential was altered between successive episodes by depolarizing or hyperpolarizing current injections. In this experiment, the membrane potential (V_m) was held at varying levels between -88 and -68 mV for 5 s before evoking the AP, and the current was turned off when the AP was evoked. The times required for 50% (●) and 90% (◆) repolarization of the AP back to the resting potential (-80 mV) were then measured as a function of the membrane potential established prior to evoking the AP (V_m) and are plotted in B. As is evident, the time required for 90% repolarization (APD_{90}) increases more than twofold when V_m is depolarized over the range -88 to -68 mV. The time required for 50% repolarization (ADP_{50}), in contrast, is relatively insensitive to changes in V_m over this potential range. Similar results were obtained in three cells.

recovery from the inactivation produced at potentials positive to -70 mV. In contrast, the peak current is reduced by $\leq 30\%$ after all conditioning potentials, consistent with the peak reflecting predominately the activation of the current component which recovers rapidly from steady-state inactivation (Fig. 13), i.e., I_{to} . The finding that the currents evoked following conditioning at ≥ -30 mV are transient is also consistent with the interpretation that, under these conditions, the measured currents reflect only the activation of I_{to} . The ($\leq 30\%$) reductions in the peak outward current amplitudes as a function of conditioning potential are attributed to steady-state inactivation of I_K . As discussed above, although I_K activates more slowly than I_{to} , it does contribute to the peak current evoked on depolarizations to $+30$ mV and, owing to the voltage dependence of steady-state inactivation of I_K (Fig. 12 A), the contribution of I_K to the peak varies as a function of the conditioning potential.

Roles of I_{to} and I_K in Action Potential Repolarization

The large differences in the voltage- and time-dependent properties of I_{to} and I_K suggest that these currents subserve different functions in AP repolarization. Because APs in adult rat ventricular myocytes have two distinct phases of repolarization (Powell et al., 1980; Mitchell et al., 1984a, b; Schouten and Ter Keurs, 1985; Meier et al., 1986) and only the early phase is affected by 4-AP (Mitchell et al., 1984b), it seemed likely that I_{to} would be active during the initial rapid decline of the AP (Fig. 15 A), and that I_K should predominate during the latter phase of repolarization back to rest. If I_K were the primary effector of the late phase of repolarization, the time required for 90% repolarization of the AP (APD_{90}) should be sensitive to changes in membrane potential near rest because I_K undergoes steady-state inactivation at all potentials positive to -120 mV and is $\approx 50\%$ inactivated at -70 mV (Fig. 12 A). The time required for 50% repolarization of the AP (APD_{50}), in contrast, should be relatively insensitive to small changes in the membrane potential near rest because 50% repolarization is reached during the early phase of AP repolarization, where I_{to} should predominate.

To test these predictions directly, we examined the waveforms of whole-cell APs evoked from different membrane potentials. APs were elicited by brief (10 ms) 200-pA depolarizing current injections, delivered via the recording pipettes, and the membrane potential between successive APs was varied over the range -88 to -68 mV by 5-s depolarizing or hyperpolarizing current injections. Evaluation of AP waveforms as a function of the membrane potential (V_m) established before evoking the AP revealed that APs elicited from depolarized membrane potentials were broader (Fig. 15 A). As plotted in Fig. 15 B, the APD_{90} for this cell increased from 52 to 120 ms when V_m was reduced from -88 to -68 mV. The APD_{50} , in contrast, varied only slightly (from 11 to 15 ms) over this potential range.

DISCUSSION

The experiments here have revealed the presence of two distinct components of depolarization-activated K^+ currents in isolated adult rat ventricular myocytes. Both components are activated over the same potential range and appear to be indepen-

dent of voltage-dependent Ca^{2+} influx. Differences in time- and voltage-dependent properties and pharmacologic sensitivities, however, have allowed the two components to be distinguished and separated into (a) a rapidly activating and inactivating, 4-AP-sensitive current, which we have termed I_{to} (transient outward) and (b) a slowly activating, TEA-sensitive current, which we have called I_{K} (delayed rectifier). Although both currents were evident in all ventricular myocytes examined, the relative amplitudes of I_{to} and I_{K} ($I_{\text{to}}/I_{\text{K}}$) varied markedly among cells. For currents evoked at +30 mV from a HP of -70 mV, the mean (\pm SD) $I_{\text{to}}/I_{\text{K}}$ ratio ($n = 19$) was 2.0 ± 0.6 , and $I_{\text{to}}/I_{\text{K}}$ ratios were in the range of 1.3–3.9. It may be that this variability reflects the properties of cells isolated from different regions of the ventricles which cannot be distinguished after dissociations. It has been shown that differences in outward, repolarizing K^+ currents in canine ventricular muscle strips can be correlated with the endocardial or epicardial origin of the tissue (Litkovsky and Antzelevitch, 1988). Differences in the relative amplitudes of I_{to} and I_{K} , therefore, may account for observed variations in AP waveforms in rat ventricular cells and muscle strips (Watanabe et al., 1983).

The reversal potentials for I_{to} and I_{K} estimated from tail current measurements were -75 and -65 mV, respectively, suggesting that these currents are carried predominately by K^+ . For both currents components, however, the experimental values are positive to the calculated equilibrium potential (-90 mV) for K^+ under the recording conditions employed, an observation which might suggest that the (K^+) channels activated on membrane depolarization have finite permeabilities for other ions or, alternatively, that other currents contribute to the measured tail currents. Owing to the difficulties associated with determination of reversal potentials for currents that are rapidly deactivating at hyperpolarized potentials (Kenyon and Sutko, 1987), however, it is also possible that inward current tails were not well resolved in these experiments. The apparent outward rectification of I_{to} similarly may reflect the difficulties associated with resolution of rapidly deactivating inward current tails (at hyperpolarized potentials). As a result, the true reversal potentials of these currents might actually be more negative than the reversal potentials estimated from the tail current analyses here.

The presence of a rapidly activating depolarization-activated K^+ current in isolated adult rat ventricular myocytes, termed I_{eo} (early outward), was first described by Josephson et al. (1984) and, more recently, by Ng et al. (1987). Although the properties of I_{to} (here) are similar in some respects to those of I_{eo} , there are also important distinctions. In particular, using various voltage-clamp paradigms in combination with the pharmacologic sensitivity to 4-AP, it has been possible to demonstrate that the rapidly activating phase of the total current can be attributed to a transient current which inactivates completely during brief depolarizations. It is the transient nature of this component which leads us to suggest that the term I_{to} be used to describe this current. Similar K^+ currents in other myocardial preparations are called I_{to} (Kenyon and Gibbons, 1979b; Boyett, 1981a, b; Escande et al., 1987; Tseng et al., 1987; Litovsky and Antzelevitch, 1988). In addition, in contrast, to I_{eo} (Josephson et al., 1984; Ng et al., 1987), I_{to} (here) does not undergo steady-state inactivation at HPs negative to -50 mV; inactivation of I_{to} is evident only at more depolarized potentials. Although it might be suggested that the voltage dependence

of steady-state inactivation of I_{to} was altered here owing to intracellular dialysis, this seems unlikely since Ng et al. (1987) also employed the whole-cell technique. It seems more likely that steady-state inactivation of I_{to} (Josephson et al., 1984; Ng et al., 1987) included contributions from I_K . Indeed, we also observe reductions in peak amplitudes when currents are evoked from HPs positive to -120 mV. The analyses here, however, reveal that steady-state inactivation of the peak current is a biphasic function of potential and that, at HPs negative to -50 mV, attenuation of the peak current reflects steady-state inactivation of I_K , rather than I_{to} .

Depolarization-activated, transient outward K^+ currents have been described in a number of different cell types. In neurons (Hagiwara et al., 1961; Connor and Stevens, 1971; Segal et al., 1984; Strong, 1984; Belluzzi et al., 1985; Rogawski, 1985), these currents, termed I_A (Connor and Stevens, 1971), begin to activate between -60 and -50 mV (Connor and Stevens, 1971; Strong, 1984; Belluzzi et al., 1985) and undergo steady-state inactivation at HPs positive to -90 mV (Rogawski, 1985); steady-state inactivation of I_A is generally complete at ≈ -30 mV (Connor and Stevens, 1971; Segal et al., 1984; Belluzzi et al., 1985). Although transient outward currents in some cardiac preparations, such as rabbit nodal cells (Nakayama and Irisawa, 1985) and canine ventricular myocytes (Tseng et al., 1987), display voltage-dependent properties similar to those of I_A , the voltage dependences of activation and steady-state inactivation of I_{to} in adult rat ventricular myocytes are very different. The properties of I_{to} are, however, similar to those of Ca^{2+} -independent transient outward currents in sheep Purkinje fibers (Coraboeuf and Carmeliet, 1982) and in isolated cells from human atria (Escande et al., 1987), rabbit atria, ventricles (Clark et al., 1988; Giles and Imaizumi, 1988), and crista terminalis (Giles and Van Ginneken, 1985). Transient outward currents in these preparations begin to activate at ≈ -30 mV and undergo steady-state inactivation only at potentials positive to -50 mV. Interestingly, the properties of I_{to} are also very similar to I_A in *Drosophila* flight muscle (Salkoff, 1981; Salkoff and Wyman, 1983). Recovery of I_{to} from steady-state inactivation, in contrast, is more rapid than the recovery rates reported for transient outward currents in other preparations (Boyett, 1981a, b; Giles and Van Ginneken, 1985; Tseng et al., 1987). It seems likely that differences in the voltage- and time-dependent properties of the transient outward K^+ currents account, at least in part, for differences in AP waveforms in various myocardial cell types (Watanabe et al., 1983; Cohen et al., 1986; Litovsky and Anzelevitch, 1988).

Delayed, slowly activating K^+ currents, usually referred to as I_K , have also been described in a variety of cells. In neurons, the pharmacologic and voltage-dependent properties of I_K are quite different from those of I_A . In particular, I_K begins to activate at more depolarized potentials and is less sensitive to steady-state inactivation than I_A (Connor and Stevens, 1971; Armstrong, 1975; Thompson, 1977; Latorre et al., 1984). Although demonstrated in some preparations, it is not clear if I_K is present in all myocardial cells because, in most cases, this possibility has not been explored. It seems that characterization of delayed K^+ currents have been complicated by the difficulties associated with separating I_K from I_{to} and from other voltage-activated currents. In multicellular preparations, studying slowly activating K^+ currents has been further complicated by K^+ accumulation in the extracellular space (Gintant et al., 1985). In Purkinje fibers, two slowly activating K^+ components, termed I_{x1} and I_{x2} ,

were initially described (Noble and Tsien, 1969), although these results have not been confirmed (Gintant et al., 1985; Jaeger and Gibbons, 1985*a, b*) and more recent studies on isolated Purkinje cells, suggest that a single type of slowly activating K^+ channel (with two closed states) can account for delayed rectification (Gintant et al., 1985). A similar current has been described in rabbit atrioventricular node (Shibasaki, 1987) and canine ventricle (Tseng et al., 1987), whereas in feline ventricular muscle, two components have been distinguished (McDonald and Trautwein, 1978*a, b*). The kinetic properties of I_K vary substantially in different preparations: activation time constants range from tens of milliseconds to several seconds. Nevertheless, the kinetics and the voltage dependence of activation of the delayed currents in other preparations (McDonald and Trautwein, 1978*a, b*; Gintant et al., 1985; Shibasaki, 1987; Tseng et al., 1987) are similar to those of I_K . In contrast, the voltage dependence of steady-state inactivation of I_K appears to be unique: steady-state inactivation of I_K is evident at all potentials positive to -120 mV.

The voltage- and time-dependent properties of I_{to} and I_K in adult rat ventricular myocytes suggest that these currents serve unique physiological functions. It appears, for example, that I_{to} underlies the initial rapid repolarization phase of the AP, whereas I_K determines the subsequent, slower phase of AP repolarization back to the resting membrane potential. The variations in AP durations with membrane potential described here, and the finding that 4-AP slows the rate of recovery during the early phase of AP repolarization with little or no effect on the slower repolarization phase (Mitchell et al., 1984*b*) are experimental observations consistent with this interpretation. In addition, the marked differences in the time courses of recovery of I_{to} and I_K from steady-state inactivation suggest that these two currents will play different roles in frequency-dependent modulation of the AP duration. Because I_K recovers slowly ($\tau \approx 500$ ms at -90 mV) from inactivation, for example, increasing the frequency of firing would be expected to increase the APD_{90} , which is dominated by I_K . The APD_{50} , in contrast, which is determined by I_{to} , would not be expected to vary measurably because I_{to} recovers rapidly from inactivation. The time- and voltage-dependent properties of I_K also suggest that, during the normal functioning of the rat heart, a substantial fraction of I_K channels will be inactivated and, therefore, not contribute to AP repolarization.

Taken together, the results here suggest that the macroscopic currents, I_{to} and I_K , reflect the activation of two distinct types of voltage-gated K^+ channels in adult rat ventricular myocytes. In support of this hypothesis is the recent report of the molecular cloning of a rat heart cDNA, termed RHK1, which, when expressed in *Xenopus* oocytes, voltage-clamp studies revealed a rapidly activating and inactivating, 4-AP-sensitive, transient outward current with time- and voltage-dependent properties very similar to those of I_{to} (Tseng-Crank et al., 1990). It will be most interesting to see if future molecular studies reveal the presence of an additional K^+ channel clone, distinct, from RHK1, which encodes for a channel protein with properties similar to those of I_K . Further electrophysiological support for the hypothesis that different channels underlie I_{to} and I_K would clearly be provided by the demonstration of two distinct K^+ channel types in single channel recordings. It may be that I_K represents the macroscopic correlate of the outwardly-rectifying K^+ channels described by

Schreibmayer and colleagues (1985). Ensemble averages of single-channel records suggest a macroscopic current which activates with a time constant of ≈ 60 ms and decays only slightly during 1-s depolarizations (Schreibmayer et al., 1985), i.e., properties similar to I_K .

The results here do not eliminate the possibility that other outward K^+ currents are present and participate in AP repolarization in adult rat ventricular myocytes. The analyses of outward current waveforms during prolonged depolarizations, for example, revealed the presence of a noninactivating current in one (of 10) cell, suggesting that there is a largely time-independent K^+ conductance pathway in some adult rat ventricular myocytes. The amplitude of this component is small ($\leq 10\%$ of the peak current) and no voltage-dependent properties were evident. Although we have not studied this current in any detail, it may be that it is the macroscopic correlate of the "novel" K^+ channel recently characterized in single channel recordings (Yue and Marban, 1988). In addition, we cannot rule out the possibility that Ca^{2+} -activated K^+ current(s) such as have been described in other preparations (Siegelbaum and Tsien, 1980; Coraboeuf and Carmeliet, 1982; Kenyon and Sutko, 1987; Escande et al., 1987; Tseng et al., 1987) are also present in these cells. Although preliminary studies have not revealed the presence of any Ca^{2+} -dependent components of the total outward K^+ currents (Apkon and Nerbonne, unpublished), further experiments will be necessary to evaluate this point in greater detail.

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