β-Adrenergic Modulation of the Inwardly Rectifying Potassium Channel in Isolated Human Ventricular Myocytes

Alteration in Channel Response to β-Adrenergic Stimulation in Failing Human Hearts

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Abstract

The β-adrenergic modulation of the inwardly-rectifying K^+ channel (I_{K1}) was examined in isolated human ventricular myocytes using patch-clamp techniques. Isoproterenol (ISO) reversibly depolarized the resting membrane potential and prolonged the action potential duration. Under the whole-cell Cl^--free condition, ISO applied via the bath solution reversibly inhibited macroscopic I_{K1}. The reversal potential of the ISO-sensitive current was shifted by ~ 60 mV per 10-fold change in the external K^+ concentration and was sensitive to Ba^{2+}. The ISO-induced inhibition of I_{K1} was mimicked by forskolin and dibutyryl cAMP, and was prevented by including a cAMP-dependent protein kinase (PKA) inhibitor (PKI) in the pipette solution. In single-channel recordings from cell-attached patches, bath applied ISO could suppress I_{K1} channels by decreasing open state probability. Bath application of the purified catalytic subunit of PKA to inside-out patches also inhibited I_{K1} and the inhibition could be antagonized by alkaline phosphatase. When β-adrenergic modulation of I_{K1} was compared between ventricular myocytes isolated from the failing and the nonfailing heart, channel response to ISO and PKA was significantly reduced in myocytes from the failing heart. Although ISO inhibited I_{K1} in a concentration-dependent fashion in both groups, a half-maximal concentration was greater in failing (0.12 μM) than in nonfailing hearts (0.023 μM). These results suggest that I_{K1} in human ventricular myocytes can be inhibited by a PKA-mediated phosphorylation and the modulation is significantly reduced in ventricular myocytes from the failing heart compared to the nonfailing heart. (*J. Clin. Invest.* 1995. 96:2870–2881.) Key words: human ventricular myocytes • inwardly rectifying K^+ channel • isoproterenol • β receptor • phosphorylation

Introduction

β-Adrenergic stimulation can produce positive inotropic and chronotropic effects in the heart and adrenergically mediated effects on cardiac action potentials have been studied electrophysiologically for many years. The effects on cardiac action potentials are quite numerous and are modulated by many other factors (1). In mammalian heart, as the result of a multiplicity of actions on various ionic currents, isoproterenol (ISO)[1] can increase action potential duration and reduce the resting membrane potential (2). However, after inhibition of the Ca^{2+} current by nisoldipine, ISO can shorten the action potential duration by activating the Cl^- current (I_{Cl}) but still can depolarize the resting membrane potential (3). This depolarization could result from either activation of an inward I_{Cl} or inhibition of the inwardly rectifying K^+ channel (I_{K1}), or both.

Recently, majority of ion channels in human heart were characterized in this and other laboratories (4–10). In contrast to the many reports concerned with ionic current systems in human heart, little is known about the effects of adrenergic agonists on human cardiac ion channels. Because of the lack of information, the present study was undertaken to determine if β-adrenergic receptors have a role in the regulation of ventricular I_{K1} channels and, if so, to investigate the nature of the mechanisms underlying both the β-adrenergically mediated regulation of these channels. In addition, influence of preexisting heart disease on β-adrenergic modulation was investigated by comparing channel characteristics between ventricular myocytes isolated from patients with congestive heart failure (the failing heart) and from normal subjects (the nonfailing heart).

Methods

Human cardiac specimens. Adult human ventricular specimens were obtained from patients undergoing cardiac surgery and from explanted donor hearts. Institutional and National Institutes of Health guidelines for human experimentation were followed in obtaining surgical specimens and written informed consent was obtained from all subjects. A total of 16 patients without heart failure and four donor hearts that could not be transplanted for technical reasons were studied as normal subjects (29–54 yr; median age 42 yr). Nine patients and two donors were male. All patients had no symptomatic heart failure. The averaged ejection fraction was 65±4% (n = 16) and cardiac index was 3.9±1.0 liter/min per m^2 (n = 16). In addition to these normal subjects (nonfailing hearts), adult human ventricular specimens were also obtained from patients with congestive heart failure. A total of eight patients were

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1. *Abbreviations used in this paper:* 4-AP, 4-aminopyridine; APase, alkaline phosphatase; APD_{90}, the action potential duration at 90% repolarization; C-subunit, catalytic subunit; HF, the failing heart; I–V, current–voltage; I_{K1}, the inwardly rectifying K^+ channel; ISO, isoproterenol; NF, the nonfailing heart; N, channel number; P_o, open probability; PKA, cAMP-dependent protein kinase; PKI, cAMP-dependent protein kinase inhibitor; P_O, open probability.

Table I. A Clinical Profile on Each of Patients Studied

Patient No.	Gender	Age	Disease state	EF	CI
			Nonheart failure patients		
1	M	51	CAD	64	4.0
2	M	44	CAD	61	3.2
3	F	44	CAD	59	3.1
4	M	49	CAD	67	4.9
5	M	38	CAD and VHD	59	3.0
6	F	40	CAD	66	4.2
7	M	45	CAD	67	4.9
8	F	54	CAD	58	3.0
9	F	29	VHD	66	4.4
10	M	46	CAD	64	3.1
11	M	47	CAD	66	4.2
12	F	31	CAD and VHD	68	4.6
13	M	43	CAD	69	4.9
14	F	47	CAD	68	4.7
15	F	30	VHD	64	3.1
16	M	41	CAD	67	3.7
Mean±SD		42.4±7.3		64.6±3.5	3.94±0.9
			Heart failure patients		
1	M	47	CAD	19	1.9
2	F	51	CAD	22	2.0
3	M	42	CAD and VHD	25	2.2
4	F	38	DCM	14	1.5
5	M	44	CAD	22	2.6
6	M	34	DCM	16	1.8
7	M	54	CAD	25	2.3
8	F	61	CAD	28	2.2
Mean±SD		46.4±8.8		21.4±4.8	2.06±0.3

EF, ejection fraction (%); CI, cardiac index (liter/min per m^2); CAD, coronary artery disease; DCM, dilated cardiomyopathy; VHD, valvular heart disease.

studied as heart failure group (34–61 yr; median age 46 yr). Five patients were male. All patients in this group had class IV heart failure. The averaged ejection fraction was 21±5% (n = 8) and cardiac index was 2.1±0.3 liter/min per m^2 (n = 8). Table I summarizes the patient population characteristics. The administration of cardiac drugs was stopped 48 h before surgery. Immediately after surgical explantation, the specimen was placed in a chilled nutrient solution and carried to the laboratory within 1 hr.

Isolation of ventricular myocytes. Human ventricular myocytes were isolated by an enzymatic dissociation method. The isolation was accomplished with a Langendorff-type apparatus for coronary artery perfusion. If the available coronary artery was not suitable for cannulation, the tissue was perfused using a method we have described previously (9) that uses a hypodermic needle to infiltrate the tissues. Specimens were obtained from left ventricular free wall or intraventricular septum. Ventricular specimens were trimmed to ~ 1 cm^3 using fine scissors, then infiltrated with Ca^{2+}-free Tyrode's solution via a 25 gauge surgical needle. 20 ml perfusion with Ca^{2+}-free Tyrode's solution (1 ml/min) was followed by perfusion with Ca^{2+}-free Tyrode's solution containing 125 U/ml collagenase (Type V; Sigma Chemical Co., St. Louis, MO) and 1 mg/ml of bovine serum albumin (Sigma Chemical Co.) for 20–30 min (1 ml/min). The perfusate was bubbled with 100% O_2 and warmed to 37°C. The specimen was minced with fine scissors in the same enzyme solution. Isolated cells were separated from the minced tissue by gravity filtration through 200 μm nylon mesh and were stored in a modified Kraftbrühe (KB) solution (11) at room temperature. Only Ca^{2+}-tolerant, clearly striated, rod-shaped cells without any blebs were studied. All experiments were performed within 4 h after cell isolation. Three to nine cells were examined from each patients. When the resting membrane potential of an isolated myocyte was < −60.2 mV (evaluated by the confidence interval estimation, δ = ±3.8 mV with 99.9% reliability), it was discarded. Sufficient action potential recordings and stable whole cell recordings could not be achieved in such cells.

Solutions and chemicals. The nutrient solution for human specimens contained (mM): NaCl 27, KCl 20, $MgCl_2$ 1.5, Hepes 5, glucose 274 (pH = 7.0). The Tyrode's solution contained (mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, Hepes 5, glucose 5 (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 (mM): 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 recording was (mM): K-aspartate 120, KCl 20, KH_2PO_4 1, $MgCl_2$ 6, Na_2-ATP 5, EGTA 5, Hepes 5 (pH = 7.2). Cl^--free external solution used for whole cell recordings contained (mM): Na glutamate 140, K glutamate 5.4, $CaSO_4$ 1.8, $MgSO_4$ 0.5, Hepes 5, glucose 10 (pH = 7.4). Pipette solution used for whole cell recordings contained (mM): K glutamate 140, KH_2PO_4 1, $MgSO_4$ 6, Na_2-ATP 5, EGTA 5 and Hepes 5 (pH = 7.2 with KOH). Internal-free Ca^{2+} concentration ($[Ca^{2+}]$) was calculated to be ~ 10^{-9} by Fabiato and Fabiato (12). In several experiments, free Ca^{2+} concentration was adjusted to 0.1 μM by adding $CaCl_2$ (12). Pipette and bath solutions for cell-attached patches and pipette solution for inside-out patch recordings contained (mM): KCl 150, Hepes 5.0 (pH = 7.4). In excised inside-out patch recordings, the cytosolic surface of the membrane was perfused with a bath solution containing (mM): K aspartate 120, KCl 30, Na_2-ATP 5.0, $MgCl_2$ 6.0, Hepes 5.0, and EGTA 5.0 (pH = 7.2).

The experimental chamber (0.2 ml) was continuously perfused with bath solution at a rate of 6–7 ml/min, and complete solution exchange was achieved within 5.0±1.2 s (n = 16) as determined by measuring the current change induced by changing the solution from 5.4 mM K^+ solution to 150 mM K^+ solution.

ISO (0.001–10 μM), propranolol (1 μM), forskolin dissolved in ethanol (5–10 μM), dibutyryl cAMP (1–5 mM), tetrodotoxin (TTX, 5 μM), $CoCl_2$ (2 mM), dihydroouabain (DHO, 10 μM) and 4-aminopyridine (4-AP, 2 mM) were added to the external solution. The synthetic peptide inhibitor of PKA (PKI), 5–24 amide, was prepared as described by Cheng et al. (13) and added to the pipette solution during whole cell recordings at a concentration of 50 μM. Alkaline phosphatase (APase, Boehringer Mannheim Biochemicals, Indianapolis, IN) was dialyzed against the perfusion buffer for 8 h at 0°C. All chemicals were obtained from Sigma Chemical Co.

Electrical recording. Patch-clamp recordings of whole cell and single channel currents were performed using cell-attached and excised inside-out patch configurations with an Axopatch-1C amplifier (Axon Instruments, Inc., Foster City, CA) (14–16). The pipettes were pulled in two stages from hard glass tubing (Narishige Scientific Instruments Laboratories, Tokyo, Japan) using a vertical microelectrode puller (Type PE-2; Narishige Scientific Instruments Laboratories) and then fire polished using a microforge (model MF-83; Narishige Scientific Instruments Laboratories). Electrodes had a resistances of 2–3 MΩ for action potential and whole cell recordings and 6–8 MΩ for single channel recording. The feedback resistance of the head stage was 500 MΩ for recording whole cell current and 50 GΩ for recording single channel current. The output of the voltage clamp amplifier was adjusted to give zero current when the tip of the pipette (filled with internal solution) was immersed in the bath containing control external solution.

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. Series resistance compensation was used to minimize the time course of the capacitative surge. The capacitative transient remaining after series resistance com-

pensation was constant throughout the experiments. Ramp voltage clamp pulses were applied from a holding potential of −120 to +40 mV at a rate of 100 mV/s. The current–voltage (I–V) relationships were obtained during ramps. Action potentials were measured in the whole cell current clamp mode.

Single channel currents were monitored with a storage oscilloscope (Type 5113; Tektronix, Inc., Beaverton, OR) and were stored continuously on digital audio tape (R-60DM, Maxell, Tokyo, Japan) using a PCM data recording system (RD-100T, TEAC, Tokyo, Japan). The single channel events were reproduced and filtered off-line with a cutoff frequency of 2 kHz through an eight-pole low-pass Bessel filter (48 dB/octave, model 902-LPF; Frequency Devices, Inc., Haverhill, MA), digitized with 14-bit resolution at a sample rate of 10 kHz. The data were analyzed on a computer (PC-9801; NEC, Tokyo, Japan) using locally written analysis programs that are based on the half-amplitude threshold analysis method of Colquhoun and Sigworth (17) for channel transitions. The measurements derived from the channel transitions were collected into histograms to allow an analysis of the single channel kinetics. Mean dwell times were determined from the sum of exponential fits to the distributions of open and closed times recorded from patches with only one channel. When no double openings were seen, we determined that only one functional channel was present in a patch according to the equation by Colquhoun and Hawkes (18). When we can get sufficiently long (~ 20 min) continuous recording, active channel number (N) in the patch can be predicted by using the equation:

$$P \approx \exp[\{-T(N - 1/N)m_o\}/m_s^2] \quad (1)$$

where T is the length of the observed record, m_o is the mean open lifetime, and m_s is the mean closed time. It is considered that the patch has only one functional channel when the predicted probability achieves 99.9%. When multiple channel activities are observed in a patch (more than two channels open simultaneously), we used the "averaging technique" to obtain the channel open probability using the equation:

$$I = NP_o i \quad (2)$$

where I is the averaged total channel amplitude, N is the channel number, P_o is the channel open state probability and i is the unitary amplitude of the channel. The unitary amplitude (i) can be obtained by directly measuring the individual channel magnitude. The averaged total channel amplitude (I) can be obtained by calculating the averaged level of channel open events. By using these values, P_o can be obtained from Eq. 2, when sufficiently long recording was achieved. P_o was determined and dwell time histograms were derived from data recorded at holding potential (HP) = −60 mV in symmetrical K^+ concentrations (150 mM K^+) external and internal to assess the channel gating kinetics at this potential. To avoid the effects of desensitization, experiments were performed in this period. All experiments were performed at 37±0.5°C using a Peltier thermo-electrical device.

Data analysis and statistics. The concentration-response curve was fitted by the least-squares method to the Hill equation:

$$y = P_{max}/\{1 + (IC_{50}/[M])^H\} \quad (3)$$

where P_{max} is the maximal effect, IC_{50} is the concentration at the half-maximal effect, and H is the Hill coefficient.

The results are expressed as mean±SD. Statistical analyses were performed using 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, a Mann-Whitney rank sum test (Wilcoxon rank-sum test) or a Kruskal-Wallis analysis of variance on ranks were used. Differences in the sample sizes from different patient and the variability in the data for each patient could generate the bias that could occur. To consider these inter- and intrapatient variabilities, each data comparison was also evaluated using a two-way ANOVA. In addition, an analysis of covariance was used to determine the influence of age and gender in each comparison, unless otherwise stated. A nonparametric procedure in SIGMASTAT version 1.00 was used for these analyses. Results were considered to be significant when $P < 0.05$.

Results

Action potential response to ISO in isolated human ventricular myocytes. Fig. 1 *A* shows representative action potentials recorded from an isolated human ventricular myocyte obtained in the absence (*circle*) and presence (*square*) of ISO (1 μM) in the external solution and after washout of ISO (*triangle*) recorded in the whole cell current clamp mode at physiological temperature (37°C). Bath exposure to ISO caused prolongation of the action potential with a small depolarization of the resting membrane potential. In addition, the rate of repolarization (phase 3) during exposure to ISO became slower than in the control, so that the prolongation of the action potential duration at 90% of repolarization (APD_{90}) was greater than at 50% repolarization (APD_{50}). APD_{90} was 394.6±32.4 ms (n = 12) in the control and 520.7±39.1 ms (n = 12, $P < 0.01$) during exposure to ISO. APD_{50} was 292.0±21.8 ms (n = 12) in the control and 372.5±24.4 ms (n = 12, $P < 0.01$) during exposure to ISO. The prolongation of APD_{90} by ISO was 129.9±11.5% (n = 12) of control ($P < 0.01$). Fig. 1 *B* shows the plot of APD_{90} change versus time of the ISO exposure from 11 different cells. The action potential lengthening occurred during exposure to ISO, which recovered almost to the control level after washout of ISO. The resting membrane potential was depolarized with 5.2±1.7 mV (n = 10). Desensitization was not observed during these experimental period (2-min exposure of ISO).

ISO-induced inhibition of whole cell I_{K1}. Fig. 2 shows the effect of ISO (1 μM) on whole cell I_{K1} in human ventricular myocytes. This concentration of ISO was saturating when analyzed for its effect on I_{K1}. Fig. 2 *A* shows the whole cell current–voltage (I–V) relationships obtained in the control, during superfusion and after washout of ISO at 37°C. The ISO-induced Cl^- conductance was blocked by omitting Cl^- from internal and external solutions. Tetrodotoxin (TTX, 5 μM), Co^{2+} (2 mM), dihydroouabain (DHO, 10 μM) and 4-AP (2 mM) were added to the external solution. In this condition, bath application of ISO reversibly inhibited whole-cell I_{K1}. The averaged slope conductance at the reversal potential was 70.5±6.6 nS (n = 10) in control, 4.1±0.5 nS (n = 10) during 3-min exposure to ISO and 69.2±7.3 nS (n = 10) after washout of ISO. Slope conductance during exposure to ISO was significantly different from control and after washout of ISO ($P < 0.001$). To confirm that inhibition of I_{K1} by ISO is mediated by stimulation of β-adrenergic receptors, the effect of ISO on I_{K1} was also characterized in the presence of the β-blocker, propranolol (Fig. 2 *B*). Bath application of 1 μM ISO inhibited I_{K1} similar to panel A. In contrast, after exposure to the cell with the bath solution containing 1 μM propranolol and 1 μM ISO, the current magnitude recovered to almost the control level and the I–V curves obtained in the presence and absence of ISO were superimposed (Fig. 2 *B*). The averaged slope conductance was 70.1±6.8 nS (n = 8) in control, 4.7±0.8 nS (n = 7) during exposure to ISO alone, and 69.4±7.1 nS (n = 7) during exposure to ISO and propranolol (NS). Propranolol may displace the β-agonist from its receptor site and reverse the effect on I_{K1}. These results suggest that ISO can inhibit I_{K1} via β-adrenergic receptor stimulation. Desensitization was not observed during these experimental period (3-min exposure of ISO).

The ISO-induced inhibition of I_{K1} was further assessed by

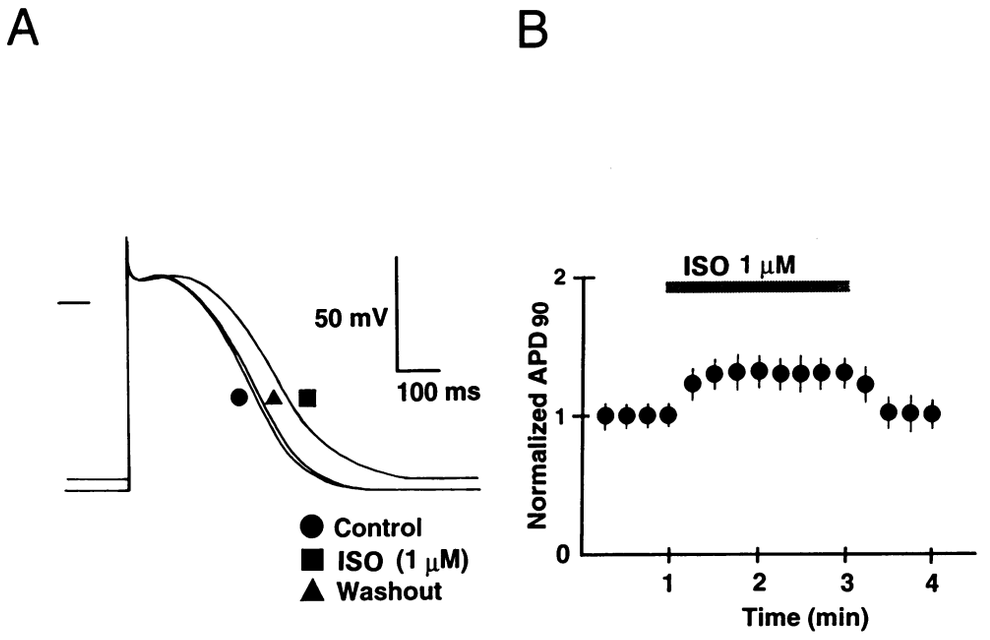

Figure 1. Effect of ISO on action potentials in isolated human ventricular myocytes. (*A*) Representative action potential and the response to ISO on an isolated human ventricular myocyte recorded under the whole cell current clamp configuration. After exposure to ISO (1 μM), prolongation of the action potential occurred with slight depolarization of the resting membrane potential. After 1 min exposure to ISO, the action potential duration prolonged to 134.1% of control measured at 90% repolarization (APD_{90}) and the resting membrane potential was depolarized (5 mV, ■). The action potential recovered partially after washout of ISO from the bath (▲). The horizontal bar indicates 0 mV. Pipette resistance is 2 MΩ. (*B*) Changes in normalized APD_{90} were plotted versus time in isolated ventricular myocytes. APD_{90} increased during exposure to ISO which was reversed following washout of ISO. The vertical bar through each point represents the standard deviation from 12 cells. The normalized values were significantly greater during exposure to ISO than in control ($P < 0.01$).

characterizing the ISO-sensitive differential currents obtained by subtracting the currents in the presence of ISO from the corresponding currents in the absence of ISO. Fig. 3 *A* illustrates ISO-sensitive currents recorded in myocytes bathed in external solutions containing K^+ concentration of 2, 5.4, and 20 mM. In each case, the I–V curve for the ISO-sensitive current closely approximates the I_{K1} I–V curves, suggesting that the ISO-sensitive current is composed predominantly of I_{K1}. The averaged reversal potentials of the ISO-sensitive current were approximately −50, −80, and −110 mV when the external [K^+] were 20, 5.4, and 2.0 mM, respectively. Reversal potential averaged from eight different cells shifted linearly with the log of the external K^+ concentration (Fig. 3 *B*), changing 60 mV for a 10-fold change in external K^+ concentration. Because the value for the rate of shift in the reversal potential at physiological temperature (37°C) calculated from the Nernst equation is 61.5 mV, the finding suggests that the ISO-sensitive current is highly selective for K^+ and provides additional evidence that the ISO-sensitive current is dominated by I_{K1}. Each of these experiments was completed after ~ 2 min application of ISO to avoid desensitization.

To confirm that the ISO-induced inhibition of macroscopic membrane current is caused by inhibition of I_{K1}, the ISO-sensitive current was compared with the Ba^{2+}-sensitive current. Fig. 4 *A* illustrates the time-course of ISO- and Ba^{2+}-induced inhibition of the whole cell current at −120 mV. The ISO-sensitive current was obtained by subtracting the currents during 2-min exposure to ISO from the corresponding currents in the absence

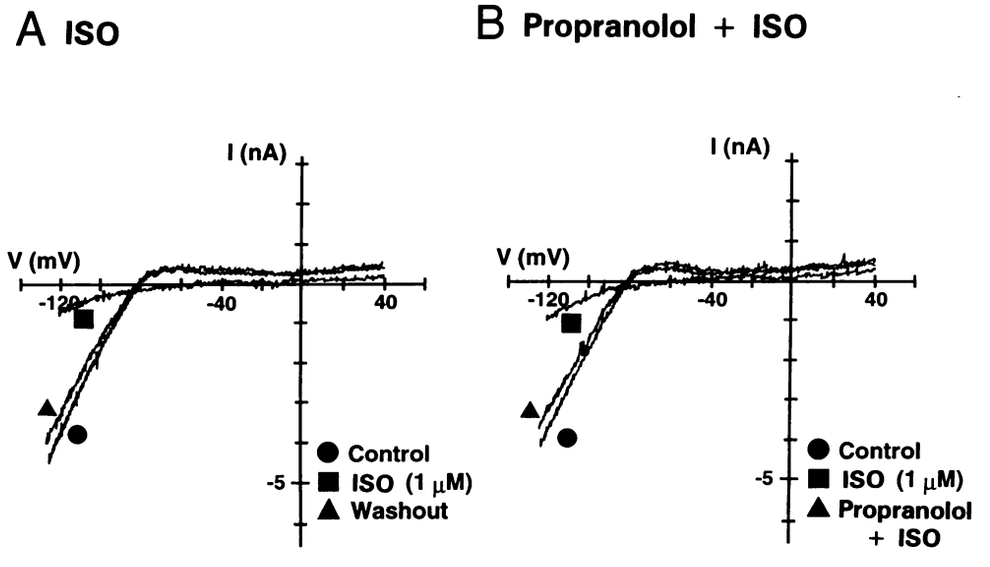

Figure 2. ISO can inhibit whole cell I_{K1} in isolated human ventricular myocytes. (*A*) Effect of ISO (1 μM) on the current–voltage (I–V) relationship measured by ramp voltage clamp in isolated ventricular myocytes. The I–V relationships for the membrane currents were measured in the control (●), during exposure to ISO (■), and after wash out (▲). (*B*) Effect of propranolol (1 μM) on the ISO-induced inhibition of I_{K1}. The I–V relationships for the membrane currents were measured during the control period (●), during exposure to ISO (■), and during exposure to propranolol in the presence of ISO (▲).

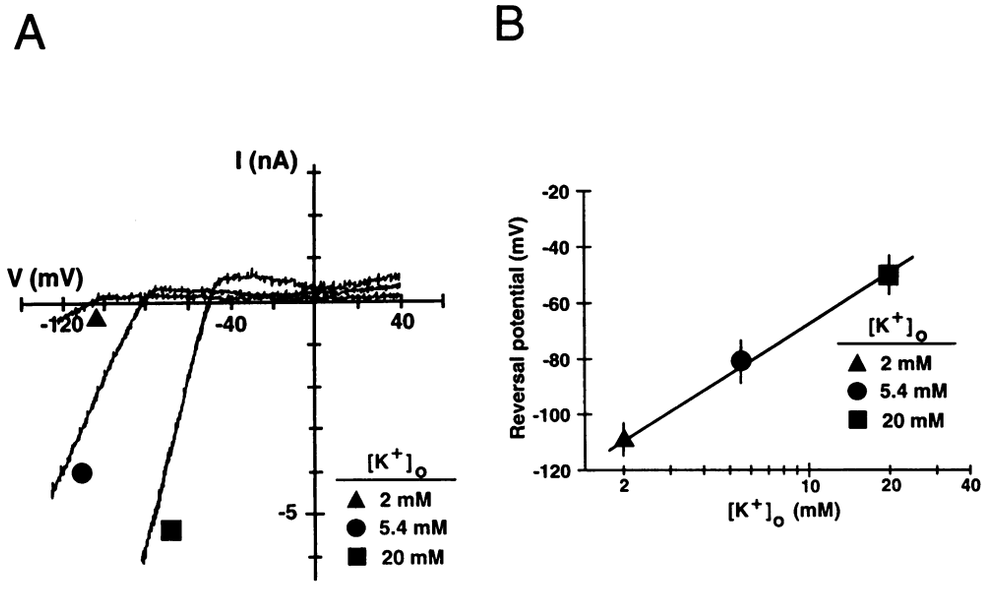

Figure 3. Effect of extracellular K^+ concentration on ISO-sensitive currents. (*A*) I–V relationships of ISO-sensitive currents obtained by subtracting the current in the presence of ISO (1 μM) from the control when external K^+ concentrations ($[K^+]_o$) were 2 mM (▲), 5.4 mM (●), and 20 mM (■). (*B*) The reversal potentials of ISO-sensitive currents (from *A*) plotted semilogarithmically as a function of the extracellular K^+ concentrations; the averaged reversal potentials were −110±7.2 mV (n = 8), −80.3±8.7 mV (n = 8), and 52.7±6.9 mV (n = 8) in 2, 5.4, and 20 mM $[K^+]_o$, respectively.

of ISO. The whole cell current was reversibly inhibited by bath application of ISO (1 μM) similar to Ba^{2+} (1 mM). Fig. 4 *B* illustrates ISO- and Ba^{2+}-sensitive currents. The averaged slope conductance was 69.5±7.4 nS (n = 9) in the ISO-sensitive current and 69.9±7.0 nS (n = 8) in the Ba^{2+}-sensitive current (NS). The ISO-sensitive current was indistinguishable from the Ba^{2+}-sensitive current, indicating that the ISO-sensitive current is I_{K1}. Application of ISO was ~ 2 min. Fig. 4 *C* shows the simultaneous effect of ISO and Ba^{2+} on I_{K1}. After application of external solution containing 1 μM ISO and 1 mM Ba^{2+}, the current was inhibited. The averaged slope conductance of the ISO- and Ba^{2+}-sensitive current was 68.8±7.8 nS (n = 7), the value did not differ from that in the ISO-sensitive current and the Ba^{2+}-sensitive current. The result indicate that the effects of Ba^{2+} and ISO are not additive.

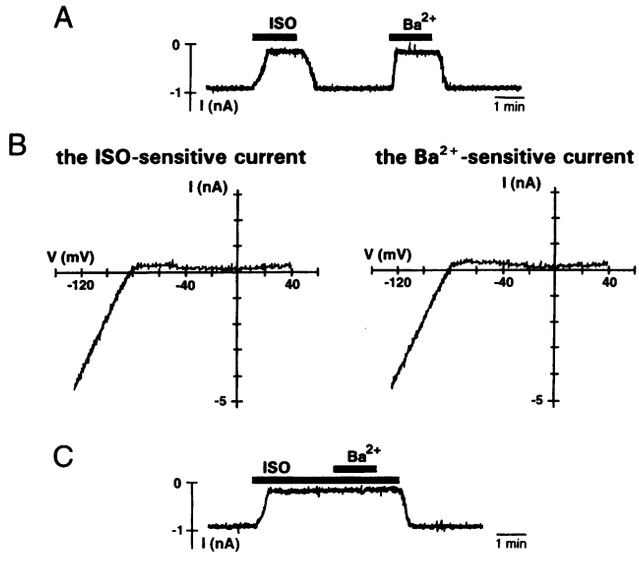

Figure 4. Effects of ISO and Ba^{2+} on whole cell I_{K1} in isolated human ventricular myocytes. (*A*) A chart record of the whole cell current response to extracellular applications of ISO (1 μM) or Ba^{2+} (1 mM) at the holding potential of −120 mV. ISO and Ba^{2+} reversibly inhibited whole cell I_{K1} to the same extent. (*B*) The whole cell I–V relationships of the ISO-sensitive current (*left panel*) and the Ba^{2+}-sensitive current (*right panel*). (*C*) A chart record of the whole cell current response to simultaneous applications of ISO (1 μM) and Ba^{2+} (1 mM).

Effects of second messengers. To address the question of whether the inhibitory effect of ISO on I_{K1} involves activation of adenylyl cyclase, the effect of forskolin on whole-cell I_{K1} was determined (Fig. 5 *A*). Bath application of forskolin (10 μM) decreased I_{K1} to the same extent as 1 μM ISO (n = 6). The averaged slope conductance was 70.7±6.7 nS (n = 6) in control and 4.2±0.6 nS (n = 6) during exposure to forskolin (n = 6, $P < 0.001$). Different from ISO, I_{K1} did not recover to the control level during 20 min washout period of forskolin. Bath application of a membrane permeable analogue of cAMP, dibutyryl cAMP (5 mM), also decreased I_{K1} (n = 6, not shown), suggesting that the effect of ISO involves activation of adenylyl cyclase and a consequent rise in cellular cAMP.

Involvement of PKA during β-adrenergic stimulation by ISO was also assessed (Fig. 5 *B*). The effects of ISO on I_{K1} were investigated after PKA was selectively inhibited with PKI: 5–24 amide applied internally via the pipette solution. Fig. 5 *B* shows a representative experiment in which bath application of ISO (1 μM) failed to inhibit I_{K1} when PKI (50 μM) was present in the pipette solution. The averaged slope conductance was 71.4±6.8 nS (n = 8) in control (in the presence of PKI) and 70.9±7.3 nS (n = 8) during exposure to ISO (NS). These results suggest that β-adrenergic inhibition of I_{K1} is caused via PKA activation.

ISO-induced inhibition of single channel I_{K1}. Fig. 6 shows that bath application of ISO (1 μM) can reversibly inhibit unitary I_{K1} channel activity recorded in the cell-attached patch condition. The unitary currents with symmetrical 150 mM internal and external $[K^+]$ concentrations exhibited slow gating properties typical for I_{K1}. The channel was active with back-ground opening without agonist and channel activity was similar to previously reported single I_{K1} channels in human and other mammalian hearts (4, 19, 20). When the myocyte was exposed to 1 μM ISO, interburst closed period (19, 20, 21) gradually

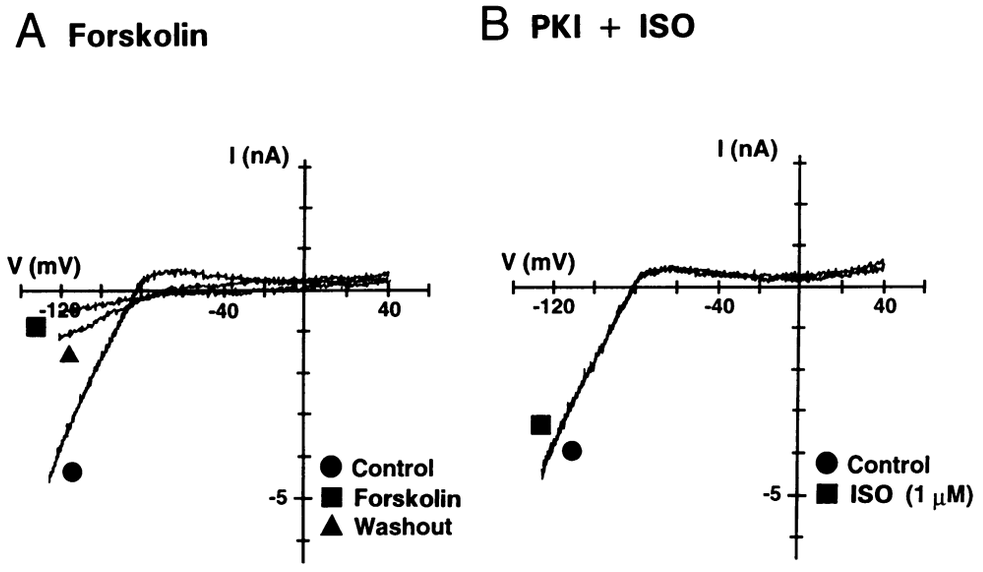

Figure 5. Effects of second messengers on β-adrenergic inhibition of whole cell I_{K1}. (*A*) Effect of forskolin (10 μM) on the I–V relationship measured by ramp voltage clamp in isolated ventricular myocytes. The I–V relationships for the membrane currents were measured during the control period (●), during exposure to (■) and after wash out of forskolin (▲). (*B*) Effect of ISO (1 μM) on the I–V relationship measured by ramp voltage clamp in the presence of the synthetic peptide PKI (5–24 amide, 50 μM) in the pipette solution. The control current (●) obtained with PKI in the pipette exhibited properties that were similar to those obtained when PKI was absent. Currents obtained after exposure of the same cell to ISO (1 μM) in the bath (■). When PKI was present in the pipette, ISO did not decrease whole cell I_{K1}.

decreased, resulted in the decrease of the channel open state probability (P_o). The unitary amplitude was unchanged by ISO. After washout of ISO, P_o recovered to almost the control level (Fig. 6 *A*). This patch contains only one channel judged by a total 25 min continuous recording period using equation (1). Continuous sampling duration was achieved for 1,390 s and sampling time after addition of agonist was 124 s. Fig. 6 *B* illustrates bursting activity with more detail on an expanded time base. After exposure to ISO, individual burst duration gradually and reversibly decreased with increasing interburst period. Fig. 6 *C* shows the effect of ISO on averaged channel activity estimated by NP_o where N is the maximal number of channels actually observed in the patch. The averaged NP_o was 1.03±0.14 (n = 12) in the control and 0.24±0.09 (n = 12, P < 0.001) during exposure to ISO (1 μM). NP_o recovered to almost the control level after washout of ISO (0.99±0.14, n

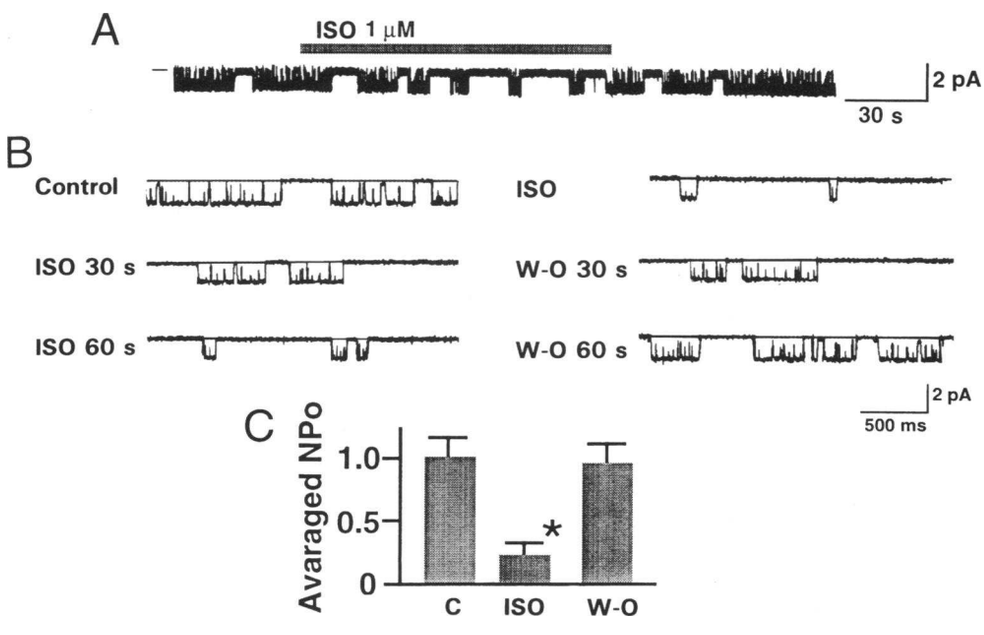

Figure 6. ISO can inhibit single channel I_{K1} in cell-attached patches. (*A*) Representative example of current recorded from unitary I_{K1} channel activity in a cell-attached patch. After introduction of ISO (1 μM) to the bath solution, individual burst duration gradually decreased with prolongation of interburst interval without be affected the unitary current amplitude. The horizontal bar indicates the zero current level. (*B*) The bursting behavior during exposure to ISO is shown with more detail on an expanded time base. ISO reversibly inhibited unitary I_{K1} channel activity. Currents shown in each panel were recorded with holding potential (HP) = −60 mV and low pass filtered at 2 kHz. (*C*) The averaged NP_O in the control (*C;* 1.03±0.14, n = 12), during exposure to ISO (*ISO;* 0.24±0.09, n = 12), and after washout of ISO (*W-O;* 0.99±0.14, n = 11). * P < 0.001 different from control and after washout.

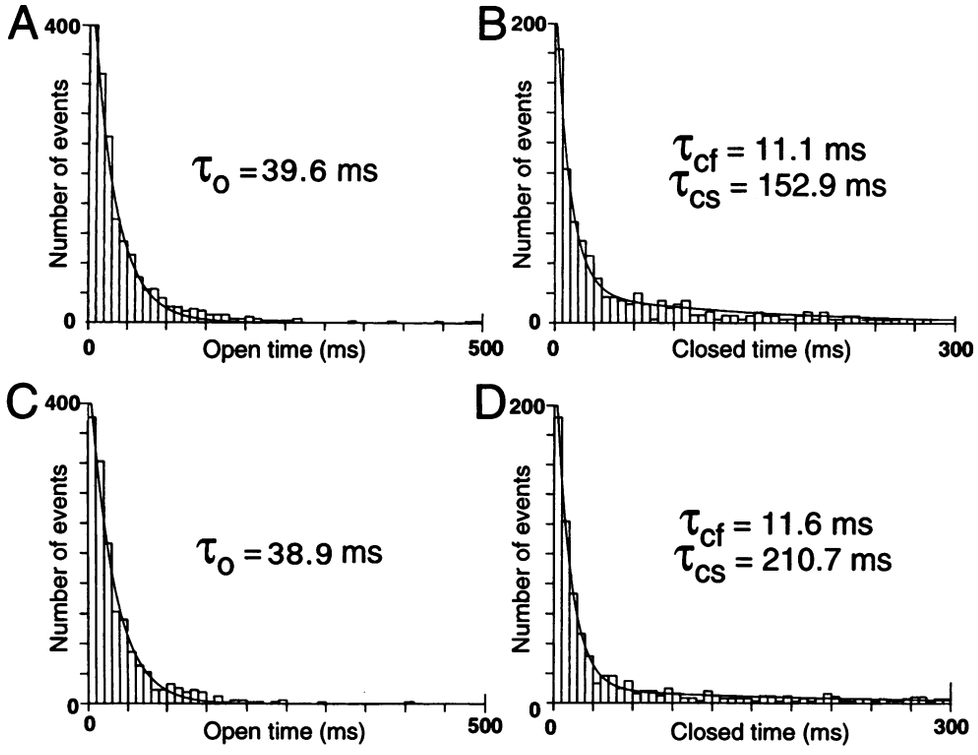

Figure 7. Representative histograms of open and closed times in the absence and presence of ISO. (*A*) Histogram of open times recorded from a representative single channel, cell-attached patch during control conditions. This patch never exhibited more than a single channel during > 30 min of continuous recording. Open state lifetimes were distributed according to a single exponential function with a time constant (τ_o) of 39.6 ms. (*B*) Control (same patch, same data as in *A*) closed times histogram. At least two exponentials were required to fit the closed time distributions with time constants of 11.1 ms for the fast component (τ_{cf}) and 152.9 ms for the slow component (τ_{cs}). (*C*) Histogram of open times in the presence of ISO (1 μM) before achievement of complete inhibition of this I_{K1} channel (same single channel patch as in *A* and *B*). The lifetime of openings was distributed according to a single exponential function with a τ_o of 38.9 ms which was similar to control. (*D*) Closed time histogram for channel activity (same data as in *C*) required two exponentials to fit the closed time distributions. τ_{cf} was 11.6 ms, a value which was similar to control; τ_{cs} was 210.7 ms, a value which was larger than control.

= 11, NS from control). Myocytes were exposed with ISO for ~ 1 min, which was too short to develop desensitization. Fig. 7 shows the open and closed time histograms before (Fig. 7, *A* and *B*) and during 3-min exposure to 1 μM ISO (Fig. 7, *C* and *D*). With both conditions, the open time histograms could be best fitted by a single exponential function having a mean open life time of 35.5±4.1 ms (n = 10) in control and 34.3±4.9 ms (n = 10) in the presence of ISO (statistically NS). The closed time histograms (Fig. 7 *B, control;* Fig. 7 *D, ISO*) were best fitted by double exponential functions in each case. The fast components of the closed time events were unchanged (10.5±1.4 ms in control, n = 10 and 11.2±1.6 ms in the presence of ISO, n = 10; statistically NS). In contrast, slow component of closed times were significantly longer in the presence of ISO (233.8±24.6 ms, n = 10) than in the control (151.9±26.7 ms, n = 10, $P < 0.001$).

The nature of the β-adrenergic inhibition of I_{K1} was further examined using excised inside-out patches (Fig. 8). When purified catalytic subunit (C-subunit) of PKA (2 μM) was applied to the bath (cytosolic side of membrane), channel activity was inhibited (Fig. 8 *A, top*) in a manner similar to that observed in cell-attached recordings. This patch contains only one channel judged by a total 21-min continuous recording period using Eq. 1. Continuous sampling duration was achieved for 1,220 s and sampling time after addition of agonist was 126 s. This effect was antagonized by alkaline phosphatase (APase, 20 U/ml) (Fig. 8 *A, bottom*). When APase was applied immediately after the C-subunit of PKA had been washed out of the bath, the recovery of channel activity was significantly faster than in the absence of APase. This patch contains only one channel judged by a total 20-min continuous recording period using Eq. 1. Continuous sampling duration was achieved for 1,160 s and sampling time after addition of agonist was 120 s. Fig. 8 *B* illustrates the averaged NP_o. The averaged NP_o in the control condition was 0.98±0.17 (n = 15), which was inhibited to 0.20±0.13 (n = 15, $P < 0.001$) during exposure to C-subunit of PKA. The PKA-induced inhibition was recovered to almost control level. At 60-s recovery period, the averaged NP_o was 0.69±0.17 (n = 8) in the absence of APase and 0.92±0.18 (n = 7) in the presence of APase ($P < 0.05$). The averaged recovery period (half-activation time) was 51.0±29.3 s (n = 8) in the absence of APase and 4.4±2.7 s (n = 7, $P < 0.01$) in the presence of APase. These results suggest that I_{K1} can be modulated by processes involving PKA-dependent phosphorylation of the channel or the channel associated protein.

Whole cell I_{K1} response to ISO in ventricular myocytes isolated from nonfailing and failing hearts. Abnormal responsiveness in cardiac function to β-adrenergic stimulation has been described in patients with heart failure (22, 23). To assess whether or not the inhibitory effect of ISO on I_{K1} is altered in heart failure, the effect of ISO on I_{K1} was compared between ventricular myocytes isolated from patients with congestive heart failure (the failing heart) and without heart failure (the nonfailing heart). Isolated human ventricular myocytes from both the nonfailing and the failing hearts were morphologically similar. The averaged cell capacitance in ventricular myocytes isolated from the nonfailing heart was generally similar to those from donors (126.2±13.4 pF, n = 25 in the nonfailing and 133.8±14.7 pF, n = 20 in the failing heart; NS). Resting membrane potentials in myocytes from the nonfailing were −79.5±6.7 mV (n = 29), while those in the failing heart were −64.0±6.9 mV (n = 24, $P < 0.05$).

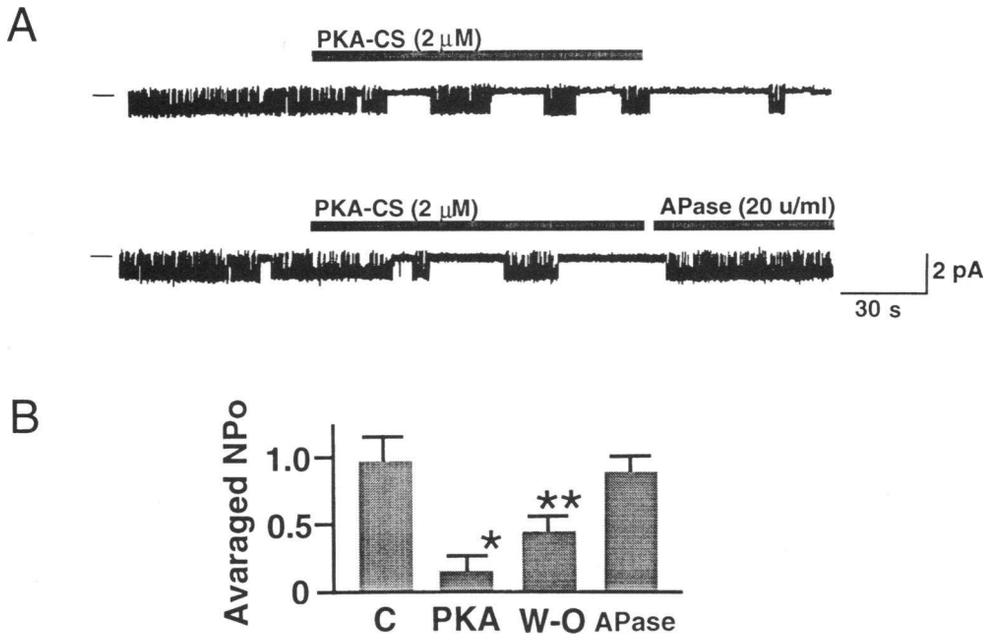

Figure 8. Effects of the catalytic subunit of cAMP-dependent protein kinase (*PKA-CS*) and protein phosphatase (*APase*) on I_{K1} channel activity recorded from excised inside-out patches. (*A, top*) Effect of catalytic subunit of PKA (*PKA-CS*) on I_{K1} channel activity in excised inside-out patches. Free Ca^{2+} concentration was adjusted to 0.1 μM in these experiments. After bath application of PKA-CS (2 μM, cytosolic surface of membrane), channel activity was inhibited. (*Bottom*) Alkaline phosphatase treatment can antagonize the inhibition of I_{K1} channel activity by the PKA-CS in an inside-out patch. After inhibition of channel activity that had been induced by exposing the cytosolic surface of an inside-out excised patch to PKA-CS (2 μM), alkaline phosphatase (*APase, 20 U/ml*), applied to the cytosolic side of the membrane, resulted in quick reactivation of the channel. The alkaline phosphatase had been dialyzed against the perfusion buffer solution for 8 h at 0°C before use. Currents were recorded with the patch held at −60 mV. (*B*) The averaged NP_O was compared between in the absence and presence of APase. The averaged NP_O in the control and during exposure to PKA-CS were 0.98±0.17 (n = 13) and 0.17±0.11 (n = 12), respectively. After 30 s washout of PKA-CS (*W-O*), the averaged NP_O recovered to 0.43±0.12 (n = 6) in the absence of APase. In contrast, the averaged NP_O recovered to 0.89±0.11 (n = 6) in the presence of APase after 30 s washout period of PKA-CS. * $P < 0.01$ different from other groups, ** $P < 0.01$ different from APase.

Fig. 9 illustrates the comparison of the effect of ISO on whole cell I_{K1} between nonfailing (Fig. 9 *A*) and the failing hearts (Fig. 9 *B*). Fig. 9, *A* and *B* (*top panels*) illustrates the averaged I–V relationships in control, during exposure to ISO (1 μM) and during exposure to Ba^{2+} (1 mM) in nonfailing (Fig. 9 *A*) and the failing hearts (Fig. 9 *B*). Table II summarizes the comparison of whole cell current parameters between nonfailing and failing hearts. The averaged slope conductance at the reversal potential in the nonfailing heart was significantly greater than that in the failing heart. In addition, the averaged slope conductance of the ISO-sensitive and the Ba^{2+}-sensitive differential currents were significantly greater in the nonfailing than in the failing heart (Fig. 9, *A* and *B, bottom panels*). The ISO-sensitive current was obtained by subtracting the currents during 2-min exposure to ISO from the corresponding currents in the absence of ISO. The current magnitudes at 0 and 10 mV positive to the reversal potential of the ISO-sensitive differential currents were not significantly different between the nonfailing (NF) and the failing heart (HF). The ISO-sensitive current was almost superimposed with the Ba^{2+}-sensitive current in NF (statistically NS in each voltage). In contrast, the ISO-sensitive current was significantly smaller than the Ba^{2+}-sensitive current in HF (61.4±7.6% of the Ba^{2+}-sensitive current). The concentration dependence of the effect of ISO to inhibit I_{K1} at −120 mV was characterized in ventricular myocytes isolated from the nonfailing and the failing hearts (Fig. 10). Using the Eq. 3, the Hill coefficient was ~ 1 in both groups. However, the half-maximal inhibition (IC_{50}) occurred at 0.023 μM in the nonfailing hearts, while it was 0.12 μM in the failing hearts. These results suggest that response to ISO on I_{K1} is significantly reduced in the failing than in the nonfailing heart.

Fig. 11 shows unitary I_{K1} channel response to C-subunit of PKA in ventricular myocytes isolated from the nonfailing and the failing heart in the inside-out patch condition. Although channel activity was inhibited by bath application of C-subunit of PKA (2 μM) in each case, channel activity during exposure to C-subunit of PKA was different between the two groups. The PKA-induced inhibition of the channel was significantly reduced in the failing heart. Although the averaged NP_o at the control period was similar to each other, the averaged NP_o during exposure to C-subunit of PKA was significantly lower in the failing than in the nonfailing heart (n = 6 for each, $P < 0.01$). These results suggest that there is not simply a problem with β-adrenergic receptor–mediated signalling in the failing hearts but that there is also a defect in the K^+ channel or other cell signalling pathways.

Discussion

The major findings in this study are as follows: (*a*) β-adrenergic stimulation by ISO caused prolongation of the action potential with a small depolarization of the resting membrane potential in human ventricular myocytes; (*b*) β-adrenergic stimulation reversibly inhibited macroscopic I_{K1} in the concentration-dependent fashion and the inhibition was prevented by PKI; (*c*) ISO and the purified PKA could suppress unitary I_{K1} channels by decreasing P_o; and (*d*) modulation of I_{K1} by ISO was significantly reduced in ventricular myocytes isolated from the failing heart compared to the nonfailing heart. These results suggest that I_{K1} in human ventricular myocytes can be inhibited by a PKA-mediated phosphorylation and the modulation is less

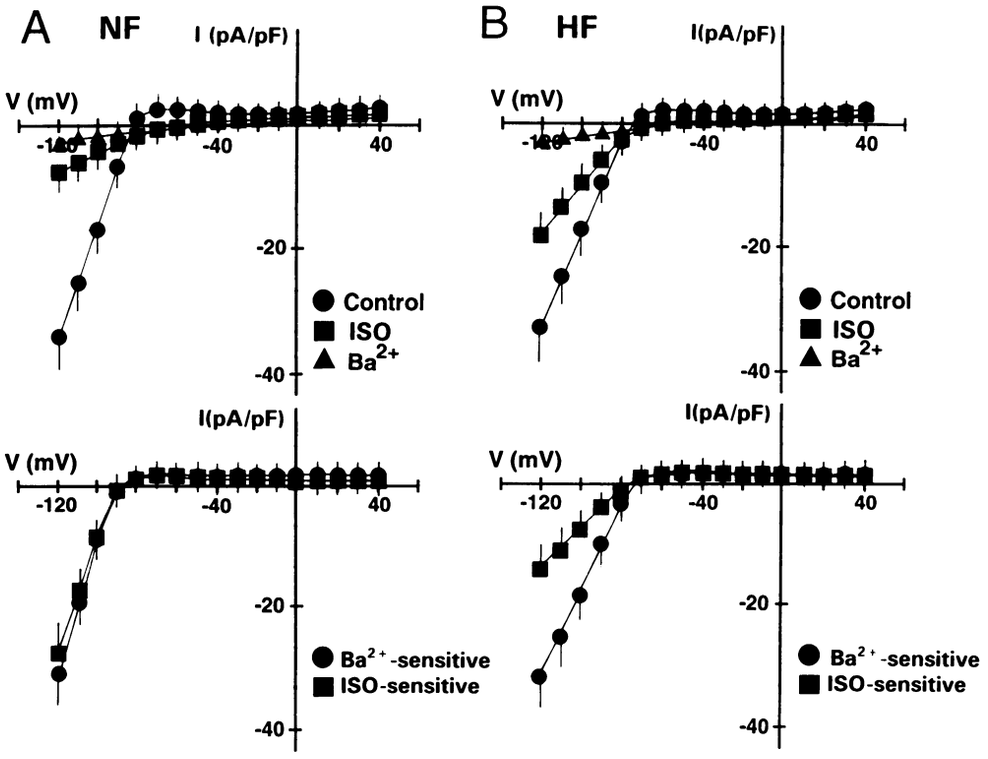

Figure 9. Effect of ISO on whole cell I_{K1} in isolated human ventricular myocytes from NF and HF. (*A, top*) The averaged I–V relationships of I_{K1} in control (●), during exposure to ISO (1 μM, ■), and during exposure to Ba^{2+} (1 mM, ▲) in an ventricular myocyte isolated from the NF and expressed as mean±SD ($n = 8$) after normalization to membrane capacitance. (*Bottom*) The averaged ISO-sensitive current obtained by subtracting the current in the presence of ISO (1 μM) from the control (■) and the Ba^{2+}-sensitive current obtained by subtracting the current in the presence of Ba^{2+} (1 mM) from the control (●) in an ventricular myocyte isolated from NF. (*B, top*) The averaged I–V relationships of I_{K1} in control (●), during exposure to ISO (1 μM, ■) and during exposure to Ba^{2+} (1 mM, ▲) in an ventricular myocyte isolated from HF and expressed as mean±SD ($n = 7$) after normalization to membrane capacitance. (*Bottom*) The averaged ISO-sensitive current (■) and the Ba^{2+}-sensitive current (●) in an ventricular myocyte isolated from HF.

significant in myocytes isolated from the failing heart compared to the nonfailing heart.

Relation to previous electrophysiological studies. Previous reports documented that various sarcolemmal channel proteins are involved in producing the positive inotropic and electrophysiological effects associated with β-adrenergic receptor activation (24) including L-type Ca^{2+} channels (25–27), delayed rectifier K^+ channels (28–30), Na^+ channels (31, 32) and Cl^- channels (33, 34). Stimulation of β receptors by catecholamines generates activated G_s protein to stimulate production of cAMP which can activate PKA. In contrast to the many reports concerned with β-adrenergic modulation of these ionic current systems, modulation of cardiac I_{K1} channels is limited to very few reports. One suggests that the background K^+ conductances of canine cardiac Purkinje fibers (35) and coronary sinus tissues (36) have been reported to increase during exposure to ISO. However, these ISO-induced increase in background K^+ conductance is thought not to be mediated by I_{K1}, because it showed no evidence of inward rectification and I–V curves of ISO-induced current obtained at different external K^+ concentrations did not cross each other (35). We did not observe such currents in either whole cell or single channel recordings in human ven-

Table II. Comparison of the Effect of ISO on Whole Cell I_{K1} between Nonfailing and the Failing Hearts

	NF	HF
Slope conductance (nS)		
n	8	7
control	71.7±6.9	61.4±6.9
ISO (1 μM)	6.5±1.1	36.4±4.1*
Ba^{2+} (1 mM)	4.7±0.9	4.9±1.0
ISO-sensitive current	68.5±7.0	35.0±5.2*
Ba^{2+}-sensitive current	70.8±7.9	60.4±7.1
Current magnitude (pA/pF)		
n	8	7
0 mV	1.2±0.3	1.2±0.2
+10 mV of E_{rev}	1.3±0.3	1.2±0.3

* $P < 0.001$ different from NF. E_{rev}, reversal potential.

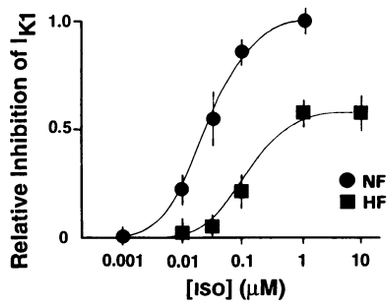

Figure 10. Concentration dependence of the inhibition of whole cell I_{K1} by ISO in isolated myocytes from NF and HF. The normalized ISO concentration dependence of the inhibition of the whole cell I_{K1} at −120 mV. Inhibition by ISO was maximal at 1 μM in myocytes from the normal subject. A relative inhibition of 1.0 was obtained by normalizing to this value. Analysis of the concentration dependence yielded an apparent dissociation constant (IC_{50}) of 0.12 μM and a Hill coefficient of ~ 1 in NF (●), while IC_{50} was 0.023 μM (Hill coefficient of ~ 1) in HF (■). Data plotted as mean±SD (n = 6–14 cells).

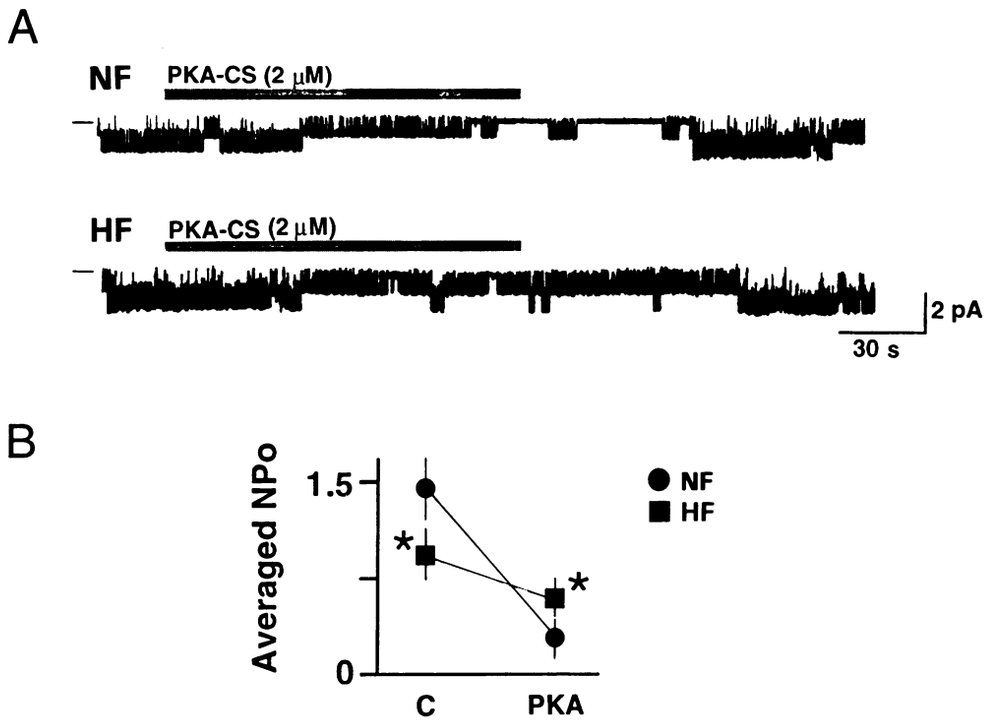

Figure 11. Effect of the catalytic subunit of cAMP-dependent protein kinase (*PKA-CS*) on unitary I_{K1} channel activity in isolated human ventricular myocytes from NF and HF. (*A, top*) Representative unitary I_{K1} channel activity during exposure to PKA-CS (2 μM) in an ventricular myocyte isolated from NF. This patch contained at least two channels both of which were inhibited. (*Bottom*) I_{K1} channel activity in an ventricular myocyte isolated from HF. Channel inhibition occurred with PKA-CS (2 μM) but was not prominent as that in the nonfailing heart. (*B*) The averaged NP_O in the control and during exposure to PKA-CS in ventricular myocytes isolated from the nonfailing (●) and the failing heart (■). The averaged NP_O in NF during control period was significantly greater than that in HF (n = 6). In contrast, the value during exposure to PKA-CS was significantly greater in HF (0.66±0.15, n = 6) than in NF (0.18±0.11, n = 6). * P < 0.01 different from NF.

tricular myocytes. Another report suggests that ISO can inactivate I_{K1} in canine cardiac Purkinje myocytes through a non β-adrenoreceptor–mediated mechanism (37). They demonstrated that ISO reduced whole cell I_{K1} magnitude and the effect was not prevented by propranolol. The present results are clearly different from those in Purkinje myocytes because of the sensitivity to propranolol. In addition to these reports, several previous studies did not observe β-adrenergic effects on I_{K1} in guinea pig ventricular myocytes (26, 30). These negative findings may be caused by contamination of overlapping current systems (see below).

β-Adrenergic modulation of cardiac action potentials and I_{K1}. I_{K1} is thought to influence the time course of cardiac action potentials, mainly during late repolarization (phase 3 of the action potential), and the resting membrane potential during the diastolic period. Inhibiting I_{K1} as a result of sympathetic nervous activity would be expected to modify those aspects of the action potential ordinarily influenced by this current. However, β-adrenergic stimulation can modify the activity of several other sarcolemmal ion channels and perhaps even the chemical gradients providing the driving forces for several components of the ionic current. Accordingly, adrenergically mediated effects on the action potential are quite numerous, vary from species to species, cell type to cell type, and are modulated by many other factors. Despite the multiplicity of the possibilities, several reports have shown that ISO can increase action potential duration and depolarize the resting membrane potential (38, 39). Since these are among the effects expected when I_{K1} is inhibited, it is tempting to suggest that they are the result of an ISO-induced inhibition of I_{K1} channels.

Because ISO can affect several whole cell ionic current systems in the heart, it was first necessary to demonstrate that the specific component of the whole cell membrane current and the ion channels affected by ISO in the present study were indeed I_{K1}. The experimental solutions and conditions were selected to remove contaminations of other current systems. To eliminate sodium (I_{Na}) and L-type calcium current (I_{Ca}), tetrodotoxin (TTX, 5 μM) and Co^{2+} (2 mM) were added to the external solution. Dihydroouabain (DHO, 10 μM) was added to the external solution to block the Na^+/K^+ pump current (40). Na^+/K^+ pump is known to be enhanced by β-adrenergic stimulation (1, 41, 42). Although it is unclear whether or not the PKA-induced Cl^- current ($CFTR_{cardiac}$) is present in human ventricular myocytes (43), I_{Cl} was eliminated by replacing Cl^- ions with other anions from internal and external solutions. The delayed rectifier K^+ channel was also eliminated by the acute ramp voltage clamp protocol (see Methods). 4-AP (2 mM) was added to the external solution to inhibit two types of the delayed rectifier K^+ current (I_{Kur} and I_{Ks}) (43–45). These current systems are known to be enhanced by β-adrenergic stimulation. Because the effects of ISO on these currents on I_{K1} can be essentially offsetting, contamination of these currents may mask the ISO-induced inhibition of I_{K1}. In canine Purkinje cells, the transient outward current (I_{to}) has also been modulated by β-adrenergic stimulation (46). I_{to} has been described in human heart (47–49) which has two components, a relatively long lasting one suppressed by 4-AP, and a briefer Ca^{2+}-dependent one suppressed by caffeine or Co^{2+} (47). External 4-AP and Co^{2+} can effectively inhibit both component of I_{to} in human heart in the present study. In addition to remove possible contaminations of these ISO-induced currents other than I_{K1}, the ISO-sensitive differential current characteristics in Figs. 3 and 4 support that the ISO-sensitive whole cell current is composed predominantly of I_{K1}.

The outward conductance of I_{K1} may be modulated by intracellular Ca^{2+} ($[Ca^{2+}]_i$) (50, 51); repetitive activation of the membrane currents can decrease outward I_{K1} in association with an elevation in $[Ca^{2+}]_i$ (52). However, it is unlikely that this effect of $[Ca^{2+}]_i$ has an appreciable role in the ISO-induced inhibition of I_{K1} because $[Ca^{2+}]_i$ should have been well below the threshold concentration for this effect with our internal solution, even in the presence of ISO. The free Ca^{2+} in the internal solution was adjusted to the level at $\sim 10^{-9}$ M by adding EGTA (see Methods).

Altered sensitivity to β-adrenergic modulation on I_{K1} in ventricular myocytes isolated from the failing heart. Abnormal response in cardiac function to β-adrenergic stimulation has been described in the failing heart (22, 23). Reduced sensitivity to the chronotropic effects of ISO and a selective loss of β-adrenergic receptors (down-regulation) were documented in human hearts with terminal heart failure (53–56). Decreased response to ISO was demonstrated in relation to β-adrenergic receptor down-regulation in these studies. An apparent loss of functional G_{sa} in cardiac cell membranes as well as deficient production of cAMP have been shown in patients with heart failure compared with control subjects (57). In contrast, ISO-stimulated adenylyl cyclase has been shown to be specifically depressed in the failing human heart (53, 58). These studies suggest that β-adrenergic receptor down-regulation, an apparent loss of functional G_{sa}, depressed cAMP production and reduced adenylyl cyclase activity are essential for the reduced response to β-adrenergic stimulation in patients with heart failure. Consistent with these previous studies, our results have shown that I_{K1} channel response to ISO was reduced in ventricular myocytes isolated from the failing heart compared to the nonfailing heart. Our results provided new information that I_{K1} channel response itself to C-subunit of PKA was reduced in the failing heart. Because excised patch measurements were achieved in essentially similar conditions between the two groups and C-subunit of PKA was directly introduced to the bath solution, altered response to PKA in I_{K1} channel in heart failure may be caused by purely reduced response of the channel to PKA. The exact reason for the lower sensitivity of I_{K1} to PKA in the failing heart relative to the nonfailing heart is unclear at present. Because the Hill coefficient of the concentration–response relationship of the channels was close to 1 in both failing and nonfailing heart, single phosphorylation sites for PKA may exist on the channel protein in both groups. Protein-phosphatase induced dephosphorylation process may be accelerated in the failing heart. Other unknown factors may cause the reduced intrinsic sensitivity of the channels to PKA. These results suggest that reduced sensitivity to β-adrenergic stimulation in patients with heart failure may be caused by β-adrenergic receptor down-regulation, the reduced intracellular signal transduction, as well as the reduced channel response to PKA.

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