Comparison of I_{to} in young and adult human atrial myocytes: evidence for developmental changes

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Crumb, William J., Jr., John D. Pigott, and Craig W. Clarkson. Comparison of I_{to} in young and adult human atrial myocytes: evidence for developmental changes. *Am. J. Physiol.* 268 (*Heart Circ. Physiol.* 37): H1335–H1342, 1995.—In an effort to understand the ionic basis for the developmental changes that have been reported to occur in the configuration of the human atrial action potential, we characterized the transient outward current (I_{to}) and the inward rectifier current in atrial myocytes isolated from 20 young (ages 1 day-2.5 yr) and 8 adult (11–68 yr) human hearts using the whole cell patch-clamp technique. We found evidence for statistically significant ($P < 0.05$) age-related changes in the I_{to}, including *1*) the presence of an I_{to} in only 67% of the cells isolated from young hearts vs. 100% of the cells isolated from adult hearts, *2*) an almost twofold increase in the current density of I_{to} in adult cells vs. young cells, and *3*) recovery kinetics that are approximately twofold slower in young myocytes relative to adult myocytes. In contrast, there were no age-related changes found in the current density of the inward rectifier current or the sustained current measured after the decay of I_{to}. These results suggest important current-dependent changes that occur with age in human atria.

potassium currents; transient outward current; inward rectifier

STUDIES PUBLISHED during the past few years have documented that there are significant developmental changes in the density and characteristics of potassium currents observed in mammalian cardiac tissue. For example, in 1990, Kilborn and Fedida (10) documented that there are significant developmental changes in the transient outward current (I_{to}) in rat ventricular myocytes, with cells from 1-day-old rats displaying a significantly smaller I_{to} compared with cells from adult rats. Similarly, in 1992, Jeck and Boyden (9) showed that neonatal canine ventricular myocytes completely lack a definable I_{to}, in contrast to adult myocytes. Developmental changes have also been observed for the inward rectifier (I_{K1}), with significant increases in the current density of I_{K1} occurring during postnatal development in both rat (14) and rabbit (8, 13) ventricular myocytes. Although these studies collectively suggest that there may be a common pattern of developmental changes in potassium channel expression in cardiac tissue in lower mammals, it is still unclear whether a similar pattern also occurs in higher primates or humans. Perhaps the best evidence for developmental changes in potassium currents in humans comes from the work of Escande et al. (4), who in 1985 documented that there are developmental changes in the shape, duration, and rate dependence of the duration of the atrial action potential, as well as a developmental change in the sensitivity of *phase 1* of the action potential to alteration by the potassium channel blocker, 4-aminopyridine. Escande et al. (4) also found that the shape of action potentials recorded from adult tissue could be made to mimic the shape of neonatal action potentials during exposure to concentrations of 4-aminopyridine known to block I_{to}. These results seem consistent with there being significant developmental changes in I_{to} in humans, similar to other species. However, to date, the only direct supporting evidence for this hypothesis has been the recent report that I_{to} is absent in atrial cells isolated from the hearts of clearly diseased young individuals (ages 2 mo-5 yr) in contrast to cells from adult patients, which contain a robust I_{to} (11). Unfortunately, cardiac disease (e.g., cardiac hypertrophy) is also known to reduce significantly the amplitude of I_{to} (2, 11). Therefore, it remains unclear whether the observation of an absence of I_{to} in young diseased atrial cells is due to disease, stage of development, or a combination of these two factors. In light of this controversy, we investigated whether developmental changes could be defined in potassium currents expressed in human tissue free from significant pathology. The primary purpose of this developmental study using human atrial cells was to answer four basic questions: *1*) Are there significant developmental changes in the amplitude dependence of I_{to}? *2*) Are there developmental changes in the time dependence of I_{to}? *3*) Are there developmental changes in the voltage-dependent behavior of I_{to}? and *4*) Are there developmental changes in the I_{K1}?

MATERIALS AND METHODS

Isolation of cardiac myocytes. Human myocytes were obtained from specimens of human right atrial appendage obtained during surgery from hearts of patients undergoing cardiopulmonary bypass. Tissue was obtained in accordance with Tulane University School of Medicine institutional guidelines. All atrial specimens were described as grossly normal at the time of excision. The cell isolation procedure was similar to that described in Fermini et al. (5) based on an earlier method by Escande et al. (3). Briefly, samples were quickly immersed in a cardioplegia solution consisting of (in mM) 50 KH_2PO_4, 8 $MgSO_4$, 10 $NaHCO_3$, 5 adenosine, 25 taurine, 140 glucose, and 100 mannitol, titrated to a pH of 7.4 and bubbled with 100% O_2 at 0–4°C. Specimens were minced into 0.5- to 1-mm cubes and were transferred to a 50-ml conical tube containing an ultra-low calcium wash solution containing (in mM) 137 NaCl, 5 KH_2PO_4, 1 $MgSO_4$, 10 taurine, 10 glucose, 5 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 100 μM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA); pH = 7.4 (22–24°C). The tissue was gently agitated by continuous bubbling with 100% O_2 for 5 min. The tissue was next incubated in 5 ml of solution containing (in mM) 137 NaCl, 5 KH_2PO_4, 1 $MgSO_4$, 10 taurine, 10 glucose, 5 HEPES, supplemented with 0.1% bovine albumin, 2.2 mg/ml collagenase (type V), and 1.0 mg/ml protease (type XXIV; Sigma Chemical), pH = 7.4 (37°C) and bubbled continuously

with 100% O_2. The supernatant was removed after 40 min and was discarded. The chunks were then incubated in a solution of the same ionic composition supplemented with only collagenase and 100 μM $CaCl_2$. Microscopic examination of the medium was performed every 10–20 min to determine the number and quality of the isolated cells. When the yield appeared to be maximal, the cell suspension was centrifuged for 2 min, and the resulting pellet was resuspended in a modified Kraftbrühe solution containing (in mM) 25 KCl, 10 KH_2PO_4, 25 taurine, 0.5 EGTA, 22 glucose, 55 glutamic acid, and 0.1% bovine albumin, pH = 7.3 (22–24°C). In general, the isolation procedure produced an initial yield of ~40–60% rod-shaped, calcium-tolerant cells. Cells were used within 8 h after isolation.

Solutions. When recording from human myocytes, cells were perfused with an "external" solution that consisted of (in mM) 137 NaCl, 4 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 11 glucose, and 10 HEPES; adjusted to a pH of 7.4 with NaOH. Glass pipettes (electrodes) were filled with an "internal" solution that consisted of (in mM) 120 K-aspartate, 20 KCl, 4 Na-ATP, 5 EGTA, and 5 HEPES; adjusted to a pH of 7.2 with KOH. Experiments were performed in the presence of 200 μM Cd^{2+} to block L-type calcium channels. All experiments were performed at room temperature (22–23°C).

Data acquisition and analysis. Acceptable atrial myocytes were rod shaped and lacked any visible blebs on the surface. Currents were measured using the whole cell variant of the patch-clamp method (7). Pipette tip resistance was ~1.0–2.0 MΩ when the pipettes were filled with the internal solution. The mean total series resistance (R_s) for the pathway between the pipette and cell membrane following the rupture of the cell membrane was estimated according to the equation: $R_s = \tau_c/C_m$, where the time constant (τ_c) was obtained by fitting the decay of the capacitive transient, and cell capacitance (C_m) was obtained by integration of the area under the capacitive transient. Before R_s compensation, the decay of the capacitive transient could be well fit by a single exponential having a time constant of 121.27 ± 9.83 μs (neonatal myocytes, n = 31) and 204.95 ± 14.37 μs (adult myocytes, n = 27). Mean cell capacitance was 28.91 ± 1.09 pF for neonatal myocytes and 64.85 ± 4.68 pF for adult myocytes. This yielded an uncompensated R_s of 4.28 ± 0.34 and 3.42 ± 0.26 MΩ for neonatal and adult myocytes, respectively. This value was further reduced by electronic compensation (typically 30–60%) to yield voltage drops across uncompensated R_s of <3 mV. Unless otherwise indicated, the amplitude of the I_{to} was measured as the difference between the peak current and the sustained level of current remaining at the end of the voltage pulse as has been described elsewhere (12, 15). Liquid junction potentials resulting from the substitution of pipette Cl^- with aspartate were typically 2–3 mV and were not corrected for.

For exponential fits of data (e.g., fits of decaying phase of I_{to} and I_{to} recovery from inactivation), a single-exponential fit was accepted as the fit of choice whenever the following criteria were met: *1*) the amplitude parameters obtained from the least-squares fit were all of the same sign, and *2*) when a negative value for the asymptotic information criteria statistic was obtained when comparing a one- vs. a two-exponential fit (1, 7). An unpaired Student's t-test or a χ^2 analysis (Fisher's test) was used for statistical analysis. Data are presented as means ± SE.

Human atrial specimens. Myocytes from very young patients were obtained from the right atrial appendages of 10 neonates, ages 1–14 days, 7 infants, ages 2 and 10 mo, and 3 young patients ages 2–2.5 yr undergoing corrective surgery for congenital defects (Table 1). None of the pediatric patients had received calcium channel blocking agents or antiarrhythmic agents. Adult myocytes were obtained from eight hearts of patients (range 11–68 yr, mean ± SE = 53.9 ± 6.4 yr) undergoing bypass graft surgery for coronary artery disease or surgery for repair of an atrial septal defect (Table 1). Some of the adult patients had received cardioactive drugs, including digoxin. Tissue was considered free from significant pathology if all of the following criteria were met: *1*) the tissue appeared grossly normal on removal, *2*) there was no evidence of right atrial enlargement (e.g., P wave amplitudes >2.5 mm), and *3*) there was no evidence for significant right atrial pressure overload (right atrial pressures >7 mmHg). Sixteen of the twenty very young patients from which atrial tissue was obtained had no evidence of right atrial enlargement or right atrial pressure overload. Although four patients had electrocardiographic evidence of right atrial enlargement, no patient had evidence of significant pressure overload (e.g., right atrial pressures ranged from 4–7 mmHg). Adult patients were determined to be free from right atrial enlargement.

Table 1. *Patient population characteristics*

Diagnosis	Age/Sex	Medications	P Wave
ToF	1 day/Female	Digoxin	None
TAPVR	3 days/Male	None	None
PA	3 days/Male	None	None
ToF	3 days/Male	None	None
VSD	5 day/Male	None	RAE
HLHS	7 days/Female	None	None
Transposition	7 days/Male	None	RAE
HLHS	8 days/Female	None	RAE
PA	14 days/Female	Digoxin	None
VSD	18 days/Female	None	None
AV canal	2 mo/Male	Digoxin	None
ASVD	2 mo/Female	Digoxin	None
ALC	4.5 mo/Female	None	RAE
MI	5 mo/Female	Digoxin, captopril	None
ToF	9 mo/Female	None	None
AV canal	9 mo/Female	None	None
DORV	10 mo/Female	Digoxin	None
VSD	2 yr/Male	None	None
ASD	22 mo/Male	None	None
VSD	2.5 yr/Male	None	None
ASD	11 yr/Male	None	None
CAD	51 yr/Male	Digoxin, nifedipine	None
CAD	56 yr/Male	None	None
CAD	58 yr/Male	Digoxin, amiodurone	None
CAD	61 yr/Male	Digoxin	None
CAD	62 yr/Female	None	None
CAD	64 yr/Male	Digoxin	None
CAD	68 yr/Female	Digoxin	None

ToF, tetralogy of Fallot; TAPVR, total anomalous pulmonary venous return; PA, pulmonary atresia; VSD, ventricular septal defect; HLHS, hypoplastic left heart syndrome; ASD, atrial septal defect; AV canal, atrioventricular canal; ASVD, anomalous systemic venous drainage; ALC, anomalous left coronary artery; MI, mitral insufficiency; DORV, double-outlet right ventricle; CAD, coronary artery disease. P wave indicates the presence of P wave abnormalities. RAE is right atrial enlargement as defined in MATERIALS AND METHODS.

RESULTS

Voltage dependence of I_{to}. We observed a rapidly decaying I_{to} in 67% (49 of 73) of the atrial myocytes isolated from the hearts of 17 patients ages 1 day-10 mo that were free of significant pathology (see MATERIALS AND METHODS). In contrast, an I_{to} was observed in 100% (14 of 14) of the adult cells tested ($P < 0.05$, χ^2 analysis). The I_{to} in both cell types was calcium independent, since all experiments were performed in the presence of 200

μM Cd^{2+} in the bath solution and 5 mM EGTA in the internal solution.

The current-voltage relationship for the I_{to} recorded from young and adult atrial myocytes is shown in Fig. 1. Application of depolarizing voltage pulses positive to −20 mV from a holding potential of −40 mV to cells from either age group elicited a rapidly activating current, which decayed to an apparent steady state over the course of an 800-ms voltage pulse. A holding potential of −40 mV was chosen to inactivate the sodium current. As indicated in Fig. 1*A*, the current density for the peak current (at +60 mV) measured in young myocytes (8.08 ± 0.88 pA/pF, n = 9) was ~40% smaller than that measured in adult myocytes (12.52 ± 0.68 pA/pF, n = 14) ($P < 0.05$), while the density of the currents measured at the end of the voltage pulse are not significantly different (young = 4.14 ± 0.69 pA/pF, adult = 5.23 ± 0.62 pA/pF). However, under these conditions the total peak current may reflect the contribution of the sum of both I_{to} and the noninactivating or sustained current. To separate these two components, we subtracted the amplitude of the sustained current from the maximum peak current. As indicated in Fig. 1*D*, the current density of I_{to} measured at +60 mV in myocytes isolated from the hearts of young patients ages 1 day-10 mo (3.94 ± 0.35 pA/pF, n = 9) was roughly twofold smaller than that measured in myocytes isolated from the hearts of adult patients (8.07 ± 0.54 pA/pF, n = 14) ($P < 0.05$). As illustrated in Fig. 2, these age-related changes in I_{to} density appear to occur between the ages of 10 mo and 2 yr of age. For example, the current density for I_{to} measured in cells isolated from three hearts ages 2–2.5 yr (7.24 ± 0.69 pA/pF, n = 12) was not significantly different from that measured in adult cells but is significantly greater than the current density measured in cells isolated from the hearts of patients with ages between 1 day and 10 mo ($P < 0.05$). We were not able to resolve a difference in the amplitude of I_{to} measured at +60 mV in cells isolated from the hearts of two 1-day-old patients (5.36 pA/pF and 4.59 pA/pF) compared with cells from two 10-mo-old patients (3.76 and 3.66 pA/pF, n = 2 cells). Further studies with a larger sample size are required to confirm this later observation.

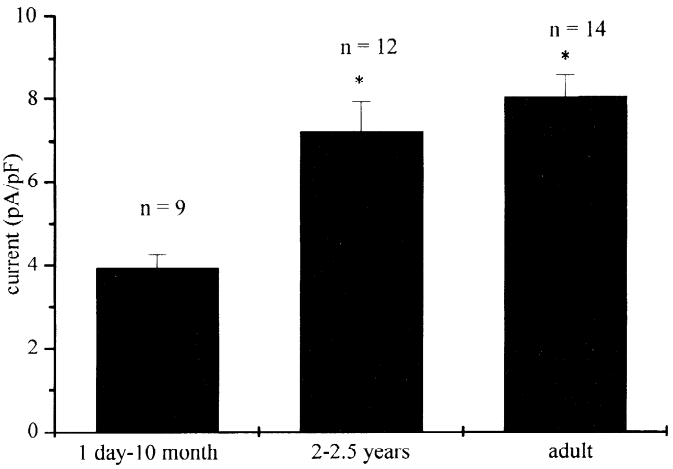

Fig. 2. Relationship between age and I_{to} amplitude. Values are peak current minus sustained current obtained from an 800-ms voltage pulse to +60 mV. Values were 3.94 ± 0.35 pA/pF (1 day-10 mo), 7.24 ± 0.69 (2–2.5 yr), and 8.07 ± 0.54 (adult). Values are means ± SE; n, no. of cells. *Significantly different from values obtained from cells 1 day-10 mo.

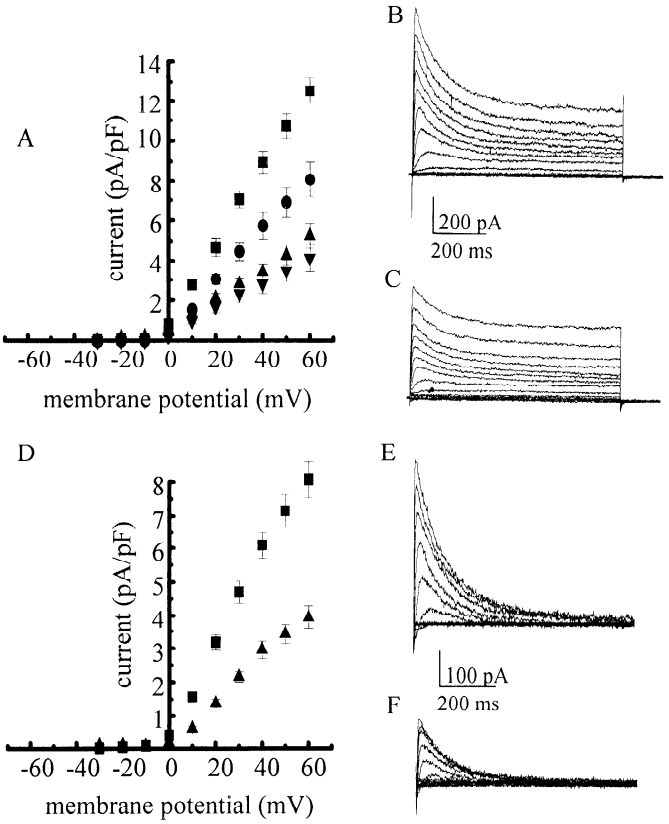

Fig. 1. Voltage dependence of transient outward current (I_{to}) in young and adult atrial myocytes. *A*: mean current-voltage relationship for peak and end of pulse current measured in young (n = 9; ● and ▼, respectively) and adult (n = 14; ■ and ▲, respectively) myocytes. Values are means ± SE. *B* and *C*: family of current traces recorded from an adult (*B*) and young (*C*) atrial myocyte. Currents were elicited by a series of 800-ms voltage pulses from −30 to +60 mV from a holding potential of −40 mV. Cycle length, 0.2 Hz. *D*: mean current-voltage relationship for peak current corrected for sustained current. Plot was constructed by subtracting current measured at the end of an 800-ms voltage pulse from the peak current. ■, Adult; ▲, young. *E* and *F*: examples of corrected currents from adult (*E*) and young (*F*) cells.

Activation and inactivation parameters. To determine whether the age-related increase in I_{to} density was the result of a shift in the voltage dependence of inactivation and/or activation, we characterized the steady-state activation and inactivation parameters of I_{to} in young and adult cells. Figure 3 shows mean values defining the steady-state activation and inactivation curves obtained from young and adult myocytes. As illustrated in Fig. 3, *A-C*, after a depolarizing prepulse, young cells exhibited a significantly smaller tail current amplitude (normalized to cell capacitance) compared with adult cells. For example, tail current amplitude following a pulse to +60 mV in cells obtained from young hearts was 0.81 ± 0.21 pA/pF (n = 5), twofold smaller than that measured in cells obtained from adult hearts (1.68 ± 0.16 pA/pF, n = 7) ($P < 0.05$). The relationship between prepulse potential and tail current amplitude (Fig. 3*C*), which defines

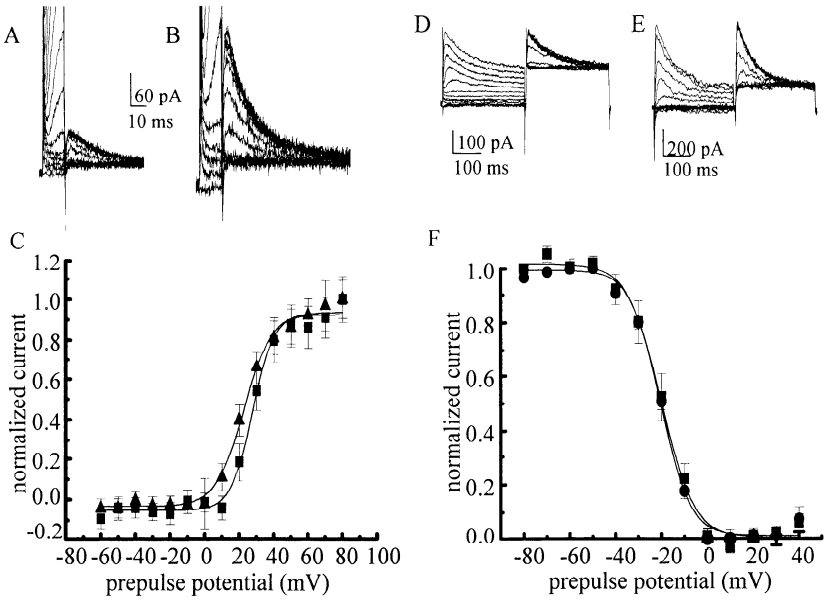

Fig. 3. Voltage dependence of steady-state activation and inactivation for young and adult atrial myocytes. *A* and *B*: example of original current recordings obtained from young (*A*) and adult (*B*) cells used to construct steady-state activation curve. Voltage protocol consisted of a 15-ms voltage pulse to potentials between −60 and +80 mV from a holding potential of −40 mV (cycle length = 0.2 Hz). On repolarization to −20 mV, tail currents were observed that were taken as a measure of activation of I_{to}. These values were normalized to the largest tail current and were plotted as a function of prepulse potential (*C*; ▲, adult; ■, young). Values indicate means ± SE. Curves shown are fits of mean data to a Boltzmann distribution. *D* and *E*: original current recordings to study steady-state inactivation in young (*D*) and adult (*E*) cells. Voltage protocol consisted of a 320-ms prepulse to potentials between −80 and +40 mV from a holding potential of −40 mV. This was followed by a 300-ms pulse to +60 mV after a 10-ms return to −40 mV. *F*: values measured at +60 mV were corrected for the sustained current (●, adult; ■, young), which remained after inactivation of I_{to} and was plotted as ratio of current at the respective potential to the largest test current. Values are means ± SE.

the voltage dependence of I_{to} activation, could be well described by a Boltzmann distribution of the form

$$I = I_{max}/\{1 + \exp[(V_{0.5} - V_m)/k]\}$$

where I is tail current amplitude at a given prepulse potential, I_{max} is the maximum current amplitude at positive potentials, $V_{0.5}$ is the voltage at half-maximal activation, V_m is the membrane potential, and k is the slope factor. When comparing mean values for $V_{0.5}$ and k between cells obtained from young hearts ($V_{0.5}$ = 24.3 ± 2.25 mV and k = 5.29 ± 0.71 mV, n = 5) and cells obtained from adult hearts ($V_{0.5}$ = 21.77 ± 5.14 mV and k = 7.86 ± 1.33 mV, n = 7), no significant differences were found. A two-pulse protocol was used to define the voltage dependence of steady-state inactivation (Fig. 3, *D* and *E*). As illustrated in Fig. 3*F*, the mean steady-state inactivation curves for young and adult cells were not significantly different. The $V_{0.5}$ and k for steady-state inactivation obtained from Boltzmann fits were −17.97 ± 5.23 and 5.72 ± 1.48 mV (n = 13) for cells isolated from young hearts and −19.19 ± 2.64 and 5.95 ± 0.39 mV (n = 7) for cells isolated from adult hearts.

The time course of inactivation (current decay) was also very similar in young and adult atrial myocytes and could be well fit by a single-exponential function at voltages between +20 and +60 mV. At +60 mV, the mean time constant for young myocytes was 81.6 ± 10.04 ms (n = 20), which was not significantly different from that obtained for adult cells (81.2 ± 6.2 ms, n = 20). There was no obvious voltage dependence for the time constant of current decay over the voltage range of +20 to +60 mV (Fig. 4). At voltages negative to +20 mV,

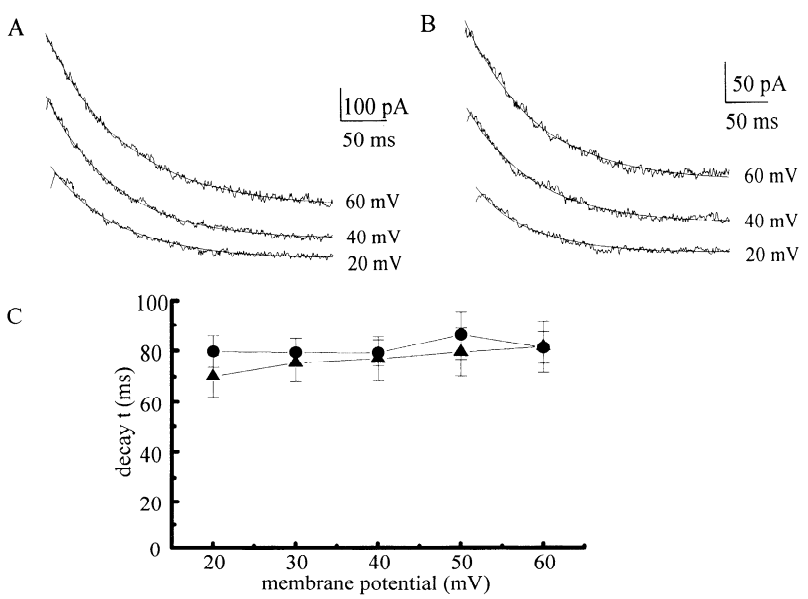

Fig. 4. Voltage dependence of I_{to} decay. *A* and *B*: examples of single-exponential fits to data obtained at test potentials of +20, +40, and +60 mV from an adult cell (*A*) and a young cell (*B*). Currents were elicited by 320-ms voltage pulses from a holding potential of −40 mV. Calculated time constants were 67.9 ms (+20 mV), 75.0 ms (+40 mV), and 83.32 ms (+60 mV) for *A* and 57.8 ms (+20 mV), 72.4 ms (+40 mV), and 81.7 ms (+60 mV) for *B*. *C*: effect of voltage on current decay time (t). ▲, Young; ●, adult. Values are means ± SE (n = 20).

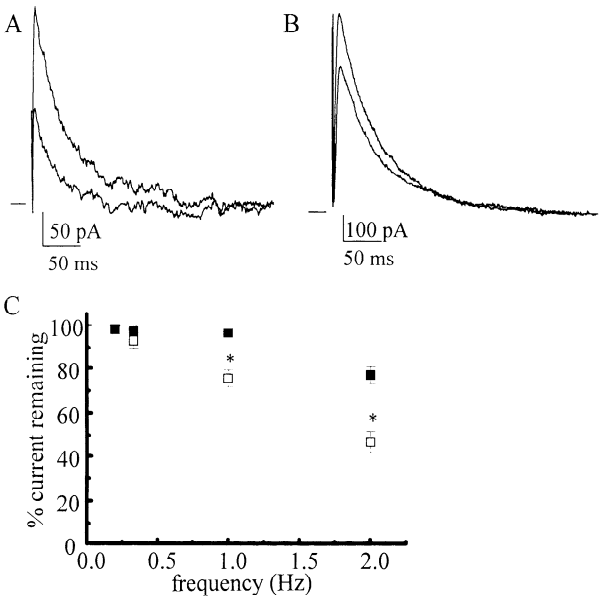

Fig. 5. Rate dependence of I_{to}. *A*: superimposed current traces recorded from a young atrial myocyte. Smaller trace represents steady-state level of current reduction after a 2-Hz rate train. Holding potential was −40 mV, test potential +60 mV, pulse duration 320 ms. Horizontal line indicates zero current level. *B*: first and last current traces recorded from an adult myocyte after a 2-Hz rate train. *C*: plot of rate-dependent reduction of I_{to} over a physiological range of frequencies (0.2, 0.33, 1.0, 2.0 Hz) (n = 5–8). ■, Adult; □, young. Values are means ± SE; * significantly different from adult ($P < 0.05$).

currents were typically too small to obtain meaningful fits.

Effect of rate on I_{to}. Previous recordings documenting a rate-dependent reduction in the amplitude of *phase 1* of the human atrial action potential suggests that the I_{to} in young atrial fibers may have a relatively slow reprimming rate compared with adult tissue (4). We therefore examined the effects of stimulation rates over the range of 0.2 to 2.0 Hz on peak I_{to}. Stimulation rates faster than 2.0 Hz were not examined, since it was necessary to use pulse durations of 320 ms to allow for near complete decay of current. Figure 5 shows the effects of a 2.0-Hz rate train on the I_{to} from a young (Fig. 5*A*) and from an adult (Fig. 5*B*) myocyte. As illustrated, the reduction in peak current during a 2.0-Hz rate train was approximately twofold greater in young vs. adult myocytes (53.4 ± 5.2 vs. 23.2 ± 3.8%, n = 5–6). At stimulation rates >0.33 Hz, the reduction of I_{to} measured in myocytes isolated from young hearts was significantly greater than that observed for adult cells ($P < 0.05$) (Fig. 5*C*). This difference in the rate dependence of I_{to} magnitude suggests that there are differences in the kinetics of recovery from inactivation in young and adult cells. To test this hypothesis, we defined the time course of recovery from inactivation using a two-pulse protocol consisting of a 500-ms conditioning pulse followed by a 300-ms test pulse after a variable recovery interval at potentials between −40 and −80 mV. As illustrated in Fig. 6, the time course of recovery of I_{to} was significantly slower in young cells compared with adult atrial myocytes and was best described by a single-exponential relationship (Fig. 6*C*). At −40 mV, the time constant for recovery (τ) obtained from young myocytes was 293.5 ± 39.9 ms (n = 8), approximately twofold slower than that for adult cells (τ = 137.9 ± 11.9 ms, n = 8) ($P < 0.05$). Recovery from inactivation was voltage dependent, with recovery being faster at more negative potentials. At −60 mV, the time constant obtained from young myocytes was 125.5 ± 12.8 ms (n = 10) and at −80 mV was 83.7 ± 9.1 ms (n = 6). Although I_{to} recovery kinetics measured in young cells were faster at more negative potentials, at all potentials tested, recovery time constants were roughly two times greater in young cells than those measured in adult cells (at −60 mV, τ =

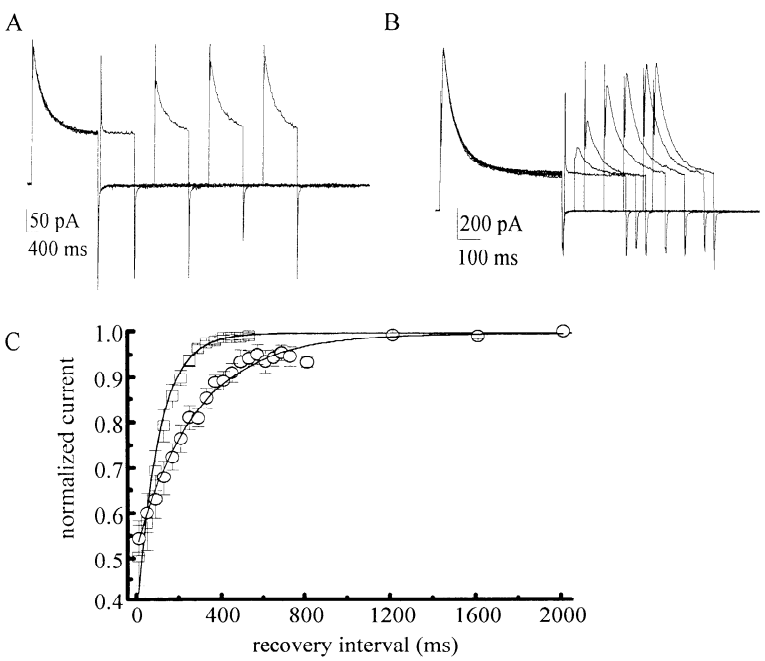

Fig. 6. Recovery from inactivation determined by a 2-pulse protocol. Protocol consisted of a 500-ms prepulse to +60 mV followed by a 300-ms pulse to +60 mV after a variable recovery period at the holding potential (−40 mV). *A*: example of current traces recorded from a young atrial mycoyte obtained 10 ms (*trace 1*), 410 ms (*trace 2*), 810 ms (*trace 3*), and 1,210 ms (*trace 4*) after prepulse (traces numbered from *left* to *right*). Currents were not corrected for sustained level of remaining current. Initial trace recorded after prepulse solely reflects the sustained current. *B*: example of current traces recorded from an adult cell obtained 10 ms (*trace 1*), 50 ms (*trace 2*), 90 ms (*trace 3*), 170 ms (*trace 4*), 250 ms (*trace 5*), 330 ms (*trace 6*), and 370 ms (*trace 7*) after prepulse. *C*: plot of recovery from inactivation values obtained from young (○) and adult (□) myocytes (n = 8). Data points are means ± SE. Curve is a single-exponential function of the form $-0.473 \cdot \exp(-x/282.5\ \text{ms}) + 0.99461$ for young cells and $-0.6419 \cdot \exp(-x/102.65) + 0.996$ for adult cells.

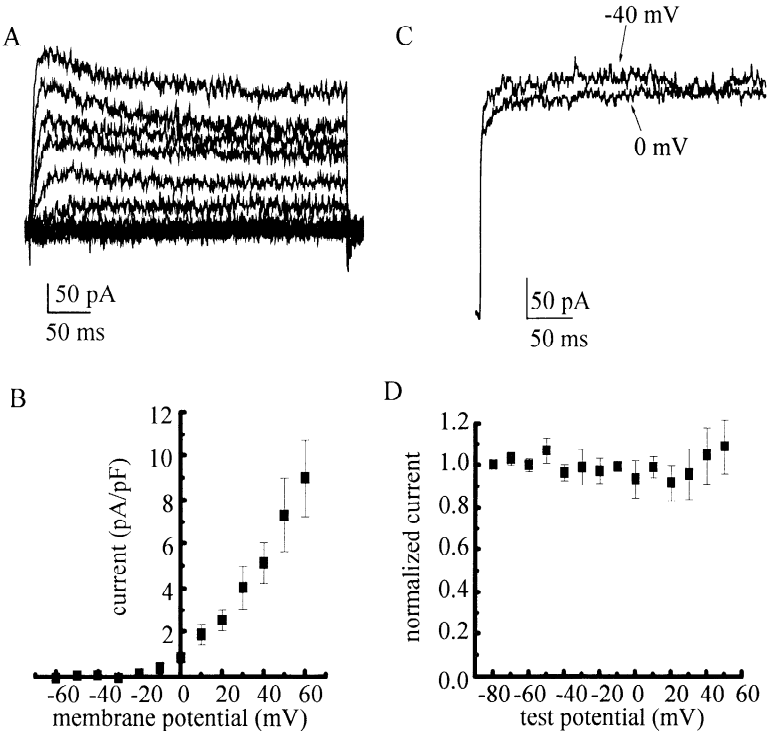

Fig. 7. Family of currents recorded from a young human atrial myocyte that did not possess an I_{to}. *A*: currents elicited by a series of 320-ms voltage pulses from −60 to +60 mV; holding potential was −40 mV (cycle length = 0.2 Hz). *B*: plot of current density vs. voltage for neonatal outward current. Symbols are means ± SE (n = 13). *C*: lack of sensitivity to holding potential of partially inactivating current in young human atrial myocytes. Outward currents elicited by a pulse to +60 mV from a holding potential of either −40 or 0 mV. *D*: plot of voltage dependence of steady-state inactivation. Symbols are means ± SE (n = 5). Voltage protocol consisted of 300-ms prepulses to potentials ranging from −80 to +50 mV. Test pulses to +40 mV followed a brief (6 ms) return to holding potential (−60 mV).

77.5 ± 12.1 ms, n = 7, and at −80 mV, τ = 51.4 ± 7.2 ms, n = 5).

Current in cells without I_{to}. During this study, 24 out of 73 (33%) of those cells isolated from young hearts were found to lack completely a current similar to I_{to} on depolarization to potentials as positive as +80 mV. These cells, which appeared otherwise normal, were isolated from the hearts of eight patients. The lack of an I_{to} was unrelated to the chronological age or sex of the patient from which the atrial tissue was obtained. In these cells, depolarizing pulses elicited a rapidly activating current, which partially decayed (15.3 ± 2.2% at +60 mV, n = 22) over the course of a 320-ms voltage step (Fig. 7*A*). The threshold for the activation of this current was between −30 and −20 mV (Fig. 7*B*), and the mean peak current amplitude at +60 mV was 8.9 ± 1.7 pA/pF (n = 13) when current was normalized to cell surface area. The amplitude of this current is very similar to the sustained current observed in cells that have an I_{to} (compare Figs. 1*A* and 7*B*). Unlike I_{to}, this partially-inactivating current was not very sensitive to holding potential, resulting in a nearly linear steady-state inactivation "curve" (Fig. 7*D*).

I_{K1}. On hyperpolarization to potentials negative to −70 mV, a current with pronounced inward rectification, typical of I_{K1}, could be recorded from both young and adult myocytes (Fig. 8, *A* and *B*). The current-voltage relationship of this current is shown in Fig. 8*C*. The outward component of this current observed at potentials between −70 and −40 mV was relatively small, typically being <15 pA. When currents were normalized to cell surface area, the density of I_{K1} in young and adult cells was very similar (Fig. 8*C*). Current density measured at −100 mV in young cells (−3.19 ± 0.43 pA/pF, n = 8) was not significantly different from that measured in adult cells (−2.50 ± 0.45 pA/pF, n = 11). The maximal slope conductance obtained from normalized values of I_{K1} (pA/pF) at potentials between −80 and −100 mV was also found to be virtually identical in young (0.12 ± 0.01 nS/pF, n = 8) and adult cells (0.10 ± 0.03 nS/pF, n = 6).

DISCUSSION

The present study utilizes direct measurements of I_{to} in young and adult human atrial myocytes to confirm the existence of marked age-related changes in this current. These changes include *1*) the presence of an I_{to} in only 67% of the cells isolated from young hearts (1 day-10 mo) vs. 100% of the cells isolated from adult hearts, *2*) an almost twofold increase in the current density of I_{to} during development from young to adult (Fig. 1), and *3*) recovery kinetics that are approximately twofold slower in young myocytes relative to adult myocytes (Fig. 6). The increase in I_{to} amplitude, which occurs with age, does not appear to be due to shifts in the voltage dependence of either steady-state activation or inactivation, which showed no age-related changes (Fig. 3), but can be explained by a twofold increase in total conductance observed for this current (Fig. 3, *A* and *B*). We believe these age-related changes in I_{to} may underlie the developmental changes observed in atrial action potential configuration previously defined by Escande et al. (4).

It is interesting to note that the age-related changes in I_{to} density appear to occur most significantly between the ages of 10 mo and 2 yr. Over this age range, the I_{to} current density in cells isolated from the hearts of patients 1 day to 10 mo of age is significantly smaller than that observed in cells isolated from the hearts of

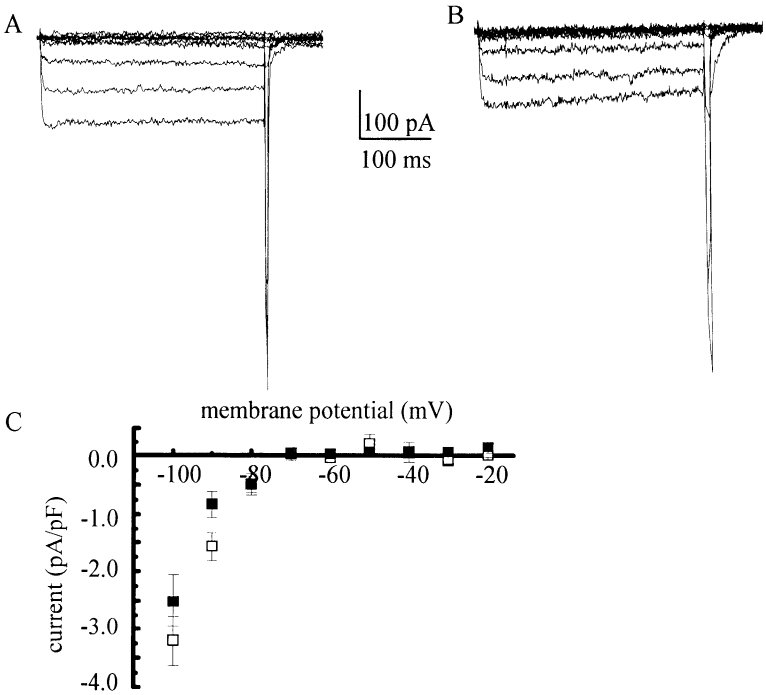

Fig. 8. Inward rectifier potassium currents (I_{K1}) recorded from young and adult atrial myocytes. *A*: inward currents recorded from a young myocyte. Currents were elicited by 320-ms pulses from −100 to −20 mV in 10-mV steps (holding potential = −40 mV). *B*: recording of I_{K1} from an adult myocyte. Pulse protocol same as in *A*. *C*: plot of current density vs. voltage for young (□) and adult (■) I_{K1}. Symbols are means ± SE (n = 8, young; n = 11, adult).

patients 2–2.5 yr of age (Fig. 2). The reasons for these changes in I_{to} density over this age range are unknown. The presence of an I_{to} in a majority of neonatal and infant myocytes was somewhat surprising, since other species show an age-related appearance of this current in which myocytes isolated from neonatal hearts exhibit either no or a very small I_{to} (9, 10, 13). In addition, a recent study characterizing currents in atrial myocytes isolated from diseased human hearts from patients ages 3 mo to 5 yr likewise exhibited no rapidly decaying component similar to I_{to} (11). The atria from which these myocytes were isolated were classified as "clearly dilated" (11). Interestingly, in the present study, the myocytes possessing an I_{to} were isolated from both enlarged, although hemodynamically normal (right atrial pressure <7 mmHg), and nonenlarged atria. There were no quantitative differences in the I_{to} measured from either enlarged or nonenlarged atria. Our ability to record a pronounced I_{to} in our cell preparations may possibly reflect changes in current density due to differences in the level of atrial pathology and/or atrial hemodynamics. This speculation requires further investigation.

When the voltage dependence of I_{to} was examined, a sustained current component was routinely observed after the complete decay of I_{to} (Fig. 1). The amplitude of this sustained current was virtually identical in myocytes isolated from young and adult hearts. Although the voltage dependence of this current was similar to the transient component, the sustained current did not share the characteristics of either a time-dependent decay or sensitivity to holding potential (data not shown) typical of I_{to}. Therefore, it seems unlikely that the sustained current is related to the transient component. In fact, in 33% (24 of 73) of the young cells tested, a transient component could not be recorded. In these cells, only a rapidly activating, partially inactivating current was recorded (Fig. 7). This current was similar to the sustained current recorded from cells with an I_{to} in terms of both its current-voltage relationship and its insensitivity to holding potential (Fig. 7*D*). It is therefore possible that the partially inactivating current observed in the absence of I_{to} may reflect the behavior of the sustained current that is observed after the decay of I_{to}. Although the sustained current observed in adult atrial myocytes has been attributed to a Kv1.5-like current (15), the nature of this current in young atrial myocytes has yet to be clarified.

I_{K1} in human atrial myocytes. In contrast to I_{to}, I_{K1} density was very similar in myocytes isolated from young and adult human atria (Fig. 8). The current density measured at −100 mV and slope conductance of this current were virtually identical in young and adult cells. The lack of age-related changes in the current density of I_{K1} recorded from human atrial myocytes is in contrast to the age-related changes that occur with the I_{K1} of rat and rabbit ventricular myocytes. For example, Wahler (14) reported an approximately threefold increase in the current density of rat ventricle I_{K1} between neonatal *day 1* and adult. Similarly, Huynh et al. (8) and Sanchez-Chapula et al. (13) reported a significant increase in the density of I_{K1} recorded from neonatal vs. adult rabbit ventricular myocytes. Nevertheless, the virtually identical current density and slope conductance observed for young and adult human I_{K1} is entirely consistent with the observation by Escande et al. (4) that the mean diastolic potential recorded from myocytes of young human atria is not significantly different from that of adult atrial myocytes. These results suggest that the pattern for developmental changes in ion channel physiology in humans may not be identical to those present in other animal species.

Potential limitations. One potential limitation of the current study results from the finite number of adult

cells (limited sample size) that was studied. For example, our data from adult atrial cells was obtained from a total of 14 cells isolated from 8 hearts. In each of these 14 adult cells we observed a measurable I_{to}, in marked contrast to results obtained from young hearts, in which 24 out of 73 cells (33%) did not exhibit a definable I_{to}. However, due to the limited sample size, we cannot be certain that a small fraction (e.g., <1 out of 14, or 7%) of adult cells may also lack an I_{to}, similar to $\sim 33\%$ of neonatal cells. Consistent with this observation, Wang et al. (15) have reported that ~ 10 out of 100 adult atrial cells lack a definable I_{to} under similar experimental conditions (15). The difference in the observed incidence of cells lacking an I_{to} in adult tissue in the current study and that by Wang et al. (15) could result from differences in sample size, cell isolation techniques, patient characteristics, or a combination of factors. Nevertheless, both χ^2 analysis of the data obtained in the current study and comparison of the percentage of cells lacking an I_{to} in young hearts (33%) vs. those from adult hearts [0% in this study, or $\sim 10\%$ in the study by Wang et al. (15)] suggest that there are significant age-related changes in the percentage of cells lacking an I_{to} in human atrial myocytes.

An additional limitation of this study is that all experiments were conducted at room temperature (22–23°C), compared with normal physiological body temperature (37°C). This was considered a necessary condition for our study, since pilot experiments confirmed a consistent inability to separate accurately the peak of the I_{to} from the capacitative transient at temperatures near or above 35°C. This limitation means that we cannot quantitatively predict the rates of I_{to} recovery kinetics at 37°C. Nonetheless, the results of this study indicate that there are significant age-related changes in I_{to} properties in human atrial cells (under conditions in which I_{to} can be experimentally defined) and provide the first direct experimental support for the hypothesis that changes in I_{to} underly developmental changes in early (*phase 1*) repolarization characteristics in human atrial myocardium (4).

Summary. This study constitutes the first characterization of I_{to} in myocytes isolated from the atria of young humans. The presence of an I_{to} in neonatal cells is in contrast to that reported in other species and diseased human atria (9, 11). The age-related changes in I_{to} amplitude, percentage of cells possessing an I_{to}, and repriming kinetics observed provide an explanation for the changes reported to occur with age in the morphology of the atrial action potential. In contrast to I_{to}, no age-related changes were observed for either the sustained current measured after the decay of I_{to} or I_{K1}. This lack of age-related change in I_{K1} is in contrast to work done in other species (8, 13, 14). These results indicate that there are significant current-specific changes that occur with age in human atria and emphasize the potential pitfalls in extrapolating results obtained in other species to human cardiac physiology.

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