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Alterations in Outward K^+ Currents on Removal of External Ca^{2+} in Human Atrial Myocytes

Federica Bertaso, Bruce M. Hendry, Paul Donohoe, and Andrew F. James
Cell Signalling Group, Department of Renal Medicine, GKT School of Medicine, King's College London, London, United Kingdom

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External divalent cations are known to play an important role in the function of voltage-gated ion channels. The purpose of this study was to examine the sensitivity of the voltage-gated K^+ currents of human atrial myocytes to external Ca^{2+} ions. Myocytes were isolated by collagenase digestion of atrial appendages taken from patients undergoing coronary artery-bypass surgery. Currents were recorded from single isolated myocytes at 37°C using the whole-cell patch-clamp technique. With 0.5 mM external Ca^{2+}, voltage pulses positive to −20 mV (holding potential = −60 mV) activated outward currents which very rapidly reached a peak (I_{peak}) and subsequently inactivated (τ = 7.5 ± 0.7 msec at +60 mV) to a sustained level, demonstrating the contribution of both rapidly inactivating transient (I_{to1}) and non-inactivating sustained (I_{so}) outward currents. The I_{to1} component of I_{peak}, but not I_{so}, showed voltage-dependent inactivation using 100 msec prepulses ($V_{1/2}$ = −35.2 ± 0.5 mV). The K^+ channel blocker, 4-aminopyridine (4-AP, 2 mM), inhibited I_{to1} by ~76% and reduced I_{so} by ~33%. Removal of external Ca^{2+} had several effects: (i) I_{peak} was reduced in a manner consistent with an ~13 mV shift to negative voltages in the voltage-dependent inactivation of I_{to1}. (ii) I_{so} was increased over the entire voltage range and this was associated with an increase in a non-inactivating 4-AP-sensitive current. (iii) In 79% cells (11/14), a slowly inactivating component was revealed such that the time-dependent inactivation was described by a double exponential time course (τ_1 = 7.0 ± 0.7, τ_2 = 90 ± 21 msec at +60 mV) with no effect on the fast time constant. Removal of external Ca^{2+} was associated with an additional component to the voltage-dependent inactivation of I_{peak} and I_{so} ($V_{1/2}$ = −20.5 ± 1.5 mV). The slowly inactivating component was seen only in the absence of external Ca^{2+} ions and was insensitive to 4-AP (2 mM). Experiments with Cs^+-rich pipette solutions suggested that the Ca^{2+}-sensitive currents were carried predominantly by K^+ ions. External Ca^{2+} ions are important to voltage-gated K^+ channel function in human atrial myocytes and removal of external Ca^{2+} ions affects I_{to1} and 4-AP-sensitive I_{so} in distinct ways.

Key Words: **cardiac myocytes; potassium current; transient outward current; I_{to1}; sustained outward current; external divalent cations; calcium.**

Voltage-gated potassium currents play a prominent role in the repolarization of cardiac myocytes and thereby contribute to the refractory period and inotropic state of the myocardium. To date, at least two distinct components to the voltage-gated potassium currents of human atrial myocytes have been identified on the basis of their kinetics and sensitivity to pharmacological blockers; a 4-aminopyridine-sensitive K^+-selective transient outward current (I_{to1}) which is thought to be important to early phase repolarization (1–7) and a highly 4-AP-sensitive ultrarapidly activating outwardly-rectifying current (I_{Kur}) (3, 4, 7). In addition, there are reports of a 4-aminopyridine (4-AP)-insensitive slowly activating delayed-rectifier current (I_K) in a sub-population of isolated cells (3, 6, 8). A 4-AP-resistant, Cl^--selective Ca^{2+}-dependent transient outward current (I_{to2}) also contributes to the outward currents observed on depolarization (2, 6). This current can be abolished by Ca^{2+} current blockade or inhibition of the sarcoplasmic reticulum (2, 6).

External divalent cations are known to play an important role in the function of voltage-gated ion channels. Possible actions of extracellular cations include the screening of surface charge, the voltage-dependent block of the pore or the stabilization of native conformations of the protein (9). Removal of extracellular Ca^{2+} ions has been shown to result in a loss of voltage- and time-dependent gating and selectivity of K^+ currents in squid neurons and in Shaker (Kv1 family) voltage-gated K^+ channels expressed in a heterologous system (10, 11). It was suggested that Ca^{2+} ions were essential to stabilise the native conformation of voltage-gated K^+ channel proteins (11). External divalent cations also modulate the voltage-gated K^+ cur-

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rents of mammalian cardiac myocytes. For example, the voltage-dependent activation and inactivation of I_{to} in rat ventricular myocytes was shown to be modulated by various external divalent cations (12, 13). Despite the sensitivity of K^+ currents to external divalent cations, ions such as Cd^{2+}, Co^{2+} or Ni^{2+} are often used to block Ca^{2+} currents in investigations of voltage-gated outward currents in cardiac myocytes. The purpose of our study was to examine the sensitivity of the voltage-gated K^+ currents of isolated human atrial myocytes to removal of external calcium at physiological temperatures in the presence of the L-type Ca^{2+} channel blocker, verapamil.

MATERIALS AND METHODS

Cell isolation. Single human atrial myocytes were isolated from right atrial appendages by enzymatic dispersion (14). Specimens were obtained from 24 patients (19 males, 5 females, average age 63 ± 2 years) undergoing coronary artery bypass surgery. The study was approved by the Ethical Committee of King's College Hospital, London. Tissue samples were quickly immersed in cardioplegic solution (100% O_2, ice cold) containing (mM): KH_2PO_4 50, $MgSO_4$ 8, HEPES 10, adenosine 5, glucose 140, mannitol 100; pH adjusted to 7.4 with KOH. The samples were chopped into small chunks and washed with an EGTA-containing solution composed of (mM): NaCl 137, KH_2PO_4 5, $MgSO_4$ 1, glucose 10, HEPES 5, EGTA 0.2; pH adjusted to 7.4 with NaOH; gassed with 100% O_2 for 5 min at 37°C. The chunks were then incubated in the same solution from which EGTA was excluded and protease type XXIV (3 U/ml, Sigma) and collagenase type V (250 U/ml, Sigma) were added. The medium was continuously gassed with 100 O_2 at 37°C. After 15 min the incubation medium was substituted for the same solution containing collagenase only. Myocytes were progressively released from the chunks into the supernatant and their yield monitored under a microscope. Only quiescent, striated, rod shaped myocytes were used for patch-clamp recording. The viability of the isolated myocytes was typically between 2–10%. The suspension was washed in the enzyme-free solution and the myocytes stored at room temperature until use.

Voltage-clamp recording. The whole-cell configuration of patch-clamp technique was used to measure outward currents in single atrial myocytes. Borosilicate glass pipettes (2–4 MΩ) were filled with a pipette solution containing (mM): K Aspartate 130, NaCl 4, $CaCl_2$ 5, EGTA 10, HEPES 10, Mg_2-ATP 4, Na_2-GTP 0.2 (pH 7.4, adjusted with 1 M KOH). The free Ca^{2+}-concentration of the pipette solution was ~72 nM (15, 16). In some experiments, K^+ was completely replaced by Cs^+, using CsOH (1 M) to adjust the pH. The cells were placed in a chamber mounted on the stage of an inverted microscope (Nikon) and perfused at a rate of 2–4 ml/min with an experimental solution containing (mM): NaCl 150, KCl 5.4, $MgCl_2$ 2, HEPES 10, glucose 10, $CaCl_2$ 0.5; pH adjusted to 7.4 with NaOH (1 M). Nominally Ca^{2+}-free solution was produced by the omission of $CaCl_2$ from the above solution. A 30 msec prepulse at −40 mV was used to inactivate the voltage-gated Na^+ current. Verapamil (10 μM) was used to block the L-type Ca^{2+} current rather than Cd^{2+} or Co^{2+} to avoid possible competition of these divalent cations with Ca^{2+} ions in effects on the K^+ currents. Since verapamil has previously been shown to block K^+ currents (17–20), the effects of verapamil were examined in preliminary experiments. Verapamil at 10 μM had no significant effect on either the peak current or the current at the end of the voltage pulse (e.g., peak current at +40 mV was reduced by 3.8 ± 7.2%, $n = 4$). All the chemicals including 4-AP and the chloride transport blocker 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) were from Sigma Chemicals (USA). All experiments were performed at 37°C. Drugs were added as indicated.

Currents were recorded using an Apple Macintosh computer-driven EPC-9 patch-clamp amplifier running Pulse software (HEKA Elektronik GmbH, Germany) to generate voltage-clamp protocols and acquire data as specified in the text. The sampling frequency was 2 kHz. Junction potentials were compensated electronically prior to seal formation. Series resistance and capacitance compensation were applied electronically. Data were analysed using IgorPro software (WaveMetrics, Inc., USA). Currents were usually normalised to cell capacitance (pA/pF), unless otherwise specified. Leak current was subtracted from the current data, assuming a linear leak current-voltage relation between −60 and −40 mV. Data are presented as mean ± SEM. Statistical significance was assessed using paired or unpaired Student's *t*-test and $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Current–Voltage Relations in the Presence and Absence of Ca^{2+} Ions

The effects of removal of external Ca^{2+} on the current–voltage relations were investigated in 14 cells. The voltage-gated outward currents were examined using a set of 200 msec voltage pulses from −60 to +60 mV in 10 mV increments, from a holding potential of −60 mV. Pulses were applied every 5 s. In the presence of 0.5 mM Ca^{2+}, outward currents were elicited from voltages positive to −30 mV. The currents activated on depolarization to a peak level (I_{peak}) and subsequently inactivated to a sustained level (I_{so}). I_{peak} was defined as the maximum outward current and I_{so} as the current remaining at the end of the voltage-pulse. Removal of Ca^{2+} ions did not result in a loss of gating since the currents still showed time-dependent activation and inactivation (Fig. 1A). There was no change in the non-leak corrected holding current at −60 mV (Fig. 1A). In the presence of external Ca^{2+}, the current-voltage relations for both I_{peak} and I_{so} showed outward rectification (Figs. 1B and 1C, filled symbols). In the Ca^{2+}-free solution I_{peak} was reduced and I_{so} was increased over the entire range of potentials. I_{peak} elicited on depolarization to +60 mV was reduced from 12.4 ± 1.9 pA/pF to 9.6 ± 1.2 pA/pF ($n = 14$, $P < 0.05$, paired *t*-test). Conversely, I_{so} was increased from 1.8 ± 0.3 pA/pF to 3.4 ± 0.7 pA/pF ($n = 14$, $P < 0.05$, paired *t*-test). The time to peak current after depolarization was unaffected by removal of external Ca^{2+} (control: 3.0 ± 0.3 msec; Ca^{2+}-free: 2.8 ± 0.4 msec at +40 mV). The effects of removal of external Ca^{2+} ions were reversible (data not shown). Replacement of intracellular K^+ with Cs^+ resulted in almost complete suppression of I_{peak} and I_{so}, both in the presence and absence of external Ca^{2+} (Figs. 1B and 1C), suggesting that the voltage-gated outward currents were predominantly K^+-selective. Furthermore, removal of external Ca^{2+} had no effect on the currents recorded with Cs^+-rich pipette solutions (Fig. 1D), demonstrating that the Ca^{2+}-sensitive outward currents were carried by K^+ ions. Therefore, human atrial myocytes do not show a divalent-cation sensitive cation non-selective current

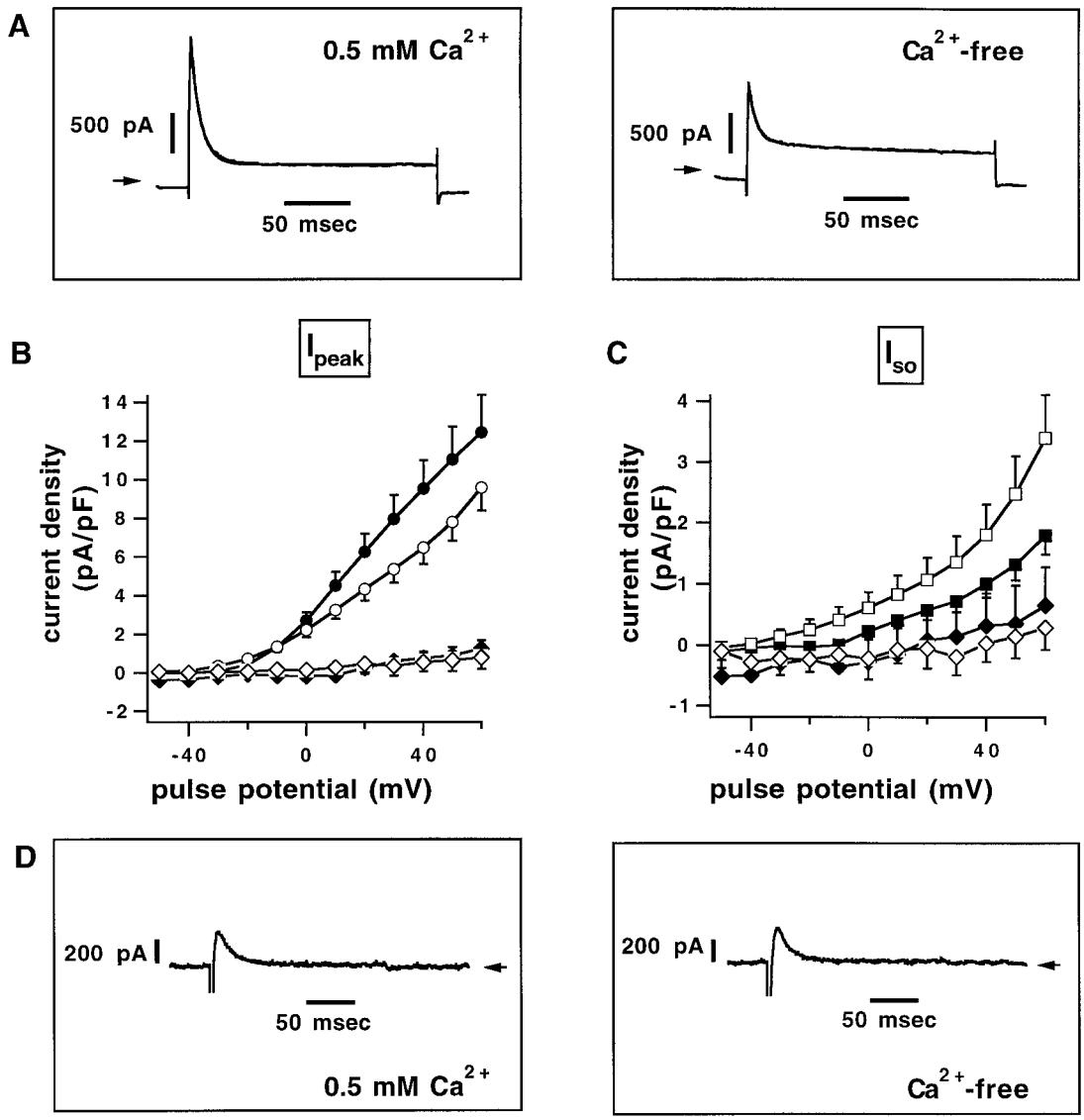

FIG. 1. Current–voltage relations of the outward currents in the presence and absence of external Ca^{2+}. The holding potential was −60 mV. (A) Example current traces recorded using the K^+-rich pipette solution on depolarization to +60 mV in the presence (left-hand panel) and nominal absence (right-hand panel) of external Ca^{2+} ions. Arrows indicate zero current level. (A) Decaying single (left-hand panel) and double (right-hand panel) exponentials are superimposed upon the current traces (see text). (B) Current density–voltage relations of I_{peak} in the presence of 0.5 mM external Ca^{2+} (filled symbols) and nominal absence of Ca^{2+} ions (open symbols). Data obtained with K^+-(circles, n = 14) or Cs^+-rich (diamonds, Ca^{2+}-free n = 3, 0.5 mM Ca^{2+} n = 4) pipette solutions. (C) Current density–voltage relations of I_{so} in the presence of 0.5 mM external Ca^{2+} (filled symbols) and nominal absence of Ca^{2+} ions (open symbols). Data obtained with K^+-(squares, n = 14) or Cs^+-rich (diamonds, Ca^{2+}-free n = 3, 0.5 mM Ca^{2+} n = 4) pipette solutions. (D) Example current traces recorded using the Cs^+-rich pipette solution on depolarization to +60 mV in the presence (left) and nominal absence (right) of external Ca^{2+} ions. Data shown in B and C are means ± SEM.

as reported recently in rat ventricular myocytes (21). It is unlikely that the Ca^{2+}-dependent Cl^- current (2, 6) contributed significantly to the outward currents since Ca^{2+} influx was blocked by verapamil in our experiments. The lack of effect of DIDS (1 mM), a blocker of swelling-activated and Ca^{2+}-dependent Cl^- channels on the currents obtained with K^+-rich pipette solutions (data not shown) confirmed the absence of Cl^- currents in our experiments.

Time- and Voltage-Dependent Inactivation

The effects of external divalent cations on the transient outward current of rat ventricular myocytes were reportedly associated with effects on the kinetics of inactivation (12, 13). The time-dependence of inactivation of the outward currents of human atrial myocytes was examined in the data shown in Fig. 1 by fitting decaying exponentials to the outward currents elicited

TABLE 1

Parameters Fitted to the Inactivation of the Outward Currents Elicited by a Pulse to +60 mV from a Holding Potential of −60 mV

Condition	A_0 (pA/pF)	A_1 (pA/pF)	τ_1 (msec)	A_2 (pA/pF)	τ_2 (msec)
All cells in the presence of Ca^{2+} (n = 14)					
$+Ca^{2+}$	1.8 ± 0.3	15.4 ± 2.6	7.5 ± 0.7	—	—
Cells showing double exponential inactivation in Ca^{2+}-free (n = 11)					
$+Ca^{2+}$	1.7 ± 0.3	14.7 ± 2.4	7.7 ± 0.7	—	—
$-Ca^{2+}$	2.8 ± 0.7*	9.0 ± 1.1*	7.0 ± 0.7	2.01 ± 0.26	90 ± 21
Cells showing single exponential inactivation in Ca^{2+}-free (n = 3)					
$+Ca^{2+}$	1.9 ± 1.0	18.0 ± 9.4	6.9 ± 1.5	—	—
$-Ca^{2+}$	5.0 ± 2.3*	5.9 ± 1.5	7.2 ± 2.8	—	—

* $P < 0.05$, paired t test.

by depolarization to +60 mV. Fitted curves superimposed on the current traces are shown in Fig. 1A. In the presence of external Ca^{2+} (left-hand panel of Fig. 1A), the inactivation from I_{peak} to I_{so} could be described by a single exponential equation: $I(t) = A_0 + A_1 \cdot \exp(-t/\tau)$, where A_0 is the amplitude of the time-independent, non-inactivating component, A_1 is the amplitude of the inactivating component at $t = 0$, and τ is the time constant of inactivation. Thus, the A_1 component corresponds to the transient outward current (I_{to1}). The mean fitted parameters are shown in Table 1. The value of τ (τ = 7.5 ± 0.7 msec, n = 14) corresponds closely with those reported by other groups for I_{to1} in human atrial myocytes (1, 3, 4, 6).

On removal of external Ca^{2+}, the majority of cells (11/14) showed a slowly inactivating component of the outward current which was not apparent in the presence of external Ca^{2+} in addition to the rapidly inactivating component (e.g., right-hand panel, Fig. 1A). Thus, in these cells an additional exponential component was necessary to fit the inactivating phase of the data in the absence of external Ca^{2+}: $I(t) = A_0 + A_1 \cdot \exp(-t/\tau_1) + A_2 \cdot \exp(-t/\tau_2)$. The faster time constant was not significantly affected by removal of external Ca^{2+} (Table 1). The slower component had a time constant (τ_2) in the range of 50 to 160 msec. For either group of cells, the amplitude of the rapidly inactivating component, A_1, was reduced and the amplitude of the non-inactivating component, A_0, was increased by removal of external Ca^{2+} ions (Table 1). Thus, the decrease in I_{peak} on removal of external Ca^{2+} was due to a reduction in the rapidly inactivating transient outward current, I_{to1}, and the increase in I_{so} on removal of external Ca^{2+} is associated with an increase in non-inactivating, or very slowly inactivating, current components.

The components of the outward currents were studied further by examining their voltage-dependent inactivation. From a holding potential of −60 mV, a series of 100 msec prepulses (P1) between −80 and +15 mV increasing in 5 mV increments were applied, each followed by a test pulse (P2, 200 msec) to +40 mV (Fig. 2A). The relative current elicited during the test pulse was plotted against the P1 potential, giving a description of the voltage-dependence of inactivation for both I_{peak} and I_{so} (Fig. 2B). In the presence of Ca^{2+}, I_{so} measured at the end of the P2 pulse did not show any voltage-dependent inactivation. I_{peak} showed voltage-dependent inactivation which could be fitted by a single Boltzmann equation ($I/I_{max}(V) = [k_0/(1 + \exp(V - V_{1/2})/V_{slope}] + k_1$, where $I/I_{max}(V)$ is the current normalized to the maximal current obtained after a prepulse to −80 mV, $V_{1/2}$ is the voltage of half-maximal inactivation and V_{slope} is the slope factor, k_0 and k_1 are the proportions of the maximal current accounted for by the inactivating and non-inactivating components, respectively. The fitted parameters were: $V_{1/2}$ = −35.2 ± 0.5 mV and V_{slope} = 9.4 ± 0.5 mV (n = 15). After positive prepulses, 24 ± 4% of I_{peak} was not inactivated (i.e., k_1 was 0.24) and I_{peak} was approximately equal to I_{so}, indicating that the transient outward current component (I_{to1}) accounted for the inactivation of I_{peak}. Moreover, the $V_{1/2}$ (∼−35 mV) corresponded closely with those reported by other groups (−40 to −20 mV) for I_{to1} of human atrial myocytes (1, 3, 4). Other groups have reported the existence of a slowly-inactivating component to I_{so} in human atrial myocytes (I_{Kur}) (1, 4, 7, 22). We did not detect significant inactivation of I_{so} in the presence of external Ca^{2+} ions. In preliminary experiments, we tested whether inactivation of I_{so} might be detected with longer voltage-pulse durations. No further inactivation of I_{so} was observed when the duration of the P1 pre-pulse was increased from 100 msec to 500 msec in the presence of Ca^{2+} (data not shown). Other workers have used pre-pulse durations of longer than 2.5 sec to detect significant voltage-dependent inactivation of I_{so} (3, 22).

In the absence of calcium, the currents showed more complex inactivation characteristics (Fig. 2C and 2D). The I_{peak} curve showed two Boltzmann components to its voltage-dependent inactivation. The half-maximal voltages were $V_{1/2}$ = −47.8 ± 1.1 mV (V_{slope} = 13.0 ± 1.2 mV) and $V_{1/2}$ = −20.0 ± 0.4 mV (V_{slope} = 9.4 ± 0.3 mV, n = 10). The most striking effect was the acquisition of voltage-dependent inactivation by I_{so} ($V_{1/2}$ = −20.5 ± 1.5 mV, V_{slope} = 7.9 ± 1.5). The component of I_{peak} inactivating with $V_{1/2}$ ∼ −20 mV contributed to both I_{peak} and I_{so} and may correspond to the relatively slowly inactivating A_2 current component fitted to the time course of inactivation. Thus, a slowly inactivating component contributed to I_{so} in the absence of external Ca^{2+}. However, even at the most depolarized prepulses I_{so} inactivation was partial with only 17.8 ± 1.4% of the current being inactivated. Despite its partial inactivation, the I_{so} elicited from a pre-pulse potential of +15 mV was greater ($P < 0.05$) in the absence (5.9 ± 1.3 pA/pF, n = 10) than in the presence of external Ca^{2+}

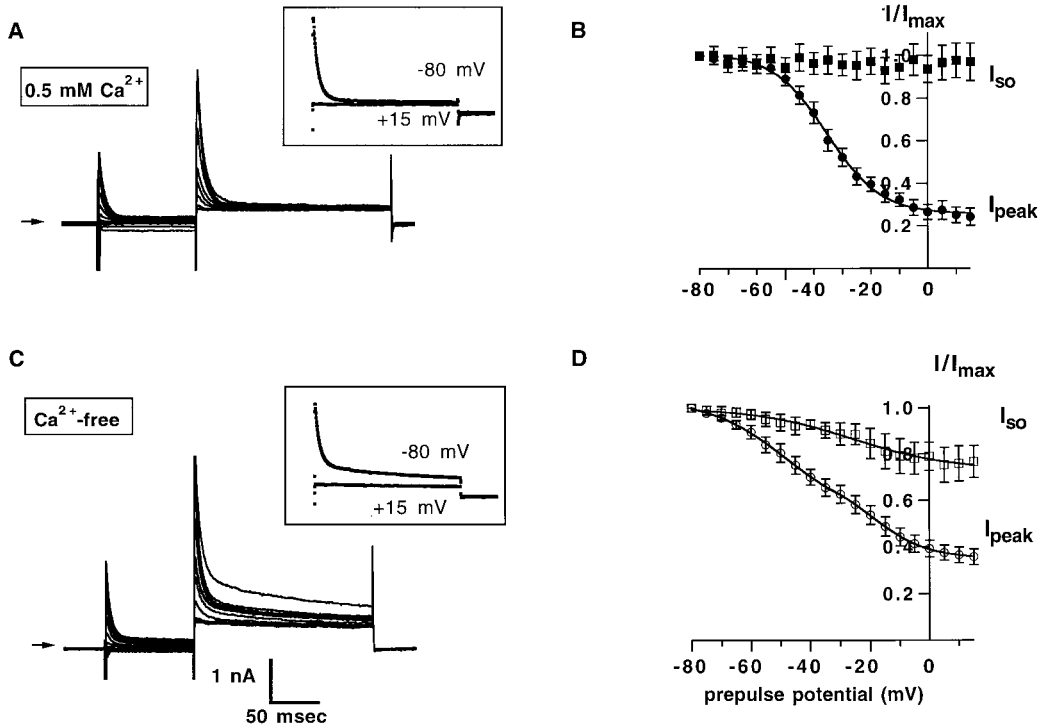

FIG. 2. Steady-state inactivation of the outward currents in the presence and absence of 0.5 mM external Ca^{2+}. (A) Current traces obtained with a K^+-rich pipette solution using a two-pulse protocol in the presence of 0.5 mM external Ca^{2+}. Inset shows current traces during test pulse to +60 mV from a prepulse potential of −80 mV and +15 mV. Inactivating phase of current elicited from a pre-pulse potential of −80 mV was fitted by a single exponential (solid line). (B) Dependence of mean current during test pulse on the pre-pulse potential for I_{peak} (filled circles) and I_{so} (filled squares). Solid line represents fit to a Boltzmann equation (see text). $n = 15$. (C) Current traces obtained with a K^+-rich pipette solution using a two-pulse protocol in the absence of external Ca^{2+}. Inset shows current traces during test pulse to +60 mV from a prepulse potential of −80 mV and +15 mV. Inactivating phase of current elicited from a prepulse potential of −80 mV was fitted by a double exponential (solid line). Same cell as shown in A. (D) Dependence of current during test pulse on the pre-pulse potential for I_{peak} (open circles) and I_{so} (open squares). Solid lines represents fits to a Boltzmann equation (see text). $n = 10$. Error bars shown in B and D are SEM.

(2.9 ± 0.7 pA/pF, $n = 15$). Thus, in addition to the appearance of the relatively slowly inactivating component, the increase in I_{so} in the nominal absence of external Ca^{2+} ions was associated with an increase in a distinct component, corresponding to A_0, showing very little voltage-dependent inactivation.

The component of I_{peak} inactivating with $V_{1/2} \sim -48$ mV corresponds to I_{to1} (Fig. 2D). Thus, the reduction in I_{peak} and A_1 elicited from a holding potential of −60 mV may partly be accounted for by the shift in voltage-dependent inactivation of I_{to1} to more negative voltages (Figs. 2B and 2D). Reduction of the external Ca^{2+} concentration has also been shown to shift the voltage-dependent inactivation of I_{to1} in rat ventricular myocytes to negative voltages (12). However, the concentration range (0.5–10 mM) at which these shifts in voltage-dependent inactivation were observed were higher than that used in the present study (nominally Ca^{2+}-free, 0.5 mM).

Effects of the K^+ Channel Blocker, 4-Aminopyridine (4-AP)

Previous reports indicate that both I_{to1} and I_{Kur} of human atrial myocytes are blocked by the K^+ channel blocker, 4-aminopyridine (4-AP) (1, 4, 7, 23). In order to examine the effects of the K^+ channel blocker on the various components of the currents, the time course of inactivation of the currents were fitted by decaying exponentials in the presence and absence of 4-AP (Fig. 3). The inactivation of three of the five cells in which the effects of the K^+ channel blocker were examined in the absence of external Ca^{2+} were described by a double exponential. Data from these three cells are shown in Fig. 3C. The fast time constant of inactivation (τ_1) was not significantly affected by 2 mM 4-AP. On the other hand, the A_1 component of the outward current, corresponding to I_{to1}, was strongly inhibited by 2 mM 4-AP (Fig. 3C), consistent with reports of the sensitivity of I_{to1} to 4-AP (1, 4, 6). Removal of external Ca^{2+} ions did not affect the sensitivity of the A_1 component to 2 mM 4-AP (Fig. 3C). A_0 was only partially inhibited by 2 mM 4-AP, a concentration at which I_{Kur} would be completely blocked, suggesting the contribution of currents other than I_{Kur} to I_{so} (3, 4, 22). However, the sensitivity of the time-independent A_0 component to the K^+ channel blocker was markedly increased in the nominal absence of external Ca^{2+} ($P < 0.05$), suggest-

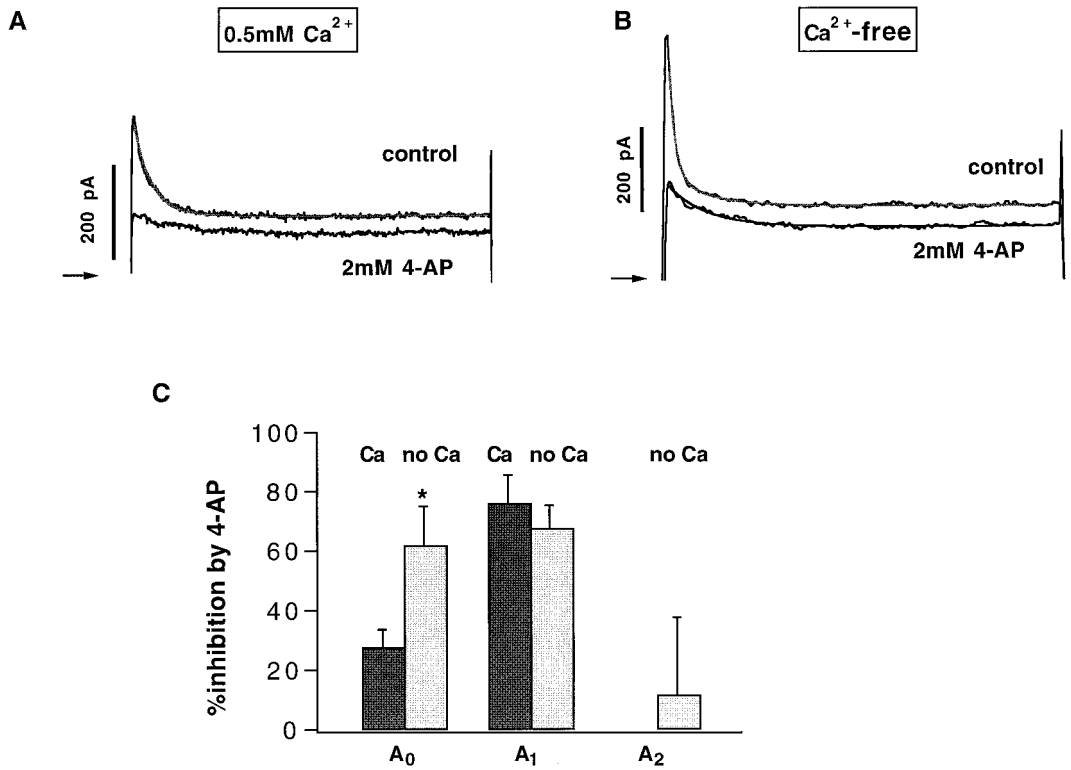

FIG. 3. Sensitivity of the outward currents to the K^+-channel blocker, 4-aminopyridine. Current traces recorded using a K^+-rich pipette solution on depolarization to +60 mV from a holding potential of −60 mV in the presence (A) and nominal absence (B) of external Ca^{2+} ions. Lower traces are the currents recorded in the presence of 2 mM 4-aminopyridine. Solid lines represent fits to either a single (A) or double (B) exponential. (C) The percentage inhibition by 4-aminopyridine of time-dependent and time-independent components of the outward currents in the presence and nominal absence of external Ca^{2+} ions. Dark bars: 0.5 mM external Ca^{2+} ($n = 5$). Light bars: nominally Ca^{2+}-free ($n = 3$). Asterisk indicates $P < 0.05$ (two-tailed t test) compared to A_0 in the presence of Ca^{2+} ions.

ing an increase in a 4-AP-sensitive component of I_{so} on removal of external Ca^{2+} ions. In contrast to the A_0 and A_1 components the slowly inactivating component, A_2, was unaffected by 4-AP (Fig. 3C).

In summary, removal of external Ca^{2+} ions produced an ~13 mV shift in the voltage-dependent inactivation of I_{to1} to more negative potential and an increase in I_{so}. The increase in I_{so} was associated with an increase in a non-inactivating, or very slowly inactivating, 4-AP-sensitive current component. In addition, removal of external Ca^{2+} ions revealed a slowly inactivating 4-AP-insensitive current which was not apparent in the presence of external Ca^{2+}. The voltage-dependent inactivation of I_{peak} of human atrial myocytes was not shifted to more positive voltages and neither was I_{so} further suppressed in the presence of higher concentrations of external Ca^{2+} (1 mM, data not shown). It is unlikely, therefore, that the effects of Ca^{2+} ions on the outward currents seen in the present study would contribute to myocardial function during changes in external Ca^{2+} likely to occur *in vivo* under physiological or pathological conditions. Since the experiments were carried out in the continuous presence of high concentrations of monovalent and divalent cations (150 mM Na^+, 5.4 mM K^+ and 2 mM Mg^{2+}), it is unlikely that a change in the non-specific masking of surface charge was responsible for the effects of removal of 0.5 mM Ca^{2+} seen in our experiments (9). Rather, our results suggest specific effects of external Ca^{2+} ions on I_{to1} and I_{so} and that external Ca^{2+} ions modulate I_{to1} and I_{so} in distinct ways.

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