Alterations in Muscarinic K^+ Channel Response to Acetylcholine and to G Protein–Mediated Activation in Atrial Myocytes Isolated From Failing Human Hearts

Shin-ichi Koumi, MD; Carl E. Arentzen, MD;
Carl L. Backer, MD; J. Andrew Wasserstrom, PhD

Background A variety of previous studies have demonstrated reduced diastolic potential and electrical activity in atrial specimens from patients with heart disease. Although K^+ channels play a major role in determining resting membrane potential and repolarization of the action potential, little is known about the effects of preexisting heart disease on human atrial K^+ channel activity.

Methods and Results We characterized the inwardly rectifying K^+ channel (I_{K1}) and the muscarinic K^+ channel [$I_{K(ACh)}$] in atrial myocytes isolated from patients with heart failure (HF) and compared electrophysiological characteristics with those from donors (control) by the patch-clamp technique. Resting membrane potentials of isolated atrial myocytes from HF were more depolarized (−51.1±9.7 mV, mean±SD, n=30 patients) than those from donors (−73.0±7.2 mV, n=4 patients, $P<.001$). The action potential duration in HF was longer than that in donors. Although acetylcholine (ACh) shortened the action potential, reduced the overshoot, and hyperpolarized the atrial cell membrane in HF, these effects were attenuated compared with those observed in donors. The whole-cell membrane current slope conductance in HF was small, the reversal potential was more positive, and the sensitivity to ACh was less compared with donors. In single-channel recordings from cell-attached patches, I_{K1} channel conductance and gating characteristics were the same in HF and donor atria. When ACh was included in the pipette solution, $I_{K(ACh)}$ was activated in both groups. Single-channel slope conductance of $I_{K(ACh)}$ averaged 42±3 pS (n=28) in HF and 44±2 pS (n=4) in donors, and mean open lifetime was 1.3±0.3 milliseconds (n=24) in HF and 1.5±0.4 milliseconds (n=4) in donors. These values were virtually identical in the two groups (not significantly different, NS), although both single I_{K1} and $I_{K(ACh)}$ channel densities were less in HF. Channel open probability of $I_{K(ACh)}$ was also less in HF (4.0±1.2%, n=24) than in donors (6.8±1.1%, n=3, $P<.01$). The concentration of ACh at half-maximal activation was 0.11 μmol/L in HF and 0.03 μmol/L in donors. In excised inside-out patches, $I_{K(ACh)}$ from HF required higher concentrations of GTP and GTPγS to activate the channel compared with donors. These results suggest a reduced $I_{K(ACh)}$ channel sensitivity to M_2 cholinergic receptor–linked G protein (G_i) in HF compared with donors.

Conclusions Atrial myocytes isolated from failing human hearts exhibited a lower resting membrane potential and reduced sensitivity to ACh compared with donor atria. Whole-cell and single-channel measurements suggest that these alterations are caused by reduced I_{K1} and $I_{K(ACh)}$ channel density and reduced $I_{K(ACh)}$ channel sensitivity to G_i-mediated channel activation in HF. (***Circulation.*** **1994;90:2213-2224.**)

Key Words • potentials • potassium • proteins

Previous studies have demonstrated that normal human atrial tissue shows normal electrophysiological characteristics similar to those from other mammals, whereas atrial specimens from patients with heart disease often exhibit low resting membrane potentials.[1-5] In addition, studies using experimental animal models with primary heart disease revealed low resting membrane potentials.[6,7] The basis for the low resting potential includes the increase and/or decrease in transmembrane ion permeabilities as well as the level of Na,K-ATPase activity in atrial tissue from diseased patients.[5]

Membrane K^+ conductance is increased by acetylcholine (ACh) in a variety of cardiac tissues from many species.[8-10] The activation mechanism of muscarinic K^+ channels [$I_{K(ACh)}$] has been studied extensively in atrial cell membranes and was shown to involve the M_2 cholinergic receptor–coupled G protein, G_i.[11-13] The electrophysiological response to ACh in human atrial myocytes has been shown to be qualitatively similar to that in guinea pig atrial cells under whole-cell voltage-clamp conditions; ACh shifted the reversal potential of the membrane current in the hyperpolarizing direction, indicating an increased contribution of the current to resting membrane potential.[14] Recently, however, our laboratory characterized the human atrial $I_{K(ACh)}$ and demonstrated that the density of $I_{K(ACh)}$ in diseased human atrial myocytes was low compared with that in other species.[15] A similar observation was made in human atrial $I_{K(ACh)}$ by Heidbuchel et al.[16] However, these studies did not evaluate the normal human heart,

Received April 29, 1994; revision accepted June 14, 1994.

From the Division of Cardiology, Department of Medicine (Reingold ECG Center) and the Feinberg Cardiovascular Research Institute (S.K., J.A.W.), the Department of Surgery (C.E.A., C.L.B.), and the Department of Molecular Pharmacology and Biological Chemistry (J.A.W.), Northwestern University School of Medicine, Chicago, Ill.

Correspondence to J. Andrew Wasserstrom, PhD, Reingold ECG Center-S203, Northwestern University School of Medicine, 303 E Chicago Ave, Chicago, IL 60611.

leaving several important questions unanswered concerning the influence of disease on electrophysiological function. In addition, the transient outward current has also been reported to be altered in myocytes dispersed from dilated and diseased atria.[17]

In this study, we focused on two major native K^+ channels in atrial myocytes isolated from adult patients with heart failure: $I_{K(ACh)}$ and the inwardly rectifying K^+ channel (I_{K1}). We compared their properties with those from normal atria to determine whether preexisting heart disease alters the behavior of these channels. This question is all the more important because these two K^+ channels provide the major current contributions to atrial resting and action potentials[16,18-20] and because $I_{K(ACh)}$ is the major effector of ACh stimulation in atrial myocytes.[8,9]

Methods

Human Cardiac Specimens

Adult human atrial specimens were obtained from explanted hearts of transplantation recipients and from patients undergoing cardiac surgery. Institutional and National Institutes of Health guidelines for human experimentation were followed in obtaining surgical specimens and informed consent was obtained from all subjects. A total of 30 congestive heart failure patients (HF) were studied (32 to 68 years old; median age, 50 years). Twenty-three patients were men and 7 were women. Twenty-two patients underwent surgery for ischemic heart disease (IHD), 5 for valvular heart disease (VHD), and 3 for ischemic heart disease with valvular heart disease (IHD+VHD). All patients had symptomatic congestive heart failure with elevated mean right atrial pressure (9.1±2.8 mm Hg, n=30, all patients >5 mm Hg), with elevated mean pulmonary artery wedge pressure (14.6±3.0 mm Hg, n=30), with elevated left ventricular end-diastolic pressure (14.1±3.2 mm Hg, n=30), and with atrial enlargement as diagnosed by ultrasound cardiography. Six patients had chronic atrial fibrillation (AF), whereas the remainder were in sinus rhythm (SR), including 4 with paroxysmal atrial dysrhythmia. All specimens were obtained from the right atrial appendage. Similar specimens were also obtained from 4 explanted donor hearts (donors 28 to 41 years old; median age, 38 years) that served as nondiseased healthy controls. All patients received diuretics and vasodilators for >2 months before surgery. Twenty-four patients received digoxin for >1 month before surgery. Three patients received calcium antagonists for 1 to 9 months. No catecholamines or β-blockers were given. The administration of cardiac drugs was stopped 48 hours before surgery. Immediately after surgical explantation of each heart, the specimen was placed in a chilled transport solution and carried to the laboratory within 1 hour.

Isolation of Atrial Myocytes

Human atrial myocytes were isolated by an enzymatic dissociation method identical to that described previously by our laboratory.[21] Specimens were minced into small pieces with a fine razor blade and washed three times, 7 minutes each time, in oxygenated Ca^{2+}-free Tyrode's solution. The tissues were then incubated in oxygenated Ca^{2+}-free Tyrode's solution containing 300 to 350 U/mL collagenase (Sigma, type V), 0.5 U/mL protease (Sigma, type XXIV), and 1 mg/mL of bovine serum albumin (Sigma) at 37°C and were gently stirred with a magnetic stirring bar until isolated myocytes appeared (≈40 minutes). The tissue was then strained through a 200-μm nylon mesh to collect the individual myocytes. Myocytes were stored at room temperature in a modified Kraftbrühe (KB) medium.[22] The residual nondigested tissue was reincubated in enzyme-containing solution for an additional 10 minutes, and isolated myocytes were collected as described above. This process was repeated until viable myocytes could no longer be isolated. Isolated cells were stored in KB solution at room temperature. Only Ca^{2+}-tolerant, clearly striated, rod-shaped cells without any blebs were studied.

Solutions

The transport solution for human specimens contained (in mmol/L): NaCl 27, KCl 20, $MgCl_2$ 1.5, HEPES 5.0, glucose 274 (pH 7.0). The Tyrode's solution contained (in mmol/L): NaCl 140.0, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 5.0, glucose 5.0 (pH 7.4 with NaOH). Ca^{2+}-free Tyrode's solution was made by omitting $CaCl_2$ from the normal Tyrode's solution. The modified KB solution had the following composition (in mmol/L): KCl 25, KH_2PO_4 10, KOH 116, glutamic acid 80, taurine 10, oxalic acid 14, HEPES 10, glucose 11 (pH 7.0 with KOH). The composition of internal solution used for the action potential and whole-cell recording was (in mmol/L): potassium aspartate 110, KCl 20, KH_2PO_4 1.0, $MgCl_2$ 1.0, Na_2-ATP 5.0, EGTA 5.0, HEPES 5.0 (pH 7.2). Pipette solution used for both cell-attached and inside-out patch recordings was (in mmol/L): KCl 150, HEPES 5.0 (pH 7.4). Normal Tyrode's solution was used for external solutions during the action potential and whole-cell recordings. Either normal Tyrode's solution or high-K^+ (150 mmol/L) solution was used for external solutions during cell-attached patch recordings. High-K^+ solution was prepared by simply adding KCl to normal Tyrode's solution. In excised inside-out patch recordings, the cytosolic surface of the membrane was perfused with a bath solution containing (in mmol/L): potassium aspartate 120, KCl 30, Na_2-ATP 5.0, $MgCl_2$ 1.0, HEPES 5.0, and EGTA 5.0 (pH 7.2).

ACh (0.001 to 10 μmol/L) was added to the bath solution during the action potential and whole-cell recordings or to the pipette solution during cell-attached patch recordings. GTP (0.1 to 500 μmol/L) and guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS, 0.1 to 200 μmol/L) were added to the bath solution in inside-out patch recordings. Pertussis toxin was dissolved in KB solution at a final concentration of 5 μg/mL with albumin (3 mg/mL) during myocyte incubations for up to 90 minutes at 35°C. All reagents were purchased from Sigma Chemical Co.

The experimental chamber (0.2 mL) was continuously perfused with bath solution at a rate of 6 to 7 mL/min, and complete solution exchange was achieved within 5.0±1.2 seconds (n=16) as determined by measuring the current change induced by changing the solution from 5.4 mmol/L K^+ solution to 150 mmol/L K^+ solution. To facilitate the rapid exchange of test solutions, the mouth of a fine-bore polyethylene inlet tube was positioned within 1 mm of the patch.

Electrophysiological Recording Technique

Patch-clamp recordings of whole-cell and single-channel unitary currents were made by use of cell-attached and excised inside-out patch configurations.[23] The amplifier and head stage were designed by Narahashi et al.[24] The feedback resistance of the head stage was 100 MΩ for recording whole-cell current and 10 GΩ for recording single-channel current. The electrodes were pulled from glass capillary tubes (Kimax-51, Kimble Products) with a programmable horizontal micropipette puller (model P-87, Sutter Instrument Co). Electrodes with tip resistances of ≈2 MΩ were used to record whole-cell currents and ≈10 MΩ for single-channel currents. Seal resistances were 10 to 100 GΩ for recording single-channel currents.

For whole-cell recording, the series resistance attributed to the pipette tip and the cell interior was compensated by summing a fraction of the converted current signal to the command potential and feeding it to the positive input of the operational amplifier. Series resistance compensation was used to minimize the time course of the capacitative surge. The capacitative transient remaining after series resistance compensation was constant throughout the experiments. The cell capacitance (C_m) was calculated from the equation

$$C_m = Q/V \quad (1)$$

TABLE 1. Action Potential Parameters in Atrial Myocytes Isolated From HF and Donor Hearts

	RP, mV	Overshoot	PI-A, mV	Notch, mV	APD_{50}, ms	APD_{90}, ms
HF, n=7 (16)						
Control	−51.9±8.8	14.8±9.7	8.7±3.6	2.7±4.1	185.4±16.5	371.0±3.1
ACh (1 μmol/L)	−52.4±8.1	15.5±9.8	8.0±3.9	2.9±4.9	153.7±14.2	320.5±20.8
Difference	0.4±0.3	0.6±0.4	0.7±2.9	0.2±0.9	80.9±11.4 (%)	83.7±12.5 (%)
Donor, n=4 (9)						
Control	−73.0±7.2†	17.5±6.4	16.8±5.0*	3.2±4.0	167.3±15.5	289.4±23.0†
ACh (1 μmol/L)	−77.4±6.1†	20.3±7.7	−8.1±4.8†	3.4±4.5	40.5±9.9†	112.6±14.5†
Difference	4.1±1.0†	2.8±0.7†	25.6±54.1†	0.2±0.6	24.8±5.6 (%)†	38.8±7.0 (%)†

HF indicates heart failure; RP, resting membrane potential; PI-A, action potential plateau amplitude; notch, depth of the notch; APD_{50} and APD_{90}, action potential duration measured at 50% and 90% repolarization, respectively; n, number of patients or donors; number in parentheses, number of cells; and ACh, acetylcholine. The percentage in APD_{50} and APD_{90} indicates the value in the presence of ACh relative to control. Values are mean±SD.

*$P<.05$, †$P<.001$ different from HF by Student's t test and two-way ANOVA.

where Q is total charge movement determined by integrating the area defined by the capacitative transient in response to +10 mV voltage step (holding potential of −40 mV). The mean cell capacitance was 84.5±11.6 pF (n=20) in human atrial myocytes. Although the averaged cell capacitance in atrial myocytes isolated from HF was generally greater than that from donors, the difference did not achieve statistical significance (84.8±11.4 pF, n=16 in HF and 83.9±8.1 pF, n=4 in donors). The sizes of myocytes under microscopic observation were also similar between the two groups. The output of the voltage-clamp amplifier was adjusted to give zero current when the tip of the patch pipette (filled with internal solution) was immersed in the bath containing control external solution. After access was gained in the whole-cell patch-clamp configuration, myocytes were allowed to "stabilize" electrophysiologically for 10 to 15 minutes before data were collected. The whole-cell membrane currents were filtered at 10 kHz with a two-pole active filter, digitized at a sampling rate of 40 kHz, and stored on the Winchester drive of an LSI 11/73 computer (Digital Equipment Corp) for subsequent analysis. Resting and action potentials were measured in the whole-cell current-clamp mode with an Axoclamp 2A amplifier (Axon Instruments, Inc).

The single-channel currents were monitored with a digital oscilloscope (7101A, Kikusui) recorded through an analog-to-digital converter and stored continuously on videotape with a PCM recording system (Unitrade, Toshiba). The recorded signals were reproduced and filtered off-line through an eight-pole low-pass Bessel filter (48 dB/octave, model 902-LPF, Frequency Devices, Inc) with a cut-off frequency of 2 kHz for I_{K1} and 5 kHz for $I_{K(ACh)}$. Data were digitized to 14-bit resolution at a sample rate of 10 kHz and stored on an LSI 11/73 computer. The data were then analyzed by use of algorithms developed locally and based on the half-amplitude threshold analysis developed by Colquhoun and Sigworth.[25] Channel transitions were calculated with an averaging technique for channel amplitudes. The measurements of single-channel transitions were collected into histograms to allow determination of single-channel kinetics. The mean dwell times were determined from the sum of exponential fits to the distribution of open and closed times recorded from patches with evidence of only one channel. When no double openings were seen, we determined that only one functional channel was present in a patch (by use of Equation 65 in Colquhoun and Hawkes[26]). Channel open probability (P_o) and dwell time histograms were derived from data recorded at HP=−60 mV in symmetrical external and internal K^+ concentrations (150 mmol/L K^+) to assess the channel gating kinetics at this potential, unless otherwise stated. Experiments were performed at room temperature (20°C to 22°C). For the action potential measurements, external solution was maintained at 37°C by use of a Peltier thermoelectrical device.

Data Analysis and Statistics

To achieve quantitative comparisons, mean values were calculated from multiple data obtained from one patient (heart) and used as one datum. We could not simply compare the data from different patient groups as a whole, because the variability of data for each patient results in a deviation from the mean value if the number of data are not balanced between patients. Thus, throughout this paper, n refers to the number of hearts, not cells, unless otherwise stated.

The results are expressed as mean±SD. Statistical analyses were performed with Student's t test or one-way ANOVA only when the data were suited for parametric tests as judged by normality and equal variance tests. When the data were not suitable for parametric tests, we used a Mann-Whitney rank-sum test (Wilcoxon rank-sum test) for data comparisons. To consider the interpatient and intrapatient variability, each data comparison was also evaluated with a two-way ANOVA. In addition, an ANCOVA was used to determine the influence of age and sex in each comparison, unless otherwise stated. A nonparametric procedure in STATISTICAL ANALYSIS SYSTEM (SAS Institute, Inc) on a NeXT computer (NeXT Computer, Inc) was used for these analyses. Results were considered to be significant when $P<.05$.

Results

Resting Membrane Potentials and Action Potential Response to ACh in Atrial Myocytes Isolated From HF and Donors

Freshly isolated Ca^{2+}-tolerant human atrial myocytes from both HF and donor atria appeared morphologically similar. As summarized in Table 1, resting membrane potentials in myocytes from HF atria were −51.1±9.7 mV (n=74 cells from 30 patients), while those in donors were −73.0±7.2 mV (n=18 cells from 4 donors, $P<.001$). When the resting membrane potential of an individual cell from HF was within −51.1±3.9 mV (evaluated by the confidence interval estimation, $\delta=\pm3.9$ mV with 99.9% reliability), we considered the cell "diseased." This voltage range of cell selection criteria in HF is similar to that reported previously in human diseased atria.[4,27,28]

Fig 1 illustrates typical action potentials and their response to ACh in atrial myocytes from HF and donor atria. In the absence of ACh, the rate of repolarization

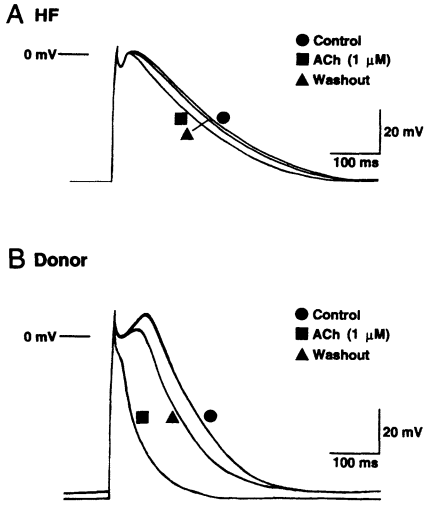

FIG 1. Effect of acetylcholine (ACh) on the action potentials recorded in atrial myocytes from heart failure (HF) and donor heart. A, Representative action potential and response to ACh in an isolated atrial myocyte from HF recorded in the whole-cell current-clamp configuration at a stimulation frequency of 1 Hz. After application of ACh (1 μmol/L) to the bath solution, shortening of the action potential occurred (to 84.8% of control measured at 90% repolarization) with a small hyperpolarization of the resting membrane potential (−1 mV, ■). The action potential recovered partially after 10 minutes of washout of ACh (▲). B, Representative action potential and response to ACh in an isolated atrial myocyte from donor heart recorded under the same conditions as A. The action potential shortened to 36.5% of control, and the resting membrane potential was hyperpolarized by −4 mV in response to ACh (■). These effects were partially reversed after 10 minutes of washout of ACh (▲).

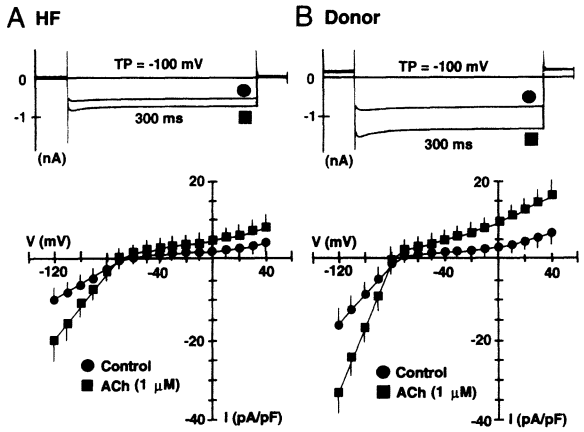

FIG 2. Effect of acetylcholine (ACh) on the whole-cell membrane current in atrial myocytes from heart failure (HF) and donor heart. A, top, Representative whole-cell membrane current and response to ACh (1 μmol/L) in an isolated atrial myocyte from HF recorded in the whole-cell voltage-clamp configuration. The membrane was voltage-clamped to −100 mV for 300 milliseconds from a holding potential of −40 mV (●). I_{Ca} was blocked by nifedipine (5 μmol/L), and I_{Na} was blocked by tetrodotoxin (10 μmol/L) in the bath solution. Application of ACh (1 μmol/L) to the bath solution slightly increased the inward current (■). Bottom, Averaged steady-state current-voltage (I-V) relations before (●) and during exposure to ACh in atrial myocytes from HF (n=7). Whole-cell current was increased by ACh, and reversal potential shifted to a more negative potential. B, top, Representative whole-cell membrane current and response to ACh in an isolated atrial myocyte from donor heart under the same conditions as A. Both the control current magnitude (●) and response to ACh (■) were greater than in HF. Bottom, Averaged steady-state I-V relations before and during exposure to ACh in atrial myocytes from donors (n=4). ACh increased membrane current and caused a greater hyperpolarizing shift in reversal potential than in HF. TP indicates test potential.

(phase 3) was slower than in the donor, so that the action potential duration at 90% of repolarization was longer in HF than in the donor. The action potential plateau amplitude, overshoot, and the depth of the notch (following the overshoot) were greater in donor than in HF. Bath application of ACh (1 μmol/L) caused a shortening of the action potential duration, shifted the plateau level to more negative potentials, and increased the resting membrane potentials in both groups. However, these responses to ACh in myocytes from HF were much less than those from donors. Table 1 summarizes the action potential parameters and their response to ACh in both groups. Characteristics of the action potential in control and in response to ACh from donors are similar to those reported previously for normal atria.[2,27-29] When a comparison was performed between transplant recipients and nontransplant patients within the HF group, the action potential parameters were not significantly different. The hyperpolarizing shift of the resting membrane potential and shortening of action potential durations at 50% and 90% repolarization by ACh were significantly reduced in myocytes from HF compared with donors ($P<.001$). These results indicate that the sensitivity of transmembrane potential characteristics to ACh is reduced in HF compared with donors.

Whole-Cell Membrane Current Response to ACh in Atrial Myocytes Isolated From HF and Donors

Since alterations in K^+ conductance could be responsible for the diminished resting potential and action potential response to ACh in HF compared with donors, the characteristics of whole-cell K^+ currents were studied. Fig 2 shows the whole-cell membrane current characteristics and response to ACh in atrial myocytes from HF and donors. Fig 2A and 2B (top panels) show examples of the response to a voltage step from a holding potential of −40 mV to −100 mV for 300 milliseconds in atrial myocytes from HF (Fig 2A) and donor (Fig 2B). Nifedipine (5 μmol/L) and tetrodotoxin (10 μmol/L) were included in the bath solution to block Ca^{2+} (I_{Ca}) and Na^+ current (I_{Na}). ACh (1 μmol/L) increased the inward current at −100 mV in both traces, but both the original current magnitude and the response to ACh were different in the two cells. The current offset at the holding potential was observed consistently in donors (n=4). Fig 2A and 2B (bottom panels) show the averaged steady-state current-voltage (I-V) relations before and during exposure to ACh (1 μmol/L) in atrial myocytes from HF (n=7) and donors (n=4). Before exposure to ACh (control), slope conductance of the membrane current at the reversal potential was 6.2±0.5 nS (n=7) in HF and 13.9±1.7 nS (n=4) in donors ($P<.001$). ACh (1 μmol/L) increased the conductance and shifted the reversal potential to more negative potentials in both groups: −2.4±0.7 mV (n=7) in HF and −5.5±0.9 mV (n=4) in donors ($P<.01$). However, the magnitude of membrane currents in HF was significantly smaller than in donors under control conditions. To compare the magnitude of

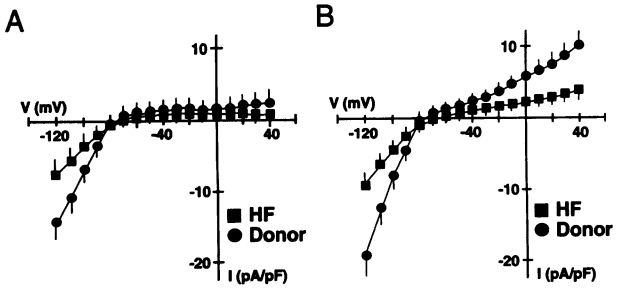

FIG 3. Whole-cell Ba^{2+}-difference (sensitive) and acetylcholine (ACh)-difference (sensitive) current in atrial myocytes from heart failure (HF) and donor heart. A, Averaged current-voltage (I-V) relations of the whole-cell Ba^{2+}-difference current in atrial myocytes from HF (■) and donors (●). Current was obtained by subtracting Ba^{2+} (1 mmol/L) -inhibited current from control current. Vertical bar through each point represents SD (n=3 for each). B, Averaged I-V relations of whole-cell ACh-sensitive current in atrial myocytes from HF (■) and donors (●). Current was obtained by subtracting control current from current during exposure to ACh (1 μmol/L). Vertical bar through each point represents SD (n=7 in HF and n=4 in donors).

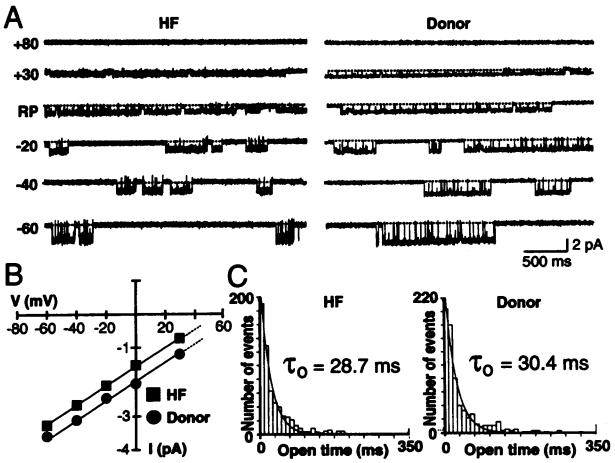

FIG 4. Characteristics of I_{K1} channel activity in atrial myocytes from heart failure (HF) and donor heart. A, Representative unitary I_{K1} currents in isolated atrial myocytes from HF and donor heart recorded under cell-attached patch conditions with 150 mmol/L K^+ in the pipette. Holding potential (HP) is expressed as voltage deviation from resting membrane potential (RP) and is indicated to the left of each current trace. Each patch contained only one channel whose openings occurred in bursts. Current recordings were low-pass filtered at 2 kHz. Dotted line running above trace indicates closed state (baseline level). B, Current-voltage relations obtained from same patches as A. Inward current showed a slope conductance of 28 pS in HF (■) and donor (●) heart. C, Histograms of open times from HF and donor in cell-attached patch recordings at HP=−60 mV. Lifetimes of openings were distributed according to a single exponential function in both cases.

the background I_{K1} between the two groups in more detail, Ba^{2+} (1 mmol/L) was introduced to the bath solution. Fig 3A illustrates the I-V relations of the Ba^{2+}-difference (sensitive) current obtained by subtracting the current in the absence of Ba^{2+} (control) from that in the presence of Ba^{2+} in both groups. The averaged slope conductance measured at the reversal potential was 6.5±0.7 nS (n=3) in HF and 14.2±1.9 nS (n=3) in donors. The slope conductance in donors was significantly greater than in HF ($P<.001$), indicating that the contribution of Ba^{2+}-sensitive K^+ conductances to the membrane current is smaller in HF than donors. In addition, the increase in whole-cell currents by ACh in HF was less than in donors. Fig 3B illustrates the I-V relations of the ACh-difference (sensitive) current obtained by subtracting the current in the presence of ACh (1 μmol/L) from control in both groups. The averaged slope conductance measured at the reversal potential was 7.1±0.7 nS (n=7) in HF and 13.7±2.0 nS (n=4) in donors. The value in donors was significantly greater than that in HF ($P<.001$). There were no significant interpatient differences associated with age and sex in both groups. These results are consistent with those obtained from the action potential measurements and indicate alterations in I_{K1} and $I_{K(ACh)}$ in HF.

Characteristics of I_{K1} Channels in Atrial Myocytes Isolated From HF and Donors

The characteristics of single atrial K^+ channels were also studied to gain further insight into the basis for the differences in whole-cell membrane currents between HF and donors and in the response to ACh.

Fig 4 shows the characteristics of unitary I_{K1} currents in isolated myocytes from HF and donor atria in cell-attached patches. I_{K1} channel activity occurred in long-lasting bursts of slow open-close events in myocytes from both groups (Fig 4A). Unitary amplitudes increased and the duration of individual open events decreased at more negative potentials. No outward currents could be detected at potentials positive to the reversal potential (estimated by zero current potential), indicating inward rectification. Channel activity was observed in 25.2% (29/115) of patches from HF atria compared with 38.7% (12/31) of patches from donor atria. The I-V relations for both groups were almost linear in the voltage range between resting potential (RP) −60 mV and RP +30 mV (Fig 4B). The estimated reversal potential was −55.0 mV in the cell from HF and −75.3 mV from the donor. The slope conductance was 28 pS in both cells.

The distributions of open times in both groups were well described by a single exponential function, indicating the presence of a single open state (Fig 4C). The mean open lifetime was 28.7 milliseconds in HF and 30.4 milliseconds in donor. Channel open probability (P_o) at HP=−60 mV was 39% in HF and 38% in donor. Table 2 summarizes the results. Channel characteristics were generally similar for the two groups (NS, not significant statistically) with the exception of the incidence (percentage of patches in which channels were found), indicating that individual single I_{K1} channel properties were unaffected by HF. These results suggest that reduced whole-cell membrane current magnitude in the absence of ACh in HF may be caused by a reduced number of functional I_{K1} channels.

Characteristics of Muscarinic K^+ Channels in Atrial Myocytes Isolated From HF and Donors

Fig 5 shows the unitary $I_{K(ACh)}$ currents in isolated atrial myocytes from HF and donor heart recorded in cell-attached patches with 1 μmol/L ACh in the pipette solution. The original current traces in Fig 5A show channel activity typical of the fast gating behavior of $I_{K(ACh)}$ with openings occurring in brief bursts superimposed on I_{K1} openings, which occur more slowly in

TABLE 2. Intergroup Comparisons of I_{K1} Channel Characteristics in Atrial Myocytes Isolated From HF and Donor Hearts

	n	γ, pS	n	τ_o, ms	P_o, %	Incidence, %
HF	23 (29)	28±2	18 (22)	27.5±4.8	40±5	25.2
Donor	4 (11)	28±2	4 (10)	29.9±4.6	41±4	38.7

HF indicates heart failure; n, number of patients or donors; number in parentheses, number of cells; γ, slope conductance; τ_o, time constant for open time distributions determined at HP=−60 mV; P_o, channel open probability measured at HP=−60 mV; and incidence, percentage of patches in which I_{K1} channels were found. Values are mean±SD. Conductance and gating parameters (γ, τ_o, and P_o) were not statistically different between the two groups (NS) by Student's *t* test. Because of the low density of I_{K1} channels, one patch (myocyte) with I_{K1} channel activity was obtained from a specimen in several cases in HF. Thus, in this comparison, comparisons were performed using the value from one cell (patient) or mean value from each patient, because neither two-way ANOVA nor ANCOVA was applicable. However, when data analysis was focused on the patients providing multiple cells, the statistical results using two-way ANOVA and ANCOVA were exactly the same as above.

long-lasting bursts. The opening of $I_{K(ACh)}$ channels occurred only when ACh was included in the pipette solution in both groups. The channel exhibited brief openings (flickering) during individual bursts. When atropine (1 μmol/L) was included in the pipette solution or myocytes were preincubated with pertussis toxin (5 μg/mL, see "Methods") and albumin, ACh failed to activate the channel even at a concentration of 20 μmol/L in the pipette in both groups (n=10 in HF, n=4

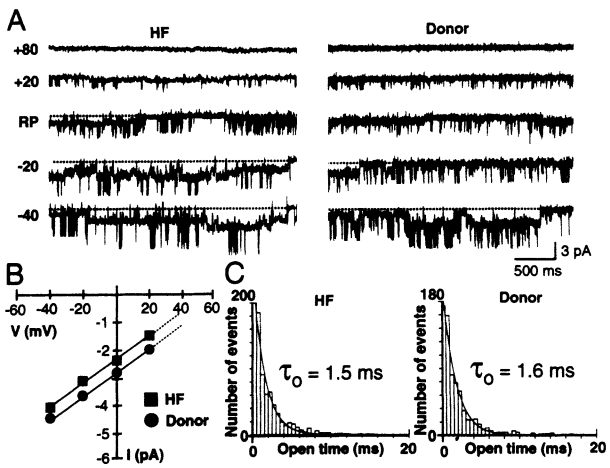

FIG 5. Muscarinic K^+ channel activity in atrial myocytes from heart failure (HF) and donor heart. A, Original single $I_{K(ACh)}$ channel recordings in atrial myocytes isolated from HF and donor heart recorded under cell-attached patch conditions with acetylcholine (ACh, 1 μmol/L) in the pipette solution. $I_{K(ACh)}$ activity is superimposed on I_{K1} activity, which displayed typical slow open-close events that occurred in long-lasting bursts with smaller unitary amplitude. In contrast, $I_{K(ACh)}$ exhibited brief openings (flickering) that occurred in short bursts. Outward currents were not detected at holding voltages more positive than RP +80 mV in both groups. Current recordings were low-pass filtered at 5 kHz. B, Current-voltage relations obtained from same patches. Single-channel slope conductance of $I_{K(ACh)}$ was 43 pS in both HF (■) and donor (●) heart. C, Open time histogram for $I_{K(ACh)}$ channels from HF and donor heart at HP=−60 mV. The lifetimes of openings were distributed according to a single exponential function in both groups.

TABLE 3. Intergroup Comparisons of $I_{K(ACh)}$ Channel Characteristics in Atrial Myocytes Isolated From HF and Donor Hearts

	n	γ, pS	n	τ_o, ms	P_o, %	Incidence, %
HF	28 (62)	42±3	24 (54)	1.3±0.3	4.0±1.2*	66.1
Donor	4 (20)	44±2	4 (16)	1.5±0.4	6.8±1.1	83.9

HF indicates heart failure; n, number of patients or donors; number in parentheses, number of cells; γ, slope conductance; τ_o, time constant for open time distributions determined at HP=−60 mV; P_o, channel open probability measured at HP=−60 mV; and incidence, percentage of patches in which $I_{K(ACh)}$ channels were found. Values are mean±SD. Conductance and the mean open lifetime (γ and τ_o) were not statistically different between the two groups (NS) by Student's *t* test and two-way ANOVA.

*$P<.01$ different from donor when comparison was made by Student's *t* test and two-way ANOVA.

in donors, data not shown). In contrast, myocyte incubation with albumin alone had no effect on channel activation (n=4). Similar results were obtained in atrial myocytes from both groups. Channel activity was observed in 66.1% (76/115) of patches from HF atria compared with 83.9% (26/31) of patches from donors. The inward I-V relations for both channels were almost linear (Fig 5B). Outward currents were not detected at holding voltages more positive than the reversal potential (estimated by zero current potential; −53.3 mV in the cell from HF and −68.7 mV from the donor). The single-channel conductance was 43 pS in both. Fig 5C shows representative histograms of single-channel open time distributions in HF and in donors. Open time distributions could be best fitted by a single exponential function in both cells. Mean open lifetime was 1.5 milliseconds in HF and 1.6 milliseconds in donor. P_o was 4.1% in HF and 6.6% in donor (at HP=−60 mV). Table 3 summarizes the gating parameters of both groups. No significant interpatient differences were detected in these comparisons. Although channel characteristics were similar in both groups, the number of functional channels (incidence) and P_o were lower in HF than in donors.

Fig 6 illustrates the dependence of single $I_{K(ACh)}$ channel activity on ACh concentration in myocytes isolated from HF and donor atria in cell-attached patches. Channel activity was estimated by NP_o, where N is the number of channels in the patch. The concentration-dependent activation of $I_{K(ACh)}$ was fitted by a least-squares method to the following form of the Hill equation:

$$(2) \qquad NP_o = V_{max}/[1+(K_D/[ACh])^H]$$

where V_{max} is the maximal NP_o value, K_D is the concentration of ACh at half-maximal channel activation, and H is the Hill coefficient. The best fit for the relation between the concentration of ACh and single-channel activity was obtained with values for the Hill coefficient of 1.7 for HF atria and 2.1 for donor atria; however, donor showed a higher sensitivity to ACh (K_D=0.03 μmol/L) than HF (K_D=0.11 μmol/L) as well as a greater maximal response to ACh. These results suggest that the reduced whole-cell membrane current response to ACh in HF may be caused by a reduced total number

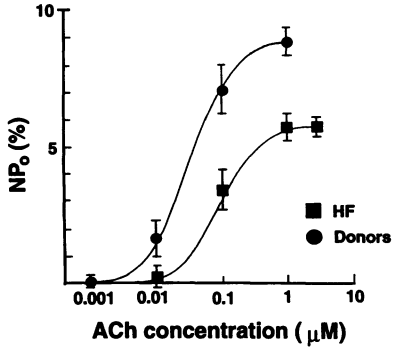

FIG 6. Concentration-dependent activation of muscarinic K^+ channels in atrial myocytes isolated from heart failure (HF) and donor heart in cell-attached patch recordings. Relation between the concentration of ACh in the pipette solution and channel NP_o (%) in HF (■) and donor (●) heart. ACh concentration at half-maximal channel activation was 0.11 μmol/L in HF and 0.03 μmol/L in donor heart. Vertical bars through each point represent SD from 3 to 6 patients (8 to 20 cells) with HF and from 3 or 4 donors (8 to 12 cells).

of functional channels (density) and/or reduced activity of individual $I_{K(ACh)}$ channels.

Coupling of G Proteins to Muscarinic K^+ Channels in Atrial Myocytes Isolated From HF and Donors

Recent studies using inside-out patch recordings showed that M_2 cholinergic receptor–linked G proteins (G_i) are involved in the activation of $I_{K(ACh)}$ in human atrial myocytes similar to other mammalian species.[15,16] G protein–mediated activation of atrial $I_{K(ACh)}$ was examined in myocytes isolated from both HF and donors by use of the excised inside-out patch configuration.

In the presence of ACh (1 μmol/L) in the pipette solution, $I_{K(ACh)}$ activity was observed in cell-attached patches in myocytes from HF and donor atria (Fig 7A).

TABLE 4. Intergroup Comparisons of Time Course of GTP-Dependent Activation and Deactivation of $I_{K(ACh)}$ in Atrial Myocytes Isolated from HF and Donor Hearts

	n	100	50	10
Half-activation time (s) in different concentrations of GTP (μmol/L)				
HF	11 (24)	94±10*	115±21*	140±27*
Donor	3 (8)	15±4	22±5	31±5
Half-decay time (s) in different concentrations of GTP (μmol/L)				
HF	10 (21)	14±4†	12±4†	9±2*
Donor	3 (8)	48±9	41±6	34±5

HF indicates heart failure; n, number of patients or donors; number in parentheses, number of cells.

*$P<.001$ compared with donor when comparisons were made by Student's t test and the two-way ANOVA; †$P<.001$ compared with donor by Mann-Whitney rank-sum test (Wilcoxon rank-sum test).

Channel activity disappeared immediately after formation of an excised inside-out patch (downward arrows). Application of GTP (100 μmol/L) to the bath solution (cytosolic side of membrane) restored channel activity in both patches. Single-channel slope conductances and mean open lifetimes of the GTP-activated channels were similar in the two groups (not shown). However, channel reactivation by GTP showed a much greater delay in the patch from HF compared with that from the donor (Table 4) evaluated by a method similar to that previously described by Okabe et al.[30] In addition, the channel open events were less frequent in myocytes from HF than from donors (see insets in Fig 7A). After washout of GTP from the bath, channel activity disappeared in both patches. In addition, the time to channel closure in HF was significantly less than that in donor (Table 4). Fig 7B illustrates a similar experiment in which the concentration of GTP was reduced to 10

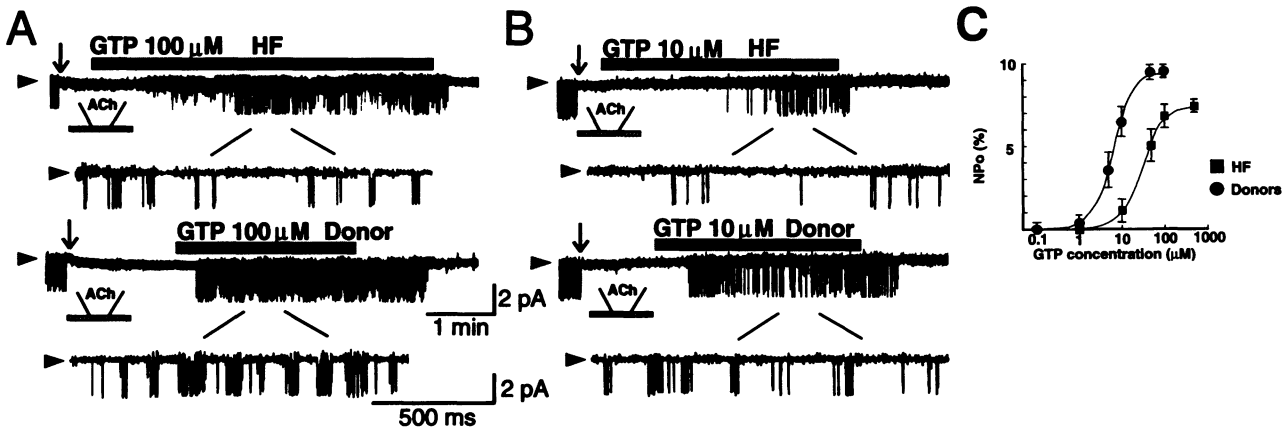

FIG 7. GTP-dependent activation of muscarinic K^+ channels in atrial myocytes isolated from HF and donor heart in excised inside-out patch recordings. A, Original current recordings in atrial myocytes from HF and donor heart at HP=−60 mV. Initially, channel activity was observed in the cell-attached patch configuration in the presence of ACh (1 μmol/L) at HP=−60 mV (150 mmol/L K^+ in both the pipette and bath). On formation of an excised inside-out patch (downward arrow), channel activity was abolished in both patches. Application of GTP (100 μmol/L) to the bath solution caused reactivation of the channel. Channel from HF activated with a significant delay after application of GTP compared with donor heart. In addition, channel activity in HF disappeared faster during washout of GTP than in donors. Arrowhead indicates baseline level (zero current, channel closed). B, Experiments similar to those in A using a lower concentration of GTP (10 μmol/L). Similar to A, the channel from HF activated with a significant delay after application of GTP compared with donor heart, and channel activity in HF disappeared faster during washout of GTP than in donors. C, Relation between concentration of GTP and channel NP_o (%) in HF (■) and donor (●) heart. GTP concentration at half-maximal channel activation was 29.8 μmol/L in HF and 6.6 μmol/L in donor heart. Vertical bars through each point represent SD from 3 to 7 patients (8 to 22 cells) with HF and from 3 or 4 donors (9 to 14 cells).

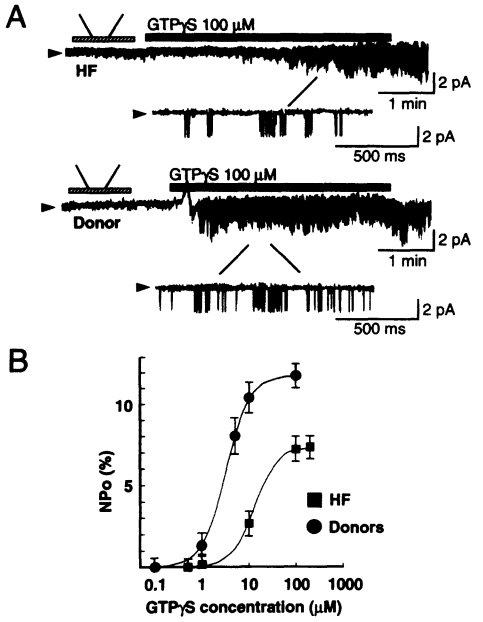

FIG 8. GTPγS-dependent activation of muscarinic K^+ channels in atrial myocytes isolated from heart failure (HF) and donor heart in excised inside-out patch recordings. A, Original current recordings of atrial myocytes isolated from HF and donor heart at HP=−60 mV under inside-out patch conditions. Acetylcholine (ACh) was absent from the pipette solution in both cases. No channel activity was detected in the initial segments of either trace. Application of GTPγS (100 μmol/L) to the bath solution caused the activation of channels in both cases in a manner similar to that which occurred with GTP. Channel activation by GTPγS in the patch from HF heart exhibited a significant delay compared with that from donor heart. B, Relation between concentration of GTPγS and channel NP_o (%) in HF (■) and donor (●) hearts. The GTPγS concentration at half-maximal channel activation was 13.1 μmol/L in HF and 3.25 μmol/L in donor hearts. Vertical bars through each point represent SD from 3 to 6 patients (8 to 19 cells) with HF and from 3 or 4 donors (9 to 13 cells).

μmol/L in both groups. Channel reactivation by GTP was significantly slower and the channel closure after washout of GTP was significantly faster in HF than in donors. These differences were observed at every concentration of GTP tested (Table 4). In addition, channel activity in HF was less than that in donor.

Fig 7C illustrates the dependence of single $I_{K(ACh)}$ channel activity on GTP concentration in myocytes isolated from HF and donor atria. Channel activity was estimated by NP_o. Overall average values of NP_o were calculated at concentrations of GTP ranging from 0.1 to 500 μmol/L in each group. The concentration-dependent activation of $I_{K(ACh)}$ was fitted by Equation 2. The relation between the concentration of GTP and single-channel activity fit with values for the Hill coefficient of 1.7 for HF atria and 1.8 for donor atria but showed a higher sensitivity to GTP in donor (K_D=6.6 μmol/L) than in HF (K_D=29.8 μmol/L) as well as a greater maximal response to GTP.

Fig 8A illustrates similar inside-out patch experiments using GTPγS instead of GTP with no ACh in the pipette solution. Although application of GTPγS (100 μmol/L) to the bath solution resulted in activation of $I_{K(ACh)}$ in both experiments, the patch from HF exhibited a delay in channel reactivation and less channel activity compared with donor heart. The half activation time by 100 μmol/L GTPγS in myocytes from HF was 231±26 seconds (n=8) compared with 37±8 seconds (n=3, P<.001) in donors. A difference in the delay of channel activation was also observed at different concentrations of GTPγS; the half activation time by 10 μmol/L GTPγS in myocytes from HF was 299±28 seconds (n=6) compared with 40±8 seconds (n=3, P<.001) in donors. In contrast to GTP, channel activity was irreversible following washout of GTPγS from the bath solution in both groups. Fig 8B illustrates the dependence of single $I_{K(ACh)}$ channel activity in HF and in donors on GTPγS concentration as estimated in a manner similar to GTP-dependent channel activation in the presence of ACh (Fig 7C). Values for the Hill coefficient were once again similar in HF (1.8) and in donor (1.8) atria. In addition, the relation between the concentration of GTPγS and single-channel activity showed that the apparent K_D in HF atria (13.1 μmol/L) was about fourfold greater than in donors (3.3 μmol/L) and that maximal NP_o was greater in donors than in HF. There were no significant interpatient differences in these comparisons. These results suggest that both sensitivity to and magnitude of G protein–mediated activation of $I_{K(ACh)}$ are diminished in HF compared with donors.

The GTP dependence of individual channel activity was directly compared between atrial myocytes isolated from HF and donor heart by estimating the P_o values for ACh- and GTP-activated channels. Fig 9 illustrates the dependence of the channel P_o on ACh in cell-attached patches and that on GTP in inside-out patches at HP=−40 mV. Throughout these experiments, 150 mmol/L K^+ external (bath) solution was used. P_o values of $I_{K(ACh)}$ were plotted versus time of single-channel recording in myocytes from HF and donor atria. In cell-attached patches, ACh (1 μmol/L) in the pipette activated the channel from HF with an overall averaged P_o value of 4.5±0.4% (n=10, Fig 9A). Upon formation of an excised inside-out patch, channel activity was completely abolished but reappeared after application of GTP (100 μmol/L) to the bath solution with a P_o value of 4.9±0.4% (n=10). Channel activity was abolished after washout of GTP from the bath solution. In myocytes from donor atria, the overall averaged P_o value in the cell-attached patch configuration was 6.7±0.6% (n=4) and the value of GTP-activated channels was 7.4±0.6% (n=4, Fig 9B). The averaged P_o values of both the ACh- and GTP-activated channels in HF were lower than those in donors (P<.001). In contrast, the level of ACh-activated channel activity was not significantly different from that of GTP-activated channel in both groups, suggesting that signal transduction between the M_2 cholinergic receptor and the G protein is not altered in HF.

Discussion

The major findings in this study are as follows: (1) Resting membrane potential in isolated atrial myocytes from HF heart was depolarized compared with donor. (2) The plateau amplitude and duration of the action potential in HF were significantly reduced compared with donors. Although ACh hyperpolarized the resting potential and shortened the action potential in HF, these effects were diminished compared with donors. (3) The whole-cell membrane current conductance was

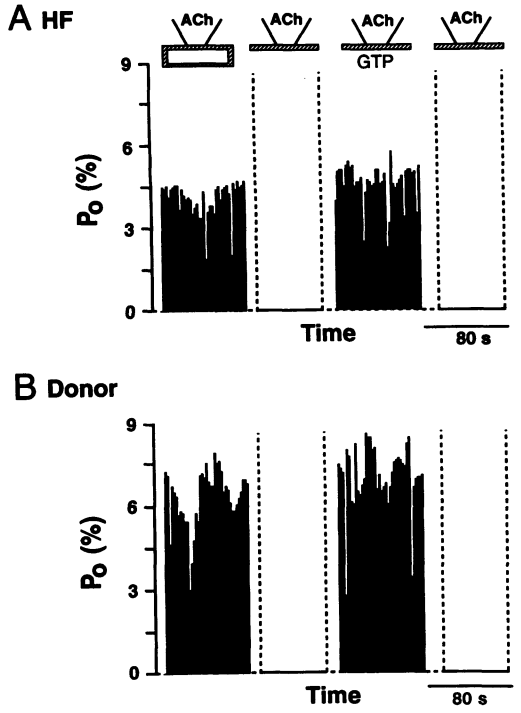

FIG 9. Dependence of $I_{K(ACh)}$ activity on ACh and GTP in cell-attached and inside-out patches in atrial myocytes from HF and donor heart. Channel activity was evaluated as P_o, which was then plotted versus time in cell-attached and in inside-out patches at HP=−40 mV. ACh (1 μmol/L) was included in the pipette solution, and measurements were made in 150 mmol/L K^+ external solution. P_o was measured every 2 seconds. Current recordings were low-pass filtered at 5 kHz. A, Data from an atrial myocyte isolated from HF. After formation of a cell-attached patch, channel activity appeared with a mean P_o value of 4.3% and was abolished after formation of the inside-out patch. Application of GTP (100 μmol/L) to the bath solution reactivated the channel with a mean P_o value of 4.9%. Channel activity disappeared after washout of GTP from the bath. B, Data from an atrial myocyte isolated from donor. P_o values were plotted as in A. Mean P_o value in the cell-attached mode was 6.5%, and that in the inside-out mode was 7.6%.

reduced in HF compared with donors. Also, the slope conductance of the ACh-difference current in HF was significantly less than that in donors. (4) Single I_{K1} channel characteristics in HF were similar to those in donors with the exception of a lower number of functional channels. (5) Single $I_{K(ACh)}$ channel activity and number of functional channels were lower and the channel sensitivity to ACh and G protein activation in HF was less than in donors. These results suggest that the low resting membrane potential and reduced electrophysiological response to ACh in atrial myocytes from HF may be caused by reduced density of I_{K1} and $I_{K(ACh)}$ channels as well as reduced $I_{K(ACh)}$ channel sensitivity to activation by ACh and G proteins.

Alteration in Resting Membrane Potential in Atrial Myocytes Isolated From HF Heart

A number of previous studies have demonstrated that human atrial specimens with low resting membrane potentials may be associated with in situ disease.[3,4,27,28,31-33] Atrial specimens obtained from primary nonatrial disease (ventricular septal defect, valvular heart disease, patent ductus arteriosus, or complex congenital heart disease) were also depolarized.[4,32,33] This may be the result of direct or indirect influences on the atrium by altered circulation and pressure and/or volume overload generated by ventricular or valvular heart disease. Using multicellular preparations, Hordof et al[34] showed that the decreased resting membrane potential is associated with atrial dilatation. Diastolic potential of atria obtained from patients with elevated right atrial pressure (≥5 mm Hg) and/or congestive heart failure was significantly depolarized compared with healthy subjects.[3] In addition, Mary-Rabine et al[4] demonstrated clear relations between atrial size, atrial pressure, cellular ultrastructural changes, and electrophysiological changes; specimens from atria with elevated pressure and/or dilatation exhibited low resting membrane potentials, alterations in the action potential, and degenerative pathological changes. Isolated myocytes from diseased atria exhibited low resting membrane potential similar to that in multicellular preparations.[14] We also found that resting potentials in atrial myocytes isolated from HF patients with elevated pressure and dilatation were depolarized compared with donors. In fact, our results are strikingly similar to those reported by Ten Eick and Singer,[3] who used multicellular specimens from diseased (−56.3±8.2 mV) and normal (−74±2.4 mV) atrial tissues.

It is unlikely that atrial myocytes isolated from HF were damaged or adversely affected by the isolation procedure or experimental conditions because of the following observations: (1) Isolated myocytes from both HF and donor atria appeared morphologically similar. They were Ca^{2+}-tolerant in normal Tyrode's solution and rod-shaped with clear striations without any blebs or granulations. (2) They generated action potentials in current-clamp mode. (3) ATP-sensitive K^+ channel activity never appeared during cell-attached patch recordings, but it appeared after the formation of excised patches in ATP-free solution in both groups (data not shown), suggesting maintained metabolic function after cell isolation. (4) Although the isolation procedure was identical between HF and donors, resting membrane potentials in myocytes from donor atria were not depolarized and were similar to the value reported previously in normal atria.[4,27,28] (5) In addition, broad study of I_{Na} in our laboratory using myocytes isolated from the same patients as in the present study demonstrated that numerous characteristics of I_{Na} were unchanged from 137 human atrial specimens.[35] This result also suggests that I_{Na} may not be a major target for modification by HF.

Although a variety of studies have addressed the origin of the low resting membrane potential in diseased human atrium,[2-4] its underlying mechanism remains unclear. However, several recent studies from our laboratory and from others have provided some clues about alterations in atrial membrane function with disease. In diseased atrium, (1) the electrogenic Na^+ pump demonstrated nearly normal function[36]; (2) low intracellular K^+ activity (measured with K^+ sensitive microelectrodes) may not be responsible for the low resting potential, which indicates that depolarization was the result of changes in ionic conductance[33]; and (3) depolarized atrial myocytes did not repolarize during exposure to 0 mmol/L Na^+, tetrodotoxin, or Ca^{2+} channel blockers, indicating that the transmembrane

Na^+ permeability (P_{Na}) is not exclusively responsible for membrane depolarization[3] (this finding also indicates that the depolarization is not caused by Na^+ ions moving through Ca^{2+} channels); and (4) I_{Na}, I_{Ca}, or Cl^- current (I_{Cl}) were not responsible for low resting membrane potential.[33] I_{Na} and I_{Ca} measured by whole-cell voltage-clamp techniques were nearly identical to other mammalian species and were unchanged in different preexisting disease states.[21,37] In contrast, other reports demonstrated that increases in permeability to ions such as Na^+ as well as reduced Na^+ pump activity also contribute to the low resting potential in diseased atria.[32,38] Another recent report demonstrated that the transient outward current is altered in myocytes isolated from dilated and diseased atria.[17]

The present study provides direct measurements of whole-cell membrane currents as well as single I_{K1} and $I_{K(ACh)}$ channel behavior. Our results suggest that low resting membrane potential may be caused at least partially by the diminished K^+ conductance associated with altered I_{K1} and $I_{K(ACh)}$, which is consistent with the notion that the absolute level of K^+ conductance is reduced in depolarized myocytes from diseased atrium.[3] However, failure to restore normal resting potential despite an increase in K^+ conductance by ACh in HF suggests that transmembrane ion conductances other than K^+ may also contribute to the low resting membrane potential in HF.

Characteristics of I_{K1} and Muscarinic K^+ Channels in Atrial Myocytes Isolated From HF Heart

Single I_{K1} and $I_{K(ACh)}$ channels in myocytes isolated from HF exhibited individual channel behavior similar to that in myocytes isolated from donor heart, with the exception of a lower P_o value of $I_{K(ACh)}$ channels in HF. The channel gating characteristics were not different from those previously reported in human atrium[15,16] and in other mammalian species.[9,13,39-41]

One of our most important observations is that the channel density of both I_{K1} and $I_{K(ACh)}$ in HF was lower than in donor heart. The lower channel incidence in single-channel recordings was consistent with a reduced whole-cell current density in HF. Previous studies suggested that the density of $I_{K(ACh)}$ in atrial myocytes isolated from diseased human hearts was lower than in other species.[15,16] However, it was unclear whether or not this difference was due simply to a species difference or to the influence of disease. In the present study, the whole-cell ACh-sensitive current magnitude in HF was significantly less than in donors, and single $I_{K(ACh)}$ channel incidence and P_o were lower than in donors. These results suggest that reduced atrial $I_{K(ACh)}$ channel density in HF may be associated with heart disease and not due to species differences.

The loss of M_2 cholinergic receptor (downregulation) may be, in part, responsible for the reduced channel density. A reduction in the density of muscarinic receptors and an alteration in agonist binding have been demonstrated in a canine model of heart failure.[42] However, aside from the possible downregulation of M_2 receptors, the major alteration in individual $I_{K(ACh)}$ activity in HF was the reduced channel sensitivity to GTP and GTPγS. Excised inside-out patch experiments with GTP and GTPγS allowed the direct comparisons of M_2 receptor–linked G protein (G_i) coupling of the channel between the two groups and excluded possible influences of receptor downregulation, agonist-receptor binding affinity, and signal transduction from receptor to G protein. The delay in channel activation, higher apparent K_D value for channel activation by GTP and GTPγS, and low NP_o value in HF may be caused by a diminished sensitivity to G_i stimulation in HF. In addition, the fast decay of $I_{K(ACh)}$ channel activity on washout of GTP from the bath in HF suggests that the intrinsic GTPase activity may be enhanced in HF. An enhanced GTPase activity would cause an apparent decrease in sensitivity of the channel to GTP.

These results suggest that the relation between G_i and the channel, but not channel gating behavior, may be altered in HF. Recent studies have shown evidence of alterations in endogenous $G_{i\alpha}$ concentrations associated with disease states in animal models.[43-45] Reduced amounts of $G_{i\alpha}$ are associated with chronic ischemia in the porcine heart.[45] In humans, increased cardiac $G_{i\alpha}$ was reported in idiopathic dilated cardiomyopathy heart but not in ischemic cardiomyopathy.[46-48] Although changes in the amount of G_i present in diseased human heart are still uncertain, the affinity of G_i for binding to intrinsic GTP may also be reduced. Our results suggest that it is unlikely that signaling between M_2 receptors and G_i is disturbed. Hence, reduced $I_{K(ACh)}$ channel activation in HF may be caused by downregulation of M_2 cholinergic receptors, the reduced function of G_i with altered GTPase activity, and/or an impaired signal transduction pathway between G_i and the channel.

Limitations and Clinical Implications

One limitation of this study was the small number of donor hearts evaluated. The availability of healthy donor hearts is usually limited in in vitro studies of human heart. However, our statistical treatment using the mean values from multiple data for each heart, the two-way ANOVA, and ANCOVA (see "Methods") effectively eliminated the statistical errors generated by using a different sample size for each heart. We found that the electrophysiological properties of myocytes obtained from donor hearts as well as HF were quite homogeneous within each group. Thus, there were no significant interpatient differences associated with age, sex, and other factors in the present study, and HF was the only factor that influenced the intergroup comparison.

The reduced sensitivity of $I_{K(ACh)}$ to ACh in HF compared with donors suggests the potential importance of parasympathetic activity in electrophysiological properties of human heart. Withdrawal of parasympathetic tone is reported in patients with congestive heart failure and a pacing-induced canine model of congestive heart failure.[49-51] Because enhanced sympathetic tone is recognized in patients with heart failure,[52-54] reduced response to ACh may influence autonomic control of the failing human heart. Study of electrophysiological responses to parasympathetic stimulation under in vivo conditions may provide further information about the changes in response to ACh in HF. Our results suggest that further study of the influence of HF and other cardiac disease on intracellular signaling is likely to provide important insights into alterations in ion channel function that may serve as the basis for altered electrophysiological properties in heart disease.

Acknowledgments

This work was supported by grants-in-aid from the National Institutes of Health (Heart, Lung, and Blood Institute, HL-30724 to Dr Wasserstrom) and by the Brinton, Reingold, and Deborah M. Cooley Trusts. The authors wish to thank Drs Donghee Kim, James E. Rosenthal, Michael F. Sheets, Ana-Maria Vites, Robert G. Tsushima, and Robert E. Ten Eick for their thoughtful discussion and comments on this work and Sundee L. Eager, Jerry S. Weiss, Feng Zhang, and Lonnie C. Harger for technical advice and assistance and Shelly D. Golden for editorial support.

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