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Inhibitory effect of endothelin-1 on the isoproterenol-induced chloride current in human cardiac myocytes

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Abstract

It is still controversial whether the cAMP-activated Cl^- current ($I_{Cl,cAMP}$) is expressed in human cardiomyocytes. The whole-cell configuration of the voltage-clamp technique was used to examine in detail the $I_{Cl,cAMP}$ in single human atrial and ventricular myocytes. Human cardiomyocytes were enzymatically isolated from atrial or ventricular specimens obtained from open-heart surgery or cardiac transplantation, respectively. Isoproterenol (1 μM) or forskolin (10 μM) was used to activate the cAMP second-messenger system. The isoproterenol- or forskolin-induced Cl^- current was elicited in 12 of 54 atrial myocytes but was completely absent from ventricular myocytes. The isoproterenol-induced Cl^- current in atrial myocytes was time-independent and had a reversal potential close to zero. Endothelin-1 (30 nM) inhibited the isoproterenol-induced Cl^- current by $75 \pm 6\%$ ($n = 4$). This inhibitory effect of endothelin-1 was attenuated by pretreating atrial myocytes with the endothelin ET_A receptor antagonist, BQ485, but not with the ET_B receptor antagonist, BQ-788. The results provide evidence that the $I_{Cl,cAMP}$ exists in human atria, but not ventricle, and is inhibited by endothelin-1.

Keywords: Cl^- channel; cAMP-activated; Atrial myocyte, human; Ventricular myocyte, Human; Endothelin-1; Voltage-clamp technique, Whole cell

1. Introduction

It has become clear that Cl^- channels play an important role in many cellular functions (Rubart et al., 2000). For example, cardiac Cl^- channels may contribute to the cardiac action potential, causing depolarization of the resting membrane potential and shortening of action potential duration (Harvey et al., 1990). Therefore, the regulation of cardiac Cl^- channels affects, more or less, the rhythm and function of the heart. It has been found that there are at least six different Cl^- conductances in the sarcolemma of various mammalian cardiac myocytes (Hume and Horowitz, 1995). These include Cl^- conductance stimulated by activation of protein kinase C, protein kinase A, intracellular Ca^{2+}, extracellular ATP, sarcolemma stretch, and a background Cl^- conductance. Moreover, the extensive progress of electrophysiological techniques and molecular biology has facilitated the identification of Cl^- channel defects in diseases such as cystic fibrosis (Welsh and Smith, 1993) and myotonia (Steinmeyer et al., 1991). For example, with regard to epithelial cells, it has been demonstrated that the debilitating symptoms of cystic fibrosis stem from the reduced Cl^- permeability, due to mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR)-dependent Cl^- channel (Gadsby and Nairn, 1999; McCarty, 2000). Recent electrophysiological data also suggest a number of similarities in the properties of protein kinase A-dependent Cl^- channels in heart and of cyclic AMP (cAMP)-dependent Cl^- channels encoded by the CFTR gene product in various epithelial cells (Gadsby et al., 1995). Furthermore, cardiac studies (Hume et al., 1994; Hart et al., 1996) have shown that the cyclic AMP-mediated Cl^- channel is the product of the CFTR gene. Molecular data also demonstrated that there is a high degree of sequence homology between CFTR and the cAMP-dependent Cl^- channel cloned from cardiac myocytes (Levesque et al., 1992). In

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human atrial myocardium, not only was the molecular expression of CFTR Cl^- channels demonstrated but electrophysiological evidence consistent with the functional expression of these channels was also provided (Sakai et al., 1995; Warth et al., 1996). In contrast, despite the presence of the CFTR gene in human cardiomyocytes (Levesque et al., 1992), Berul et al. (1997) reported that functional expression of the cyclic AMP-mediated Cl^- channel does not appear to exist in isolated pediatric human atrial myocytes. In mouse ventricular myocytes, Levesque and Hume (1995) also demonstrated that adding $[ATP]_o$, but not $[cAMP]_i$, activates a Cl^- conductance. Moreover, there is a recent, similar observation that no CFTR-like current is found in any human atrial or ventricular myocyte (Oz and Sorota, 1995; Du et al., 2000), although there is a low level of anti-CFTR immunoreactivity in non-diseased human ventricle (Du et al., 2000). Therefore, it is still controversial whether the cAMP-activated Cl^- current ($I_{Cl,cAMP}$) is expressed in human cardiomyocytes. One aim of the present study was therefore, using the whole-cell patch-clamping technique, to clarify whether the cAMP-activated Cl^- channels exist, and also can be activated in isolated human atrial and ventricular myocytes.

Endothelin is a vasoconstrictor peptide first derived from media bathing primary cultures of porcine aortic endothelial cells (Yanagisawa et al., 1988). Of several endothelin analogues, endothelin-1 consisting of 21 amino acids is understood to be the most potent active form. Endothelin-1 has been shown to have specific receptors on the sarcolemma of cardiac myocytes, including the human's (Molenaar et al., 1993). The cellular mechanisms underlying the effects of endothelin-1 have been studied in voltage-clamped guinea-pig ventricular myocytes (James et al., 1994; Ono et al., 1994). Endothelin-1, acting via endothelin ET_A (endothelin-1-selective) receptors, inhibited the cAMP-dependent Cl^- current ($I_{Cl,cAMP}$) and calcium current ($I_{Ca,L}$) by decreasing cAMP accumulation through a pertussis toxin-sensitive mechanism in guinea-pig cardiac cells (James et al., 1994; Ono et al., 1994). Our previous results showed that, in human cardiac myocytes, endothelin-1 inhibits the $I_{Ca,L}$ (Cheng et al., 1995), but it remains to be determined whether endothelin-1 also inhibits the $I_{Cl,cAMP}$ in human cardiac myocytes. Therefore, the second aim of the present study was to examine the effect of endothelin-1 on the $I_{Cl,cAMP}$ in human cardiac myocytes.

2. Materials and methods

2.1. Cell isolation

The procedure for human cardiac cell isolation was derived from those already described for human cardiac

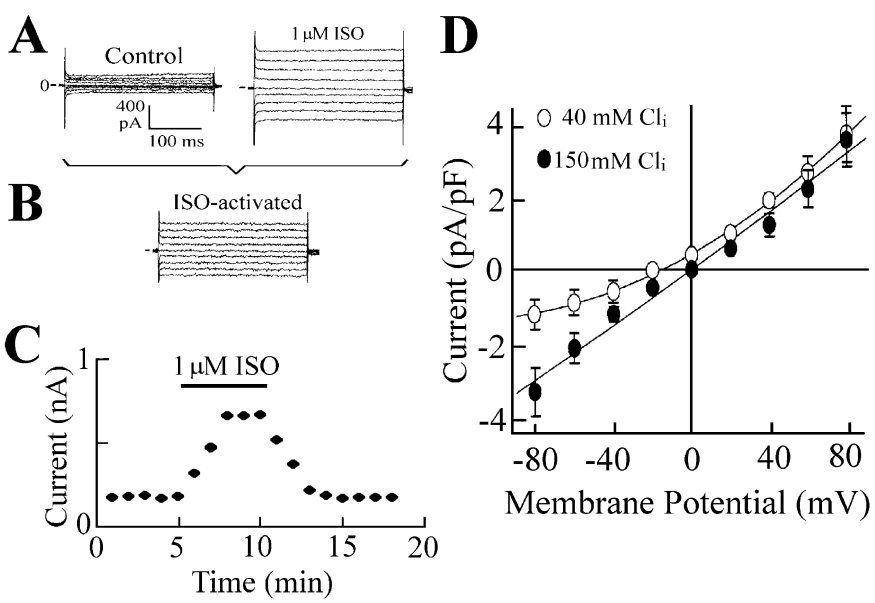

Fig. 1. Effects of isoproterenol (ISO) on Cl^- current in isolated human atrial cells. (A) Currents recorded in the presence of 150 mM extracellular Cl^- (Cl_o) before (left) and after (right) exposure to 1 μM isoproterenol. The cell was dialyzed with an intracellular solution containing 150 mM Cl^- (Cl_i). Currents were elicited by 300-ms voltage clamp steps to membrane potentials between −80 and +80 mV, in 20-mV increments (holding potential 0 mV). Total cell capacitance, 136 pF. (B) The difference currents were obtained by subtracting (A) (left) from (A) (right). For each set of traces, a bar indicates the zero current level. (C) Time course of isoproterenol-induced changes in background current elicited during 300-ms step depolarizations to +80 mV from a holding potential of 0 mV. Isoproterenol was applied during the period indicated by the solid bar. (D) Change of the intracellular Cl^- concentration on the isoproterenol-activated Cl^- currents. Cells were bathed in an extracellular solution containing 150 mM Cl^- and dialyzed with an intracellular solution containing either 150 ($n = 5$) or 40 mM Cl^- ($n = 4$); aspartate was used as the replacement anion. Isoproterenol (1 μM)-induced difference currents were normalized to cell capacitance. The solid lines represent fitting of the data using a non-linear least squares equation. Data points represent means ± S.E.M.

myocyte dispersion (Bustamante et al., 1982; Cheng et al., 1995; Escande et al., 1986). Specimens of right atrial appendages were obtained from 12 patients (five females and seven males) without apparent heart failure undergoing coronary artery bypass grafting. The mean age of patients was 47 ± 6 years (range from 20 to 77 years). The ventricular tissues were obtained from explanted hearts of five patients (45 ± 9 years; three females and two males) with dilated cardiomyopathy undergoing transplantation. Institutional rules for the protection of human subjects were observed. Prior to surgery, informed consent was obtained. Atrial or ventricular tissues were cut into chunks of approximately 1 mm^3 using scissors, and the chunks were shaken in 15–25 ml of a Ca^{2+}-free medium composed of (mM) NaCl 137.5, KCl 5.4, KH_2PO_4 1.2, $MgSO_4$ 1.2, HEPES 6, and glucose 10, at 37 °C for 15 min. The solution was adjusted to pH 7.30 with NaOH and bubbled with 100% O_2 and was changed at 5-min intervals. The chunks were then transferred to 15–20 ml Ca^{2+}-free solution, containing protease (Sigma type XXIV, 4 IU/ml) and collagenase (Sigma type I, 280 IU/ml), and were shaken gently for 40 min under a 100% O_2 atmosphere. Then, the supernatant was removed and replaced by a fresh enzyme medium having the same composition but without protease. Samples were taken every 10–15 min to check the number of cells in suspension by microscopic examination.

2.2. Voltage-clamp technique

The cells were placed in a chamber on the stage of an inverted microscope (Olympus CK2) with a gravitational perfusion system used to expose the cells to different extracellular solutions. The flow rate used was ≈5 ml/min. The experiments were performed at 37 °C with the single-electrode voltage-clamp technique by means of an Axopatch 1D amplifier (Axon Instruments, Foster City, CA, USA) using suction pipettes with resistance ranging from 3 to 5 MΩ. After the formation of a GΩ seal with a 10-ml syringe, brief strong suction was applied to the pipette interior to rupture the membrane patch. Whole-cell currents were recorded during 300-ms rectangular pulses to potentials in the range −80 to +80 mV from a holding potential of 0 mV. Data acquisition and analysis were controlled by pCLAMP software (Axon Instruments, Version 6.01) running on an IBM-compatible PC. All currents were low pass-filtered by using the 10-kHz filter built into the amplifier. Cell membrane capacitance was calculated by integration of the capacitive transient elicited by a small hyperpolarizing step from −50 to −55 mV of 10-ms duration.

2.3. Solutions

Cells were dialyzed with an intracellular solution that consisted of (in mM): CsCl 130, tetraethylammonium-Cl 20, MgATP 5, Tris–GTP 0.1, and HEPES 5; the pH was adjusted to 7.2 using CsOH. In some experiments, intracellular Cl^- was reduced to 40 mM by replacement of CsCl with Cs-aspartate so that the total concentration of Cl^- and aspartate was maintained at 150 mM. The extracellular solution consisted of (in mM): NaCl 140; $MgCl_2$ 2; HEPES 5; $BaCl_2$ 1; $CdCl_2$ 0.1; glucose 10; pH was adjusted to 7.4 using NaOH. In some experiments, 140 mM sucrose replaced 70 mM NaCl in the isoproterenol-osmotic solution. For recording the calcium currents in some human ventricular myocytes, the superfusate was changed to Tris solution containing (mM): Tris (hydroxymethyl) aminomethane 137, $MgCl_2$ 1, $CaCl_2$ 5.4, $CsCl_2$ 20, glucose 5 and brought to pH 7.4 with 1 N HCl. The isoproterenol-induced Cl^- current was activated by exposing the cells to maximally stimulating concentrations of isoproterenol (1 μM) or forskolin (10 μM) in the extracellular solution.

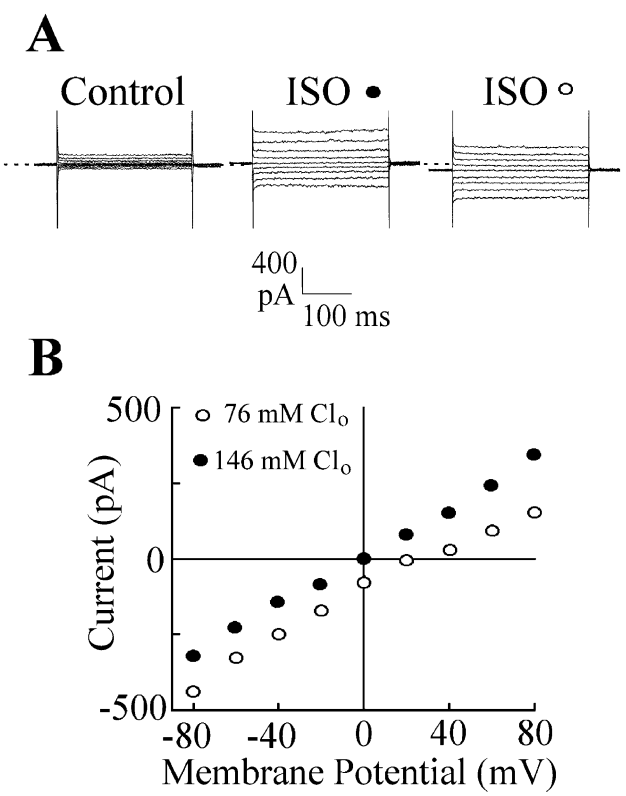

Fig. 2. Effects of changing extracellular Cl^- concentration on the isoproterenol (ISO)-activated Cl^- currents in a human atrial myocyte. (A) Membrane currents recorded from the same cell during 300-ms voltage-clamp steps to potentials ranging from −80 to +80 mV from a holding potential of 0 mV in the absence of isoproterenol (left), the external solution contains 146 (middle) or 76 mM Cl^- (right) after exposure to 1 μM isoproterenol, while the internal solution contained 150 mM Cl^-. Total cell capacitance, 86 pF. (B) Current–voltage relationship of the isoproterenol-induced current determined in 146 and 76 mM Cl^- external solutions. The isoproterenol-induced current was defined as the difference current obtained by subtraction of current traces recorded under control conditions from those recorded in the presence of isoproterenol in either 146 or 76 mM Cl^- external solutions.

Isoproterenol, forskolin, endothelin-1 and all other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.4. Data analysis

The Cl^- current was defined as the agonist-induced difference current determined by subtracting currents recorded in the absence of drug from currents recorded in the presence of drug. In some figures, current measurements were normalized to cell capacitance. Averaged data are expressed as means ± S.E.M. Statistical analyses were performed with Student's *t*-test. Changes were considered significant at $P < 0.05$. Where indicated, the data were fitted using a non-linear, least squares curve-fitting routine (Sigma Plot, Jandel, San Rafael, CA, USA).

3. Results

Under our experimental conditions, with selected external and internal solutions, most of the ionic currents known in cardiac cells were inhibited. The K^+ channels were blocked by the application of external Ba^{2+}, internal tetraethylammonium and the removal of K^+ from both external and internal solutions. The Ca^{2+} channels were blocked by adding Cd^{2+} in the superfusates and by removing Ca^{2+} from the external solution. The Na^+ channels were inactivated by using a holding potential of 0 mV. Thus, we consider that the remaining current reflects solely the background current. To examine whether there is cAMP-dependent Cl^- conductance in the human atrial cell, two different cAMP-activating agents, isoproterenol and forskolin, were applied. Isoproterenol is an unselective beta-adrenoceptor agonist while forskolin is a potent activator of adenylate cyclase.

3.1. Effects of isoproterenol and forskolin on membrane conductance in the human atrial myocytes

Fig. 1A shows the membrane currents in response to 300-ms square pulses from a holding potential of 0 mV. The external solution contained 150 mM Cl^- and the cells were dialyzed with an internal solution that contained 150 mM Cl^-. Under control conditions (Fig. 1A, left), the background current traces did not show any marked time-dependent changes. Application of isoproterenol (1 μM) resulted in a considerable increase in membrane conductance as shown in Fig. 1A (right). The current component induced by isoproterenol was obtained at various testing voltages (−80 to +80 in 20 mV steps) by subtracting the control current from the current obtained in the presence of isoproterenol (Fig. 1B). Fig. 1C shows the time course of isoproterenol-induced changes in background current activated during 300-ms step depolarizations to +80 mV from a holding potential of 0 mV. It is clearly demonstrated that the isoproterenol (1 μM)-induced rise of the background current is reversible. The ionic gradient dependence of the isoproterenol-induced Cl^- current is illustrated in Fig. 1D, which shows the *I–V* relations from experiments where

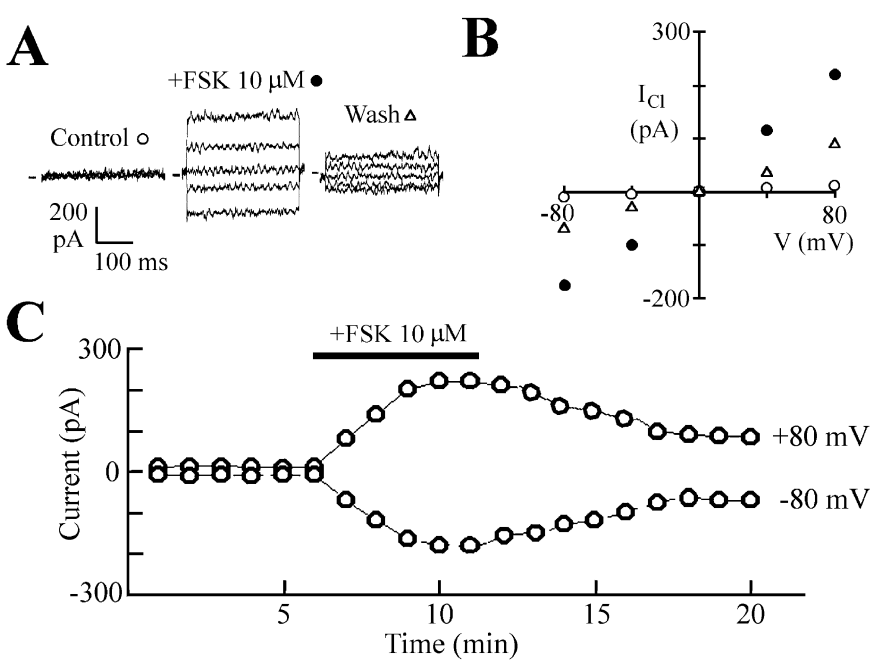

Fig. 3. Activation of I_{Cl} by forskolin (FSK) 10 μM in a human atrial myocyte. (A) Difference currents elicited by voltage pulses to ±40 and ±80 mV (holding potential 0 mV), recorded under symmetric Cl^- concentrations before (left), after (center) exposure to 10 μM forskolin and washout of forskolin. Total cell capacitance, 107 pF. (B) Current–voltage relationships from (A) showing forskolin-induced Cl^- conductance (●) and washout of forskolin (△). (C) Time course of forskolin-induced changes in membrane current monitored at −80 and +80 mV.

the external solution contained 150 mM Cl^- and cells were dialyzed with an internal solution that contained either 40 ($n = 4$) or 150 mM Cl^- ($n = 5$). Intracellular Cl^- was reduced by replacement with aspartate. The isoproterenol-induced current in atrial myocytes was time-independent and had a reversal potential close to zero in the presence of symmetrical Cl^- concentrations inside and out (solid circles, Figs. 1D and 2). When [Cl^-] in the bath solution was decreased to 76 mM, the *I–V* relationship showed an inward rectification with a reversal potential of 20 mV (open circles, Fig. 2), which is very close to the equilibrium potential for the [Cl^-] gradient (i.e. 17.5 mV). The changes in membrane conductance are attributable to the Cl^- current, since the isoproterenol-induced current–voltage (*I–V*) relations (Figs. 1D and 2) showed similar shape and a common reversal potential near the Cl^- equilibrium potential (E_{Cl}). The Cl^- current was elicited by isoproterenol (1 μM) in 5 of 22 atrial myocytes under symmetrical Cl^- concentration. The current density of the Cl^- current was 3.1 ± 0.6 pA/pF at +80 mV under symmetrical Cl^- concentration with isoproterenol (1 μM) in five human atrial myocytes (Fig. 5).

We also tested the effect of forskolin (10 μM) on the membrane current to determine whether the Cl^- current can also be activated by the adenylyl cyclase-protein kinase A pathway in human atrial myocytes. Fig. 3 shows the result of an experiment with an atrial myocyte treated with forskolin for 5 min. The Cl^- current induced was essentially time-independent during exposure to forskolin (Fig. 3A). The *I–V* relationship of the current activated by forskolin (forskolin-stimulated minus control background current) exhibited a linear relation (Fig. 3B). In the presence of 150 mM Cl^- inside and out, the reversal potential of the current was near 0 mV, the predicted equilibrium potential for Cl^-. Currents monitored at −80 and +80 mV increased to a new steady state within 1 min (Fig. 3C). The summarized results for 15 experiments similar to that in Fig. 3A are shown in the histogram of Fig. 5. The results clearly showed that the Cl^- current was elicited by forskolin (10 μM) in 3 of 15 atrial myocytes under symmetrical Cl^- concentration. The current density of the Cl^- current was 4.1 ± 0.7 pA/pF at +80 mV with forskolin (10 μM) in three human atrial myocytes (Fig. 5).

3.2. Effects of isoproterenol and forskolin on membrane conductance in human ventricular myocytes

In rabbit and guinea pig, the CFTR Cl^- current (i.e. $I_{Cl,cAMP}$) proved to be present with the highest density in ventricular myocytes (Du et al., 2000). In order to examine whether the $I_{Cl,cAMP}$ exists and can also be activated in human ventricular myocytes, the parallel experiments to that in human atrial myocytes were done. A representative experiment investigating the effect of forskolin on membrane conductance in human ventricular myocytes is shown in Fig. 4. There was no change in steady-state current in response to forskolin as shown in the upper row of Fig. 4A. To rule out the possibility of membrane malfunction

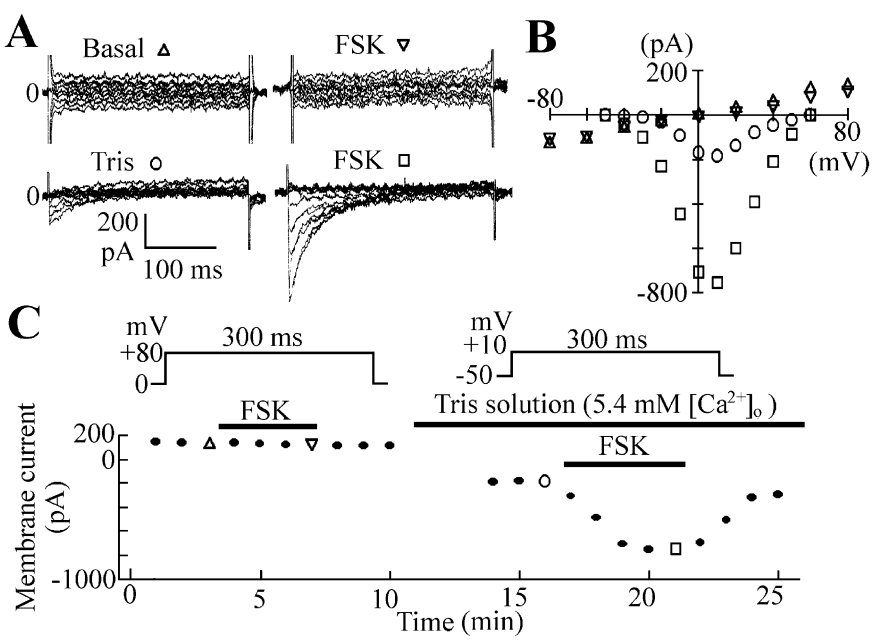

Fig. 4. Lack of effect of forskolin (FSK) on I_{Cl} in a human ventricular myocyte. (A) Upper panels: Currents recorded under symmetrical Cl^- concentration before (left) and after (right) exposure to 10 μM forskolin. Currents were elicited by 300-ms voltage clamp steps to membrane potentials between −80 and +80 mV, in 20-mV increments (holding potential 0 mV) Lower panels: Currents recorded in the Tris solutions before (left) and after (right) exposure to 10 μM forskolin. Currents were elicited by 300-ms voltage clamp steps to membrane potentials between −50 and +60 mV, in 10-mV increments (holding potential −50 mV). (B) Current–voltage relationships from (A) showing basal and forskolin-induced membrane conductance. (C) Time course of forskolin-induced changes in membrane current in the presence of various superfusates.

caused by heart disease de nova or due to the process of cell isolation, parallel experiments were repeated with changing of superfusate to a Tris bathing solution that contained 5.4 mM calcium (lower row of Fig. 4A and right part of Fig. 4C). Under these conditions, there was a dramatic increase in the peak net inward current, i.e. L-type Ca^{2+} ($I_{Ca,L}$) current, during voltage steps from −50 to +10 mV. The effects of forskolin on Cl^- current and $I_{Ca,L}$ can be seen more clearly in the *I–V* curve of Fig. 4B. In other words, the positive response to forskolin on $I_{Ca,L}$ shows that there is no forskolin-induced, i.e. AMP-dependent, Cl^- current in the human ventricular myocytes. The lack of effect of forskolin on the steady state of the Cl^- current was observed in nine of nine ventricular myocytes (Fig. 5). A similar result was obtained in the experiments using isoproterenol as agent to evoke cAMP-dependent Cl^- current (original figure not shown). As shown in Fig. 5, the isoproterenol-evoked Cl^- current was absent in all six of six ventricular myocytes. We concluded from the above results that the isoproterenol- and forskolin-induced Cl^- current exists in human atrial myocytes but not in human ventricular myocytes.

3.3. Inhibition of the isoproterenol-induced Cl^- current by endothelin-1 in human atrial myocytes

Given the existence of the $I_{Cl,cAMP}$ in human atrial myocytes, we therefore further examined the effect of endothelin-1 on the current conductance induced by isoproterenol. Since $I_{Cl,cAMP}$ evoked by isoproterenol was not found in all the human atrial myocytes (Fig. 5), only those human atrial myocytes that showed the isoproterenol-induced Cl^- current were chosen for this study. Fig. 6A shows an example of an atrial myocyte. The top trace of Fig. 6A is the control trace in which the background membrane conductances of voltage-clamped human atrial myocyte, dialysed internally with 150 mM Cl^- pipette solution and superfused with 150 mM Cl^- external solution, were elicited by a series of depolarizations from a holding potential of 0 mV. Application of isoproterenol (1 μM) resulted in an increase in membrane conductance as shown in Fig. 6A (○). This isoproterenol-induced change was inhibited significantly by the addition of endothelin-1 (30 nM; ●) and partially reversible after washout of endothelin-1 (△). The *I–V* relations of the isoproterenol- and/or endothelin-induced difference currents are shown in Fig. 6B. Exposure to 30 nM endothelin-1 for 3 min reduced the current by 77% (from 4.0 to 0.9 pA/pF at +80 mV) (Fig. 6C). Endothelin-1 did not affect the reversal potential, demonstrating that endothelin-1 reduced the membrane Cl^- conductance specifically. In four human atrial myocytes, 30 nM endothelin-1 inhibited the Cl^- currents induced by 1 μM isoproterenol by 75 ± 6% (from 3.4 ± 0.5 to 0.8 ± 0.2 pA/pF at +80 mV; Fig. 7). This

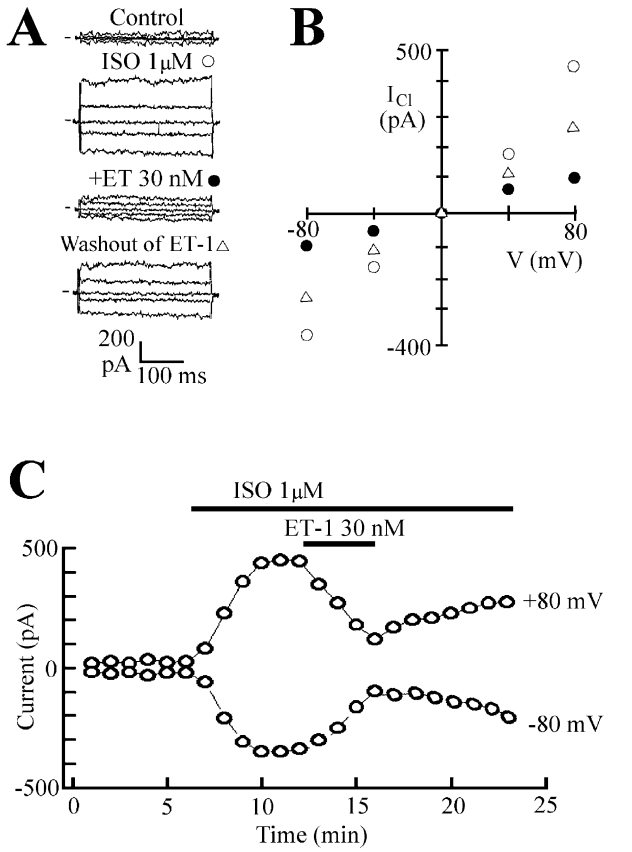

Fig. 6. Endothelin-1 (ET-1) inhibits the isoproterenol (ISO)-induced current in human atrial myocytes. (A) Difference currents elicited by voltage pulses to ±40 and ±80 mV, showing the resting conductance (control), the 1 μM isoproterenol-induced current (○), the current inhibited by 30 nM endothelin-1 (●) and washout of endothelin-1 (△) in isoproterenol-containing superfusate. Holding potential = 0 mV. Total cell capacitance, 118 pF. For each set of traces, a bar indicates the zero current level. (B) Current–voltage relationships from (A) showing isoproterenol-induced Cl^- conductance (○), its inhibition by endothelin-1 (●) and washout of endothelin-1 (△). (C) Time course of the inhibition of I_{Cl} by endothelin-1 in a myocyte pretreated with 1 μM isoproterenol. Membrane currents were monitored at −80 and +80 mV.

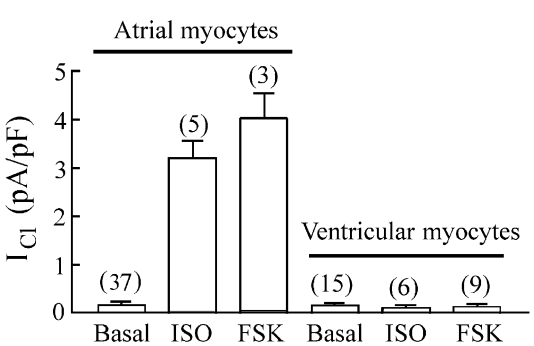

Fig. 5. Comparison of I_{Cl} in basal and isoproterenol (ISO)- or forskolin (FSK)-activated states in human cardiomyocytes. I_{Cl} was recorded under symmetrical Cl^- concentrations. Difference currents elicited by voltage pulses from 0 to +80 mV. Bar graph shows mean values (means ± S.E.M.). Numbers in parenthesis correspond to number of cells investigated.

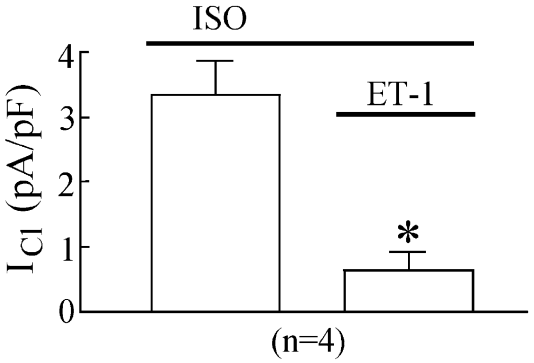

Fig. 7. Summary of results obtained with 5-min application of 30 nM endothelin-1 (ET-1) in the presence of isoproterenol (ISO). Difference currents elicited by voltage pulses from 0 to +80 mV. I_{Cl} activated by 1 μM isoproterenol was significantly reduced by the endothelin-1 ($^{*}P < 0.05$). Number of myocytes is in parentheses.

result demonstrated that endothelin-1 is able to inhibit the cAMP-dependent Cl^- current in human atrial myocytes.

Endothelin-1, acting via endothelin ET_A (endothelin-1-selective) receptors, inhibited the cAMP-dependent Cl^- current ($I_{Cl,cAMP}$) and calcium current ($I_{Ca,L}$) by decreasing cyclic AMP accumulation through a pertussis toxin-sensitive mechanism in guinea-pig cardiac cells (James et al., 1994; Ono et al., 1994). To test if the functionally important sub-receptor that contributed to the inhibitory effect of endothelin-1 on the $I_{Cl,cAMP}$ in human atrial myocyte is endothelin ET_A, we further pretreated with specific blockers of endothelin ET_A or ET_B 10 min before adding endothelin-1. In all three experiments, we found that the inhibitory effect of endothelin-1 on the isoproterenol-induced Cl^- current was attenuated by pretreating atrial myocytes with the endothelin ET_A receptor antagonist, BQ485, but not with the endothelin ET_B receptor antagonist, BQ-788 (data not shown). Therefore, our data suggest that the functional receptor for endothelin-1 in human atrial myocytes is most likely to be endothelin ET_A, instead of endothelin ET_B.

4. Discussion

Many types of Cl^- current have been reported in the hearts of various species, including an isoproterenol-induced Cl^- current identified in guinea pig and rabbit cardiac myocytes (Hume and Harvey, 1991). Recent electrophysiological data also suggested a number of similarities in the properties of protein kinase A-dependent Cl^- channels in heart and cyclic AMP (cAMP)-dependent Cl^- channels encoded by the cystic fibrosis transmembrane conductance regulator (CFTR) gene product in various epithelial cells (Gadsby et al., 1995). Molecular data also demonstrated that there is a high degree of sequence homology between CFTR and the cAMP-dependent Cl^- channel cloned from cardiac myocytes (Levesque et al., 1992). The initial goal of this study was to determine whether human cardiac cells possessed the cAMP-activated Cl^- current similar to that observed in guinea pig cardiac ventricular myocytes, and to examine the effect of endothelin-1 on the cAMP-activated Cl^- current. In the present study, isoproterenol, an unselective beta-adrenoceptor agonist, and forskolin, a potent activator of adenylate cyclase, were used in vitro on human atrial and ventricular tissues to increase the concentration of cAMP.

The isoproterenol-induced Cl^- current found in the human atrial myocyte was time-independent and had a reversal potential close to zero in the presence of symmetrical Cl^- concentrations inside and out. However, it was only elicited in 12 of 54 human atrial myocytes. Similar findings were also reported for inside-out patches, with the giant-patch technique, for human atrial myocytes where unitary Cl^--sensitive channels were activated by the catalytic subunit of protein kinase A in 3 of 12 patches examined (Warth et al., 1996). However, our previous report showed that the calcium current was elicited in almost all human atrial myocytes (Cheng et al., 1996). Our findings clearly demonstrate the presence of cAMP-dependent Cl^- currents in human atrial cells in agreement with previous reports (Hume et al., 1994; Warth et al., 1996). The data, however, contrast with several reports that show this isoproterenol-induced Cl^- conductance occurs rarely, in 1 of 20 pediatric human atrial myocytes (Berul et al., 1997) or is absent in human myocardium (Oz and Sorota, 1995; Sakai et al., 1995). Possible solutions for this discrepancy include the procedure for cell isolation after enzyme digestion, specimens from the patients with different disease status or age, or even racial differences. There is also an agreement between our electrophysiological results and the reports of mRNA for CFTR Cl^- channels in human atrium found by Northern blot analysis (Levesque et al., 1992). We have no explanation, as yet, for the finding that the cAMP-activated Cl^- current could only be elicited in $< 25\%$ of the human atrial myocytes studied. Vandenberg et al. (1994) reported that the isoproterenol-induced Cl^- current was elicited in $< 10\%$ atrial cells isolated from guinea pig hearts. Regional differences in CFTR mRNA expression in the guinea pig heart were reported to be responsible, at least in part, for the regional differences in cAMP-activated Cl^- current density (James et al., 1996). It is possible that the cAMP-activated Cl^- channels may only be expressed in a small percentage of human atrial cells.

In agreement with another report (Oz and Sorota, 1995), we saw no effect of isoproterenol or forskolin on activation of the cAMP-activated Cl^- current in 15 of 15 ventricular cells isolated from four human patients. Especially, the fact that forskolin stimulates L-type Ca^{2+} current but does not elicit $I_{Cl,cAMP}$ (Fig. 4) is a valid control experiment to show that human ventricular cells lack cAMP-activated Cl^- conductance. Because failing human

ventricle was used for these studies, we cannot exclude the possibility that the cAMP-activated Cl^- channel is only present in human ventricular cells isolated from non-failing hearts.

The anti-adrenergic action of endothelin-1 is mediated via G-protein-dependent inhibition of adenylyl cyclase and a reduced intracellular cAMP level (James et al., 1994; Ono et al., 1994; Cheng et al., 1995). Our finding of the inhibition of the isoproterenol-induced Cl^- current by endothelin-1 in human atrial myocytes is consistent with that from animal studies. The mechanisms of the endothelin-1-induced reduction of the isoproterenol-induced Cl^- current in human atrial myocytes are not completely understood. However, in our present study, we found that this inhibitory effect of endothelin-1 was attenuated by pretreating atrial myocytes with the endothelin ET_A receptor antagonist, BQ485, but not with the endothelin ET_B receptor antagonist, BQ-788. Therefore, this suggests that the functional receptor of endothelin-1 in human atrial myocytes is most likely to be endothelin ET_A. Furthermore, inhibition of the outward Cl^- current at the plateau potential range by endothelin-1 may balance a decrease in the inward current through L-type Ca^{2+} channels (Cheng et al., 1995). The action potential duration of atrial cells is therefore unchanged in the presence of endothelin-1 as observed in our previous experiments with isolated atrial tissues (Lin et al., 1994). Further studies will be necessary to get an insight into the mechanisms involved in the action of endothelin-1 on the isoproterenol-induced Cl^- current in human atrial cells.

In summary, the present study provided straightforward and convincing evidence that there is a whole-cell Cl^--selective membrane current in freshly isolated human atrial myocytes whose properties are consistent with those of the $I_{Cl,cAMP}$ (CFTR-related channel). As found by others, $I_{Cl,cAMP}$ elicited by isoproterenol or forskolin was sparsely expressed in atrial cells but was completely absent in ventricular myocytes. In atrial cells, the isoproterenol-induced Cl^- conductance could be partially attenuated by endothelin-1 through, mainly, the sub-receptor of endothelin ET_A.

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