β_1-adrenergic regulation of rapid component of delayed rectifier K^+ currents in guinea-pig cardiac myocytes

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Abstract. Human ether-à-go-go-related gene (hERG) potassium channels conduct the rapid component of the delayed rectifier potassium current (I_{Kr}), which is crucial for repolarization of cardiac action potential. Patients with hERG-associated long QT syndrome usually develop tachyarrhythmias during physical and/or emotional stress, both known to stimulate adrenergic receptors. The present study aimed to investigate a putative functional link between β_1-adrenergic stimulation and I_{Kr} in guinea-pig left ventricular myocytes and to analyze how I_{Kr} is regulated following activation of the β_1-adrenergic signaling pathway. The I_{Kr} current was measured using a whole-cell patch-clamp technique. A selective β_1-adrenergic receptor agonist, xamoterol, at concentrations of 0.01-100 μM decreased I_{Kr} in a concentration-dependent manner. The 10 μM xamoterol-induced inhibition of I_{Kr} was attenuated by the protein kinase A (PKA) inhibitor KT5720, the protein kinase C (PKC) inhibitor chelerythrine, and the phospholipase (PLC) inhibitor U73122, indicating involvement of PKA, PKC and PLC in β_1-adrenergic inhibition of I_{Kr}. The results of the present study indicate an association between I_{Kr} and the β_1-adrenergic receptor in arrhythmogenesis, involving the activation of PKA, PKC and PLC.

Introduction

Repolarization of the cardiac action potential is accomplished by several types of potassium currents. One of these, the rapid component of delayed rectifier potassium current (I_{Kr}), is unique in its ability to modify membrane repolarization at the end of each cardiac action potential (1). Activation of I_{Kr}, which is predominantly carried through the human ether-à-go-go-related gene (hERG) potassium ion channels, initiates membrane repolarization and terminates the plateau phase of the cardiac action potential (2). Mutation in hERG or pharmacological blockade of the hERG channels can produce an excessive prolongation of the action potential duration and the QT interval, leading to proarrhythmic events usually characterized by polymorphic ventricular tachycardia or torsades de pointes (3-9). Such cardiac electrical disturbances are often closely correlated with physical or emotional stress, particularly in patients with hereditary long QT syndrome, indicating a potential correlation between adrenergic stimulation and hERG potassium channel activity (10).

Previous studies have revealed that hERG/I_{Kr} currents are modulated by α- and β-adrenergic stimulation, thus providing a pathophysiological rationale for an increased incidence of arrhythmias during stress (11-14). In human hearts, there are several main subfamilies of the adrenergic receptor (adrenoceptor) family, namely α_1-, α_2-, β_1-, β_2- and β_3-adrenoceptors. Our previous study found that I_{Kr} currents in the guinea-pig left ventricular myocytes was regulated by α_1-adrenergic stimulation via protein kinase C (PKC)- and protein kinase A (PKA)-dependent pathways (15).

The β_1- and β_2-adrenoceptors are the predominant subtypes in the heart. In human myocardium, β_1-adrenoceptors constitute 70-80% of the total β-adrenoceptors abundance (16) and an altered β_1-adrenoceptor activity and/or signaling are associated with a high incidence of cardiac arrhythmias (17). β_1-adrenoceptor coupled with Gs-protein stimulates adenylate cyclase (AC), resulting in the accumulation of cyclic adenosine monophosphate (AMP) and the activation of PKA. The activation of the AC/cAMP/PKA pathway results in a complex regulation of hERG/I_{Kr}. However, whether PKC and PLC are involved in β_1-adrenoceptor-induced regulation of I_{Kr} remains unclear. The present study aimed to investigate how I_{Kr} is regulated in guinea-pig cardiomyocytes following activation of β_1-adrenergic receptors, and the involvement of activation of PKA, PKC and PLC.

Materials and methods

Animal and myocyte isolation. All experiments were approved by Animal Care Protocols of Nanjing Medical University Institutional Animal Care and Use Committee (Nanjing, China). Single left ventricular myocytes were

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enzymatically isolated from guinea-pig heart as described previously (18) with minor modifications. Briefly, male healthy guinea pigs (weight, 300-350 g; provided by the Experimental Animal Center of Jiangsu Province, China) were sacrificed by cervical dislocation, and the heart was then rapidly removed and cannulated at the aorta. Following perfusion with an enzymatic solution, the left ventricular tissue was excised from the softened hearts, minced, and simultaneously filtered cardiomyocytes were stored at 4˚C prior to patch clamp recording.

Electrophysiology recording. Cardiomyocytes were transferred to a recording chamber (Warner TC-324B; Warner Instruments, Hamden, CT, USA) continuously perfused with the bath solution. Pipettes had resistances of 3-6 MΩ subsequent to filling with the pipette solution. Whole-cell patch-clamp recordings were performed with an EPC-9 amplifier (HEKA, Lambrecht, Germany). All the recordings were conducted at 37±0.5˚C and the flow rate was maintained at ~2 ml min^{-1}.

Solutions and drugs. In order to record the I_{Kr} current, the pipette solution contained 140 mmol l^{-1} KCl, 1 mmol l^{-1} $CaCl_2$, 2 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} HEPES, 11 mmol l^{-1} EGTA, 5 mmol l^{-1} Na_2-ATP and 5 mmol l^{-1} creatine phosphate (disodium salt); pH 7. 4 adjusted with 8 M KOH. The bath solution contained 140 mmol l^{-1} NaCl, 3.5 mmol l^{-1} KCl, 1.5 mmol l^{-1} $CaCl_2$, 1.4 mmol l^{-1} $MgSO_4$ and 10 mmol l^{-1} HEPES; pH adjusted to 7.4 with 10 M NaOH. Calcium currents were blocked by 10 μM nifedipine in the bath solution and 10 μM chromanol 293B was used to ablate the slow component of the delayed rectifier potassium currents (I_{Ks}). Na_2-ATP, EGTA, nifedipine, chromanol 293B, chelerythrine, U73122 and xamoterol were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA), collagenase II from Worthington (Lakewood, NJ, USA) and KT5720 from Merck (Darmstadt, Germany). Dofetilide, a specific blocker of I_{Kr} or hERG, was provided by Pfizer (Shanghai, China). All the other reagents were purchased from Amresco (Solon, OH, USA).

For stock solutions, dofetilide was dissolved in distilled water to a concentration of 10 mM; KT5720, chelerythrine and U73122 were dissolved in dimethylsulfoxide (DMSO) to a concentration of 2.5 mM, 1 mM and 0.1 mM. These chemicals were stored at -20˚C until further use. The final concentration of DMSO was <0.5% in the bath solution and exerted no effect on the currents that were observed.

Quantification and statistics. Following initiation of the test pulse, tail currents were measured. Changes in the current amplitude were normalized prior to the application of xamoterol. All the data were acquired by Pulse + Pulsefit V8.53 (HEKA Elektronik, Lambrecht, Germany) and were analyzed by SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Statistical data were presented as the mean ± standard error of the mean. A paired-sample t-test was used for determining significant differences prior to and following the xamoterol intervention. One-way analysis of variance, with a post hoc comparison using a Newman-Keuls test was performed to compare the differences among groups. P<0.05 was considered to indicate a statistically significant difference.

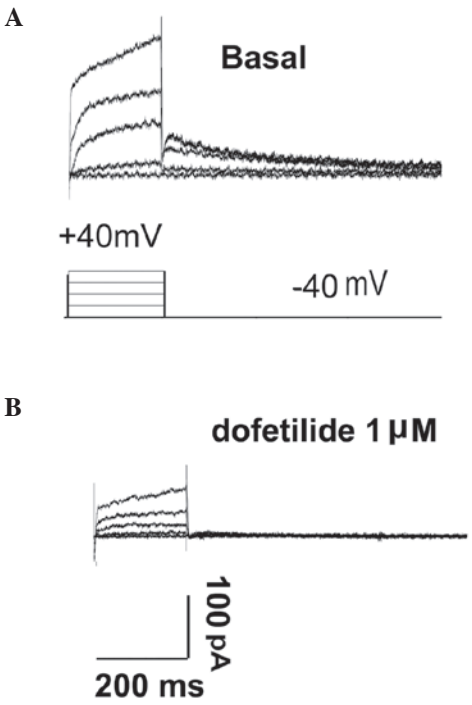

Figure 1. Recordings of I_{Kr} tail currents in the same myocytes prior to and following the administration of dofetilide. Panel A and B separately demonstrate the I_{Kr} current traces from a typical cell prior to and following 1 μM dofetilide application. During the test pulse, inward calcium currents were measured and displayed variable current amplitude. The I_{Kr} tail currents were measured following the return to a constant -40 mV and were blocked by the specific I_{Kr} blocker dofetilide, demonstrating that I_{Kr} tail currents were measured during the return pulse without contamination by other currents under the given experimental conditions. A holding potential of -40 mV, test pulses from -40 to +40 mV by 20 mV increments (duration 200 ms) and a return pulse constant of -40 mV (600 ms) were used to measure the I_{Kr} tail currents. I_{Kr}, delayed rectifier potassium current.

Results

Effects of xamoterol on I_{Kr} tail currents. A representative I_{Kr} tail current from a guinea-pig ventricular myocyte is shown in Fig. 1A. In Fig. 1B, the current was completely blocked by 1 μM dofetilide, a specific inhibitor of I_{Kr}, indicating lack of contribution from any other current to the tail current in the experimental settings of the present study. The dose-dependent effects of xamoterol, a specific β_1-adrenoceptor agonist, on I_{Kr} current amplitude were examined in freshly isolated guinea-pig ventricular myocytes.

Fig. 2A shows a representative current trace when the cardiomyocyte was treated with by 0.01 μM-100 μM xamoterol. Fig. 2B shows the concentration-dependent reduction of I_{Kr} elicited by xamoterol in cardiomyocytes. In the cardiomyocytes examined (n=5), the bath application of 0.01, 0.1, 1, 10 and 100 μM xamoterol significantly reduced the I_{Kr} current amplitude to 0.96±0.12, 0.86±0.13, 0.67±0.12, 0.59±0.10 and 0.55±0.11 respectively, compared with the basic amplitude. The concentration of 10 μM was selected for xamoterol for the rest of this study, since this concentration had almost decreased the current by the maximum degree. To examine whether the xamoterol-induced effect was β_1-adrenoreceptor-mediated, the specific β_1-adrenoceptor

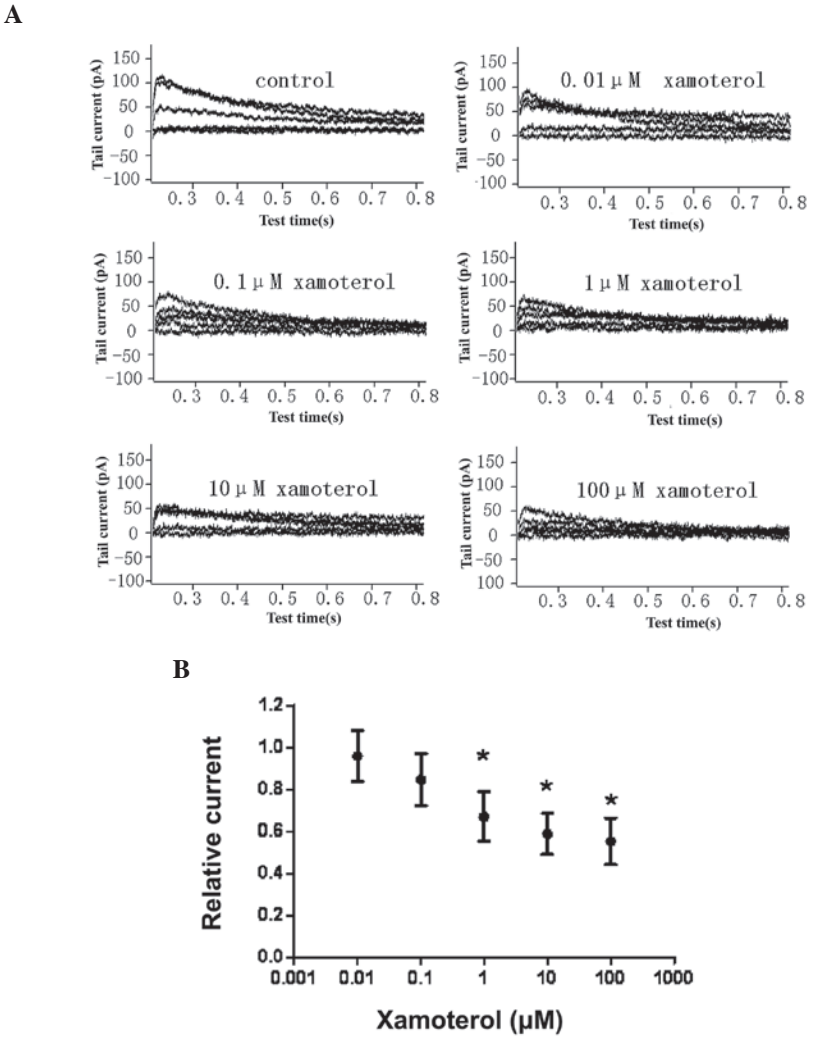

Figure 2. Concentration-dependent effects of xamoterol on the I_{Kr} tail currents. (A) The representative current traces of I_{Kr} prior to and following treatment by various concentrations of xamoterol. (B) The relative I_{Kr} tail currents following administration of 0.01-100 μM xamoterol. Current amplitudes were measured at +40 mV and were normalized to the value prior to xamoterol perfusion (n=5, *P<0.05 vs. control). I_{Kr}, delayed rectifier potassium current.

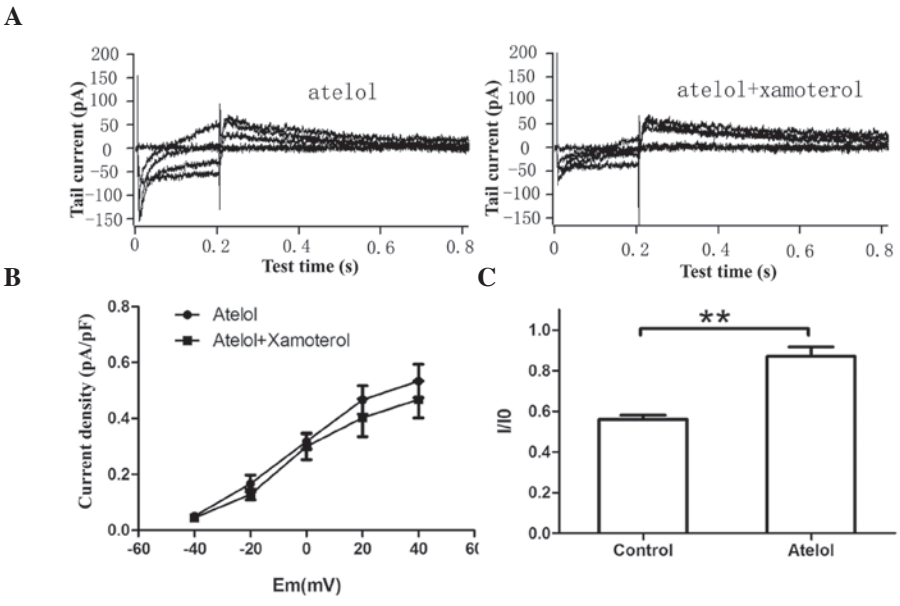

Figure 3. Effects of xamoterol in the cells pretreated with 10 μM of the selective β1-adrenoceptor blocker atenolol. (A) Representative current traces of I_{Kr} tail currents prior to and following application of 10 μM xamoterol in the presence of atenolol. (B) The tail current density-voltage correlation of the two groups. (C) The I_{Kr} reduction of xamoterol may be blocked by atenolol, I/I_0 reflect the inhibitory effects of xamoterol in the presence of empty bath solution and bath solution with atenolol (n=7 in the control group and n=5 in the atenolol group, **P<0.01 vs. control). I_{Kr}, delayed rectifier potassium current.

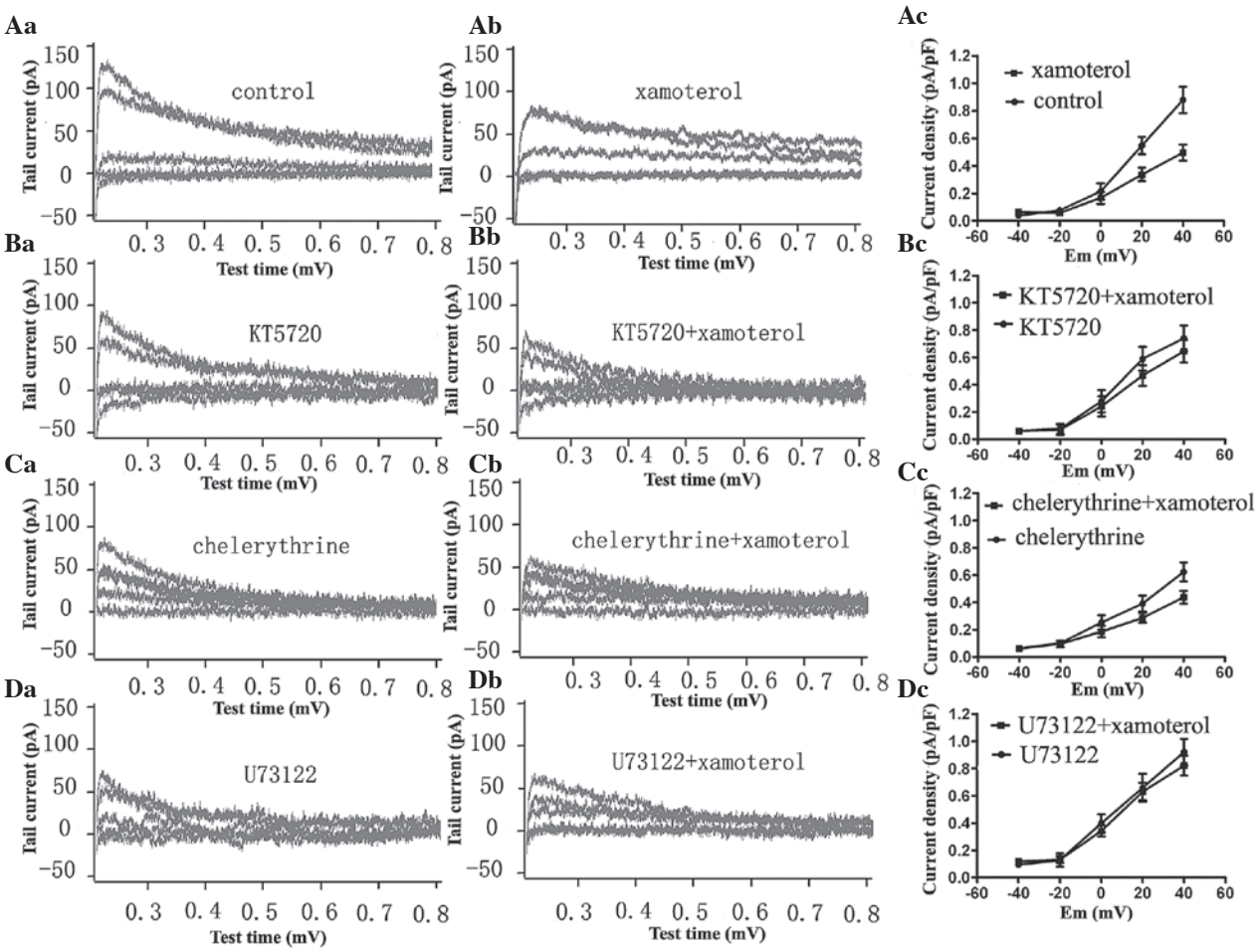

Figure 4. Effects of xamoterol on I_{Kr} tail currents in the presence of an empty bath solution, PKA inhibitor (KT5720), PKC inhibitor (chelerythrine) or PLC inhibitor (U73122). (A) The original I_{Kr} tail current traces (Aa) prior to and (Ab) following application of 10 μM xamoterol in the presence of empty bath solution and (Ac) the corresponding I_{Kr} tail current density-voltage correlation. (B) The original I_{Kr} tail currents from cells pretreated with 2.5 μM KT5720 (Ba) prior to and (Bb) following administration of 10 μM xamoterol and (Bc) the corresponding I_{Kr} tail current density-voltage correlation. (C and D) The original I_{Kr} tail currents from cells pretreated with 1 μM chelerythrine and 100 nM U73122, respectively, (Ca and Da) prior to and (Cb and Db) following perfusion of 10 μM xamoterol and (Cc and Dc) the corresponding I_{Kr} tail current density-voltage correlation (n=7 in each group).

blocker 10 μM atenolol was coincubated with 10 μM xamoterol. This resulted in a decrease in the current amplitude to only 0.87±0.05 at +40 mV, significantly different from the current treated with 10 μM xamoterol alone (0.56±0.04) (Fig. 3). These results indicate that I_{Kr} is regulated by β_1-adrenoceptors in guinea-pig cardiomyocytes.

Effects of PKA inhibitor on xamoterol-induced inhibition of I_{Kr}. Cardiomyocytes were pretreated with 2.5 μM KT5720, a specific PKA inhibitor, for 1 h prior to the examination of the 10 μM xamoterol-elicited effect on I_{Kr}. The I_{Kr} tail current and the current density-voltage curve prior to and following administration of xamoterol in cells pretreated with KT5720 is shown in Fig. 4B. In the present study, the I_{Kr} tail current density decreased from 0.74±0.09 to 0.64±0.08 pA/pF at +40 mV. However, 10 μM xamoterol was found to reduce the I_{Kr} tail current density from 0.88±0.09 to 0.50±0.05 pA/pF at +40 mV in the presence of an empty bath solution (Fig. 4A). In other words, the I_{Kr} tail current amplitude was reduced to 0.87±0.03 subsequent to 10 μM xamoterol in the presence of KT5720 (the second column in Fig. 5), which was significantly different from that in the absence of KT5720, 0.56±0.04 (the control group, the first column in Fig. 5). These data demonstrate that a xamoterol-induced decrease in I_{Kr} was reversed by KT5720.

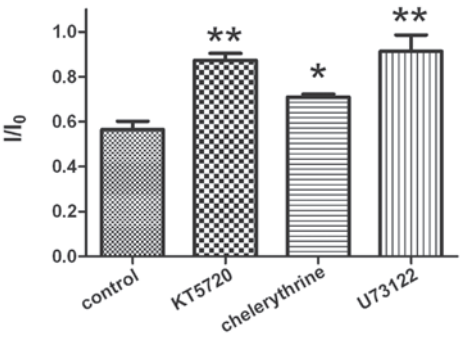

Figure 5. Ratios of I_{Kr} tail currents prior to and following administration of xamoterol in the presence of an empty bath solution, KT5720, chelerythrine or U73122. I_0 and I respectively represent the I_{Kr} tail currents at +40 mV prior to and following treatment of 10 μM xamoterol, therefore the ratios of I/I_0 reflect the inhibitory effects of xamoterol in the presence of an empty bath solution and different inhibitors (n=7, *P<0.05 and **P<0.01 vs. control group).

Xamoterol-induced inhibition of I_{Kr} is antagonized by the PKC inhibitor chelerythrine and the PLC inhibitor U73122. The guinea-pig left ventricular myocytes were pretreated with the 1 μM specific PKC inhibitor chelerythrine or 100 nM PLC inhibitor U73122 for one hour, and then the I_{Kr} tail currents prior to and following xamoterol administration were examined. Xamoterol reduced the I_{Kr} tail current density from 0.62±0.07 to 0.44±0.05 pA/pF at +40 mV (Fig. 4C), and it decreased I_{Kr} to 0.71±0.01 pA/pF in the presence of chelerythrine (Fig. 5), which was significantly different from that in the absence of chelerythrine, the control group, 0.56±0.04 pA/pF (Fig. 5). The current trace and the tail current density-voltage (Id-V) curve almost superimposed prior to and following xamoterol treatment in the presence of U73122 (Fig. 4D), from 0.92±0.09 to 0.82±0.07 pA/pF at +40 mV, indicating that xamoterol failed to suppress I_{Kr} when myocytes were pretreated with the PLC inhibitor. However, the effects of xamoterol were significantly different between the control and the U73122 group (Fig. 5).

Discussion

The present study indicated that xamoterol inhibits I_{Kr} through β-adrenoceptors in freshly isolated guinea-pig cardiomyocytes. Furthermore, this inhibitory effect was significantly attenuated by the PKA inhibitor KT5720, the PLC inhibitor U73122 and the PKC inhibitor chelerythrine. These data indicated the involvement of PKA, PKC and PLC activation in the β1-adrenoceptor-induced inhibition of the I_{Kr} current.

Activation of β_1-adrenoceptors has been demonstrated to elicit an inhibitory effect on I_{Kr} or hERG via a cAMP/PKA-dependent pathway (11,19) consistent with our data that the PKA inhibitor KT5720 attenuated the inhibitory effect of xamoterol. However, in an early report by Heath and Terrar (20), a concentration-independent increase of the I_{Kr} currents was observed at low concentrations of the β_1-adrenergic agonist isoprenaline and the stimulatory effect of isoprenaline on I_{Kr} was inhibited by the selective PKC inhibitor bisindolylmaleimide I. There may be several reasons for the disparate findings between the two laboratories, including differences in patch-clamp modes and experimental conditions, aswell as dual regulation of hERG by cAMP and PKA phosphorylation (21,22). Nonetheless, to the best of our knowledge the involvement of PKC in β_1-adrenergic regulation of I_{Kr} demonstrated in the present study has not been documented previously.

PKC is an important member of the signaling transduction pathway, capable of reducing hERG currents through a mechanism independent of PKC-elicited phosphorylation of hERG (23,24). The results of the present study indicated that xamoterol-induced inhibition of I_{Kr} is partially modulated by PKC. In addition, the decrease in I_{Kr} induced by xamoterol was also antagonized by the selective PLC inhibitor U73122. Usually PLC is linked to α_1-adrenergic stimulation leading to the PIP_2 hydrolysis and then the activation of PKC. PIP_2 depletion has been shown to alter the cardiac I_{Kr} current (25,26). PKC has also been shown to reduce the hERG current (27). Although PKC and PLC are associated with the classical α_1-adrenergic signaling pathway, the results of the present study reveal that PKC and PLC are also activated in the β_1-adrenergic signaling pathway in the regulation of the I_{Kr}/hERG currents, indicating that there may exist a 'cross-talk' between the α_1- and β_1-adrenergic signaling cascades. Therefore, our next aim is to analyze the details in this type of cross-talk and demonstrate direct evidence.

In conclusion, the present study demonstrates that I_{Kr} is regulated by β_1-adrenergic receptors in guinea-pig cardiomyocytes, via the PKA-, PKC- and/or PLC-dependent signaling pathways. These findings provide a possible correlation between stress and life-threatening arrhythmias and may provide insight into the pathogenesis and potential therapeutic strategies for clinical cardiac arrhythmias.

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