Original Article

Adrenergic regulation of the rapid component of delayed rectifier K+ currents in guinea pig cardiomyocytes

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Background: Guinea pig ventricular cardiomyocytes display the rapid component of the delayed rectifier potassium current (I_{kr}) that contributes to ventricular repolarization and promotes stress-induced arrhythmias. Adrenergic stimulation favors ventricular arrhythmogenesis but its effects on I_{kr} are poorly understood.

Methods: Adrenergic modulation of I_{kr} was studied in isolated guinea pig ventricular cardiomyocytes using whole-cell patch clamping.

Results: We found that the I_{kr} amplitude was reduced to 0.66±0.02 and 0.62±0.03 in response to 0.1 μM phenylephrine (PE), an α_1AR agonist, and 10 μM isoproterenol (ISO), a βAR agonist, respectively. The effect of PE can be blocked by the selective α_1A-adrenoceptor antagonist 5-methylurapidil, but not by the α_1B-adrenoceptor antagonist chloroethylclonidine or α_1D-adrenoceptor antagonist BMY7378. Additionally, the effect of ISO can be blocked by the β_1-selective AR antagonist CGP-20712A, but not by the β_2-selective AR antagonist ICI-118551. Although PE and ISO was continuously added to cells, ISO did not decrease the current to a greater extent when cells were first given PE. In addition, PE's effect on I_{kr} was suppressed by β1AR stimulation.

Conclusions: I_{kr} can by regulated by both the α_1 and β ARs system, and that in addition to direct regulation by each receptor system, crosstalk may exist between the two systems.

Keywords: Adrenergic receptors (ARs); potassium current; crosstalk; cardiomyocytes

Submitted Aug 12, 2014. Accepted for publication Nov 13, 2014.
doi: 10.3978/j.issn.2072-1439.2014.12.27
View this article at: http://dx.doi.org/10.3978/j.issn.2072-1439.2014.12.27

Introduction

Cardiac action potential repolarization is carried out by different potassium currents. The rapid component of the delayed rectifier potassium current (I_{kr}) is the most important component for phase 3 repolarization and is required to control orderly repolarization at the end of each cardiac action potential, particularly near the threshold potential of early afterdepolarizations that can trigger tachyarrhythmias (1). The human ether-a-go-go-related gene (*bERG*) encodes the alpha subunit of I_{kr} current (2-5). HERG channels are a primary target for the pharmacological management of cardiac arrhythmias using class III antiarrhythmic agents, such as dofetilide and amiodarone (6-8). Reduced hERG currents due to hERG mutations or excessive blockade of hERG channels by antiarrhythmic or non-antiarrhythmic drugs may lead to congenital or acquired long QT syndrome (9,10). The sympathetic nervous system modulates the I_{kr} current though both α1 and β-adrenoceptors (ARs) (11-14). Separate stimulation of α_1-ARs or β-ARs decreased I_{kr} current and prolonged action potential. In recent years, considerable progress has been made toward a detailed understanding of these two signaling pathways. Bian and colleagues revealed that hERG potassium channels or the I_{kr} current of rabbit cardiomyocytes can be regulated by the α_1-adrenoreceptor agonist phenylephrine (PE), which is consistent with our previous work in guinea pig (12,15). In an early report by Heath and Terrar, a concentration-independent increase

in I_{kr} currents was observed at low concentrations of the β-adrenergic agonist isoprenaline (16), whereas Karle found activation of β-adrenoceptors elicits an inhibitory effect on I_{kr} or hERG via a cAMP/PKA-dependent pathway (17). Morever, at least three α_1 adrenoceptor subtypes (α_{1A}, α_{1B}, and α_{1D}) and three β adrenoceptor subtypes (β_1, β_2, and β_3) have been pharmacologically identified, it is unclear which AR subtypes primarily regulate I_{kr}. In addition, the interaction between the α_1 and β-adrenergic components during sympathetic regulation of the cardiac I_{kr} current were not examined, and their functional importance was not determined.

Methods

Cardiomyocyte preparation and whole-cell patch clamp

Guinea pigs (male, weighing 300±50 g) were purchased from Nanjing Medical University Institutional Animal Care. Single left ventricular myocytes were isolated from the heart of guinea pigs using the Langendorff apparatus as described previously (12). All experiments were performed in accordance with animal care protocols approved by the Nanjing Medical University Institutional Animal Care and Use Committee.

Cells were transferred to a recording chamber that was continuously perfused with bath solution. Whole-cell patch-clamp recordings were performed with an EPC-9 amplifier (HEKA, Germany) at 37±0.5 ℃. Pipettes, filled with the pipette solution, had resistances of 3-6 MΩ. The flow rate of bath solution through the chamber was maintained at 2-3 mL/min.

Data analyses

Currents were acquired with Pulse + Pulsefit V8.53 and analyzed using SPSS 13.0 software. Statistical data are expressed as the mean ± standard error of the mean (S.E.M). Paired-sample *t*-tests were used to determine significant differences before and after PE or ISO intervention. One-way analyses of variance (ANOVA), with post-hoc comparisons using Newman-Keuls tests, were performed to compare differences among groups. Differences were considered significant if $P<0.05$.

Solutions and drug administration

For I_{kr} recordings, the solution was prepared as described by Wang *et al.* (12). The pipette solution contained 140 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, 11 mM EGTA, 5 mM Na_2-ATP, and 5 mM creatine phosphate (disodium salt). The pH was adjusted to 7.4 using 8 M KOH. The bath solution contained 140 mM NaCl, 3.5 mM KCl, 1.5 mM $CaCl_2$, 1.4 mM $MgSO_4$, and 10 mM HEPES. The pH was adjusted to 7.4 using 10 M NaOH. Nifedipine (10 μM) was added to the bath solution to block calcium currents and 10 μM chromanol 293B was added to ablate the slow component of delayed rectifier potassium currents (I_{Ks}). Na_2-ATP, EGTA, creatine phosphate, *L*-glutamic acid, HEPES, taurine, bovine serum albumin (BSA), nifedipine, chromanol 293B, dofetilide, PE, and ISO were purchased from Sigma (St. Louis, MO). Collagenase II was purchased from Worthington (Lakewood, NJ). All other reagents were obtained from Amresco.

For stock solutions, nifedipine and chromanol 293B were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 10 mM. ISO was dissolved in distilled water to 10 mM, and PE and dofetilide were dissolved in distilled water to 1 mM. All stock solutions were stored at –20 ℃, except nifedipine, which was stored at 4 ℃.

Results (*Figures 1-3*)

I_{kr} tail currents are inhibited by PE and α_{1A} subtype mediates this effect

When cell chambers were perfused with a bath solution containing 0.1 μM of the specific α_1-adrenereceptor agonist PE, the tail current amplitude significantly decreased (*Figure 1A,B*). The mean I_{kr} current density-voltage relations before and after PE are shown in *Figure 1C*. I_{kr} tail current density at +40 mV decreased to 0.66±0.02 upon the addition of 0.1 μM PE (first column of *Figure 1H*). When cells were pretreated with the non-selective α_1-adrenergic antagonist prazosin (1 μM), PE does not decrease I_{kr} current (*Figure 1D*, second column of *Figure 1H*). To further evaluate the α_1-adrenergic receptor (AR) subtype involved, selective α_1-AR blockers, α_{1A}-selective 5-methylurapidil (5-MU; 1μM), α_{1B}-selective chloroethylclonidine (CEC; 10 μM), or α_{1D}-selective BMY7378 (BMY; 1 nM) were applied together with PE (0.1 μM). When 5-MU, CEC, or BMY7378 was applied together with PE, the I_{kr} amplitudes decreased to 0.99±0.07, 0.62±0.03, and 0.70±0.04, respectively, indicating the α_{1A}-receptor antagonist 5-MU prevented PE-induced suppression of I_{kr}, whereas the α_{1B} and α_{1D}-receptor blockers did not (*Figure 1E-H*). These results suggested that stimulation of the α_1 adrenereceptor may

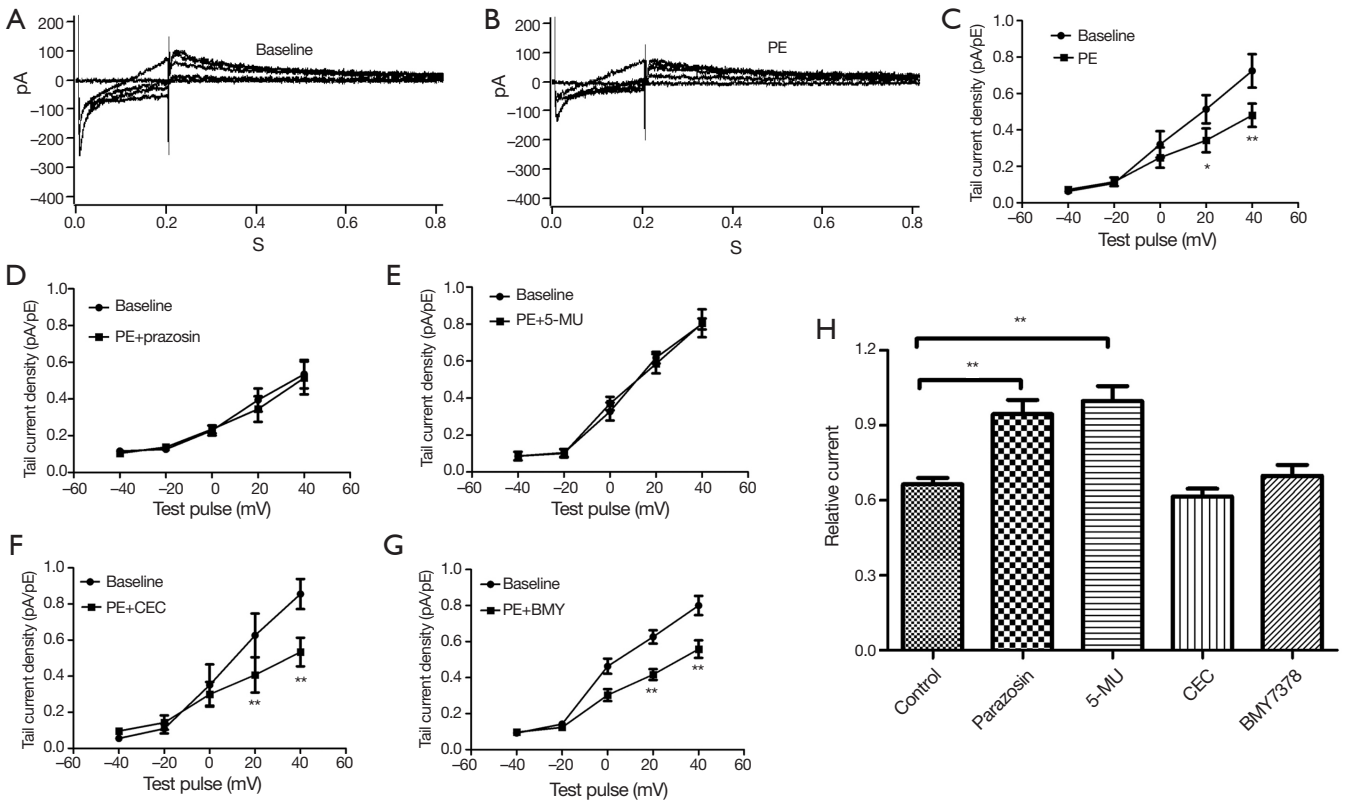

Figure 1 Effect of α-adrenoceptor and subtype on I_{kr}. (A,B) Representative I_{kr} current trace before and after PE application; (C) I_{kr} density-voltage relations (mean ± S.E.M) from five cells under control conditions (baseline) or in the presence of PE; (D-G) I_{kr} density-voltage relations (mean ± S.E.M) under control conditions and after the addition of 0.1 μM PE plus the α_1 antagonist prazosin (1 μM), α_{1A}-selective antagonist 5-MU (1 μM), α_{1B}-selective antagonist CEC (10 μM), or α_{1D}-selective antagonist BMY (1 nM); (H) current amplitudes were measured at +40 mV and amplitudes were normalized to the values before PE perfusion in the control, prazosin, 5-MU, CEC, and BMY groups (n=5, **P<0.01).

reduce I_{kr} tail current, and that α_{1A}, rather than the α_{1B} or α_{1D} receptor subtypes, is involved in this effect.

I_{kr} tail currents are inhibited by isoproterenol and β_1 subtype mediates this effect

When cell chambers were perfused with a bath solution containing 10 μM of the non-specific β-adrenereceptor agonist isoproterenol (ISO), the tail current amplitude significantly decreased (*Figure 2A*). The mean I_{kr} current density-voltage relations before and after ISO are shown in *Figure 2B*. I_{kr} tail current density at +40 mV decreased to 0.62±0.03 upon the addition of ISO (first column of *Figure 2F*). Propranolol blocked this effect because when cells were pretreated with the non-specific β-adrenereceptor blocker propranolol (10 μM), ISO no longer decreased the I_{kr} tail current (*Figure 2C*, second column of *Figure 2F*). To evaluate the β-AR subtype involved, we added selective β-AR blockers along with ISO: β_1-selective CGP-20712A (10 μM) and β_2-selective ICI-118551 (10 μM). The β_1-receptor blocker CGP completely prevented the ISO-induced reduction in I_{kr} (the I_{kr} tail current density at +40 mV decreased to 0.97±0.08), whereas the β_2-receptor blocker ICI did not alter the action of ISO (the I_{kr} tail current density at +40 mV decreased to 0.55±0.06) (*Figure 2D,E*; first, third, and fourth columns of *Figure 2F*). These results suggested that stimulation of the β-adrenereceptor reduced I_{kr} tail current, and that the β_1 rather than β2 receptor subtype is involved in the regulation of I_{kr}.

PE prevents the inhibitory effect of ISO on I_{kr}

We evaluated the effects of ISO in the presence of PE (0.1 μM). ISO (10 μM) was applied after the PE-induced changes

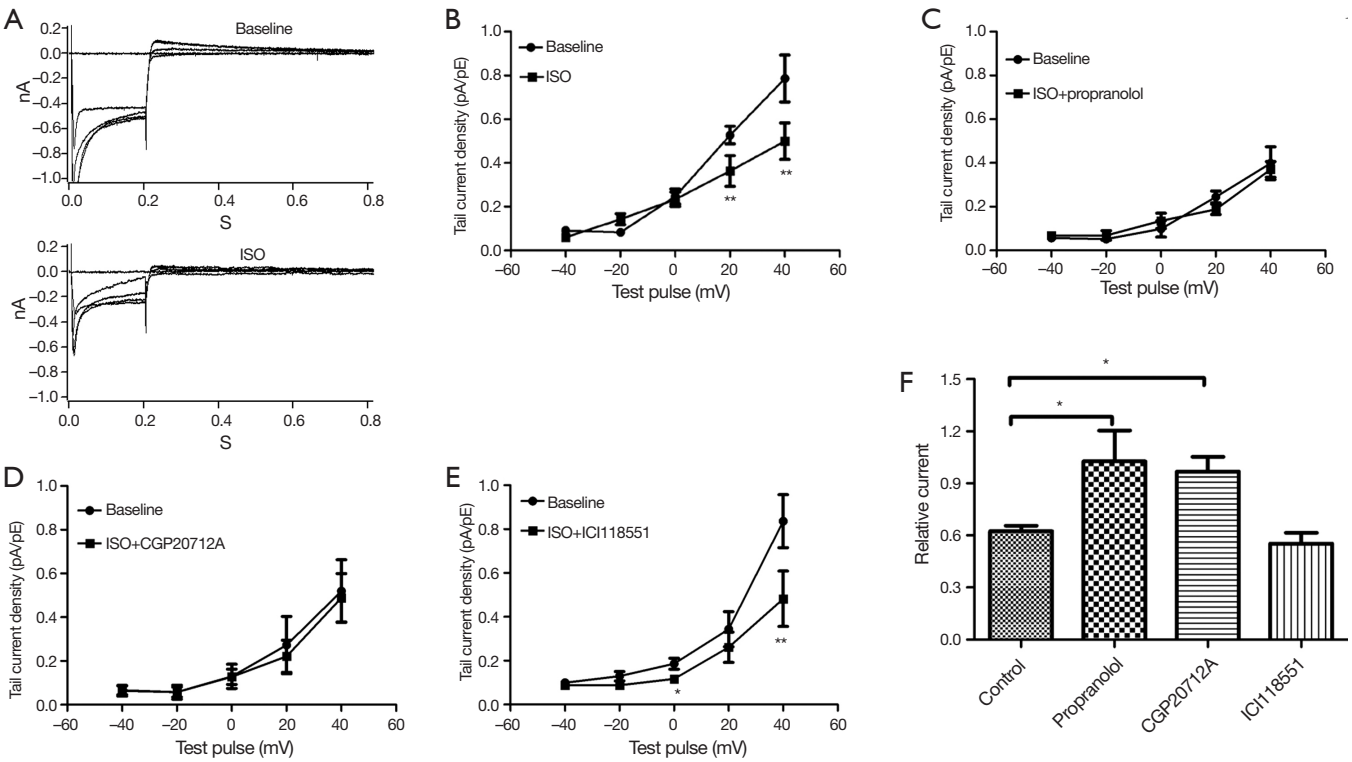

Figure 2 Effect of β-adrenoceptor and subtype on I_{kr}. (A) Representative I_{kr} current trace before and after ISO application; (B) I_{kr} density-voltage relations (mean ± S.E.M) from four cells under control conditions (baseline) and in the presence of ISO; (C-E) I_{kr} density-voltage relations (mean ± S.E.M) under control conditions and after the addition of 10 μM ISO plus the β_1 antagonist propranolol (10 μM), the β_{1A}-selective antagonist CGP-20712A (10 μM), or the β_{1B}-selective antagonist ICI-118551 (10 μM); (F) current amplitudes were measured at +40 mV and amplitudes were normalized to the value before ISO perfusion in control, propranolol, CGP-20712A, and ICI-118551 groups (n=4, *P<0.05).

stabilized (approximately 8-10 min after PE application). Changes in current amplitude are expressed relative to the amplitude before application of any drug (PE or ISO). *Figure 3A* shows a typical current trace of cells without drugs, then followed by PE and the last ISO. *Figure 3B* shows the relative current at test pulse +40 mV in each group. When cells were acutely stimulated with PE, the I_{kr} tail currents did not decrease with ISO addition (10 μM ISO after 0.1 μM PE). The relative current amplitudes were 1.01±0.12, which was significantly different from the ISO effect on I_{kr} without PE (third and fourth columns of *Figure 3E*). Thus, ISO does not reduce I_{kr} tail currents in cardiomyocytes pretreated with PE, which significantly differed from cardiomyocytes not acutely stimulated by PE. These results suggested that the PE prevents the current reduction effects of ISO on I_{kr}.

ISO attenuates the inhibitory effect of PE on I_{kr}

We also evaluated the effects of PE in the presence of ISO. PE (10 μM) was applied after the ISO-induced changes had stabilized. A typical current trace and the relative current at +40 mV test pulse in each group are shown in *Figure 3C,D*. When cardiomyocytes were acutely stimulated with ISO, the I_{kr} tail currents only decreased to 0.80±0.02 upon PE addition. This result significantly differed from cardiomyocytes that were not acutely stimulated by ISO (0.58±0.04, P<0.05) (first and second columns of *Figure 3E*). These data suggested that the inhibitory effects of PE on I_{kr} can be attenuated by ISO.

Discussion

In the present study, we report that the β_1 and α_{1A}-AR systems modulate I_{kr}. The current reduction effects of ISO can be prevented by preactivation of α_1-adrenoceptors whereas the inhibitory effects of PE can be attenuated by preactivation of β-adrenoceptors. Signaling crosstalk between the α_{1A}- and β_1-adrenergic cascades might be involved in I_{kr} tail current

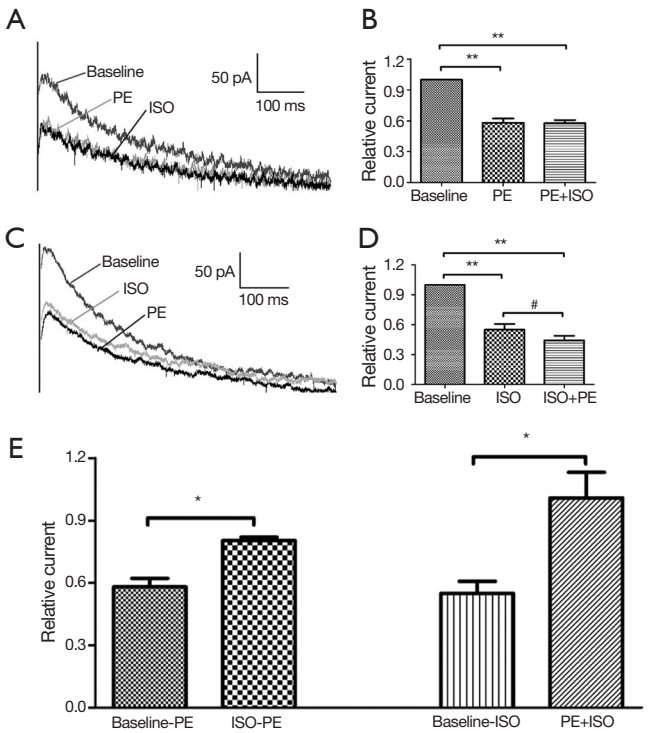

Figure 3 Interaction of α- and β-adrenoceptors on I_{kr}. (A) Representative current changes in the amplitude of peak I_{kr} tail current (test pulse at +40 mV) following exposure to PE and PE plus ISO; (B) the relative current at test pulse +40 mV in relative groups of *Figure 3A*; (C) representative current changes in the amplitude of peak I_{kr} tail current (test pulse at +40 mV) following exposure to ISO and ISO plus PE; (D) the relative current at test pulse +40 mV in relative groups of *Figure 3C*; (E) comparison of decreased I_{Kr} tail currents after application of different adrenergic agonists. Column baseline-PE represents the relative I_{Kr} tail current upon application of α_1-adrenergic agonist alone, column ISO-PE represents the α_1-AR activation-induced percentage of I_{Kr} after preactivation of β_1-AR, column baseline-ISO indicates the β_1-AR alone stimulation-induced inhibition percentage, and column PE-ISO shows the β_1-AR stimulation-induced inhibition percentage after pre-stimulation with α_1-AR (n=3; *P<0.05).

regulation in guinea pig ventricular myocytes.

ARs bind and are activated by the endogenous catecholamine hormones epinephrine and norepinephrine (NE). Epinephrine is primarily produced and released into circulation from the adrenal gland. However, NE is synthesized and released by sympathetic nerve terminals in the peripheral nervous system and brain. In the heart, the two main ARs are the β-ARs, which comprise roughly 90% of total cardiac ARs, and α_1-ARs, which account for approximately 10% (18). In this study, α_1-adrenergic activation reduced I_{kr} in guinea pig ventricular cardiomyocytes, which are similar results to those reported by Thomas *et al.* (19) and Wang *et al.* (12). At least three α_1-AR subtypes (α_{1A}, α_{1B}, α_{1D}) have been described using molecular and pharmacological techniques (20,21). The ventricular α1-adrenoceptor densities did not significantly differ between guinea pig and human (22). A previous study found that different adrenoceptor subtypes have different effects on I_{to} (23) and $I_{ca\text{-}L}$ (24). We found that α_1 adrenergic action on I_{kr} was via α_{1A}. Currently, there are no data regarding the effects of α_{1B} or α_{1D} on I_{kr}. Moreover, we also found that in addition to α_1, β-adrenergic activation also reduced I_{kr}, which is consistent with the study by Karle *et al.* (17). These results differ from the data of Heath and Terrar who reported that isoprenaline increased I_{kr} (16). This discrepancy could be related to ISO concentrations, different techniques inherent to whole-cell perforated patch clamp and switched electrode voltage clamp (SEVC), and/or different effects of β adreneoceptor subtypes on I_{kr}. The β_1 and β_2 adreneoceptor subtypes have different effects in regulating Ica-L (25) and cardiac function (26,27). Our study found that β-adrenoceptor action is mediated by the β_1-adrenoceptor subtype rather than the β_2-adrenoceptor subtype.

It is noteworthy that α_1-AR activation-induced inhibition of I_{kr} tail current in the presence of β-AR activation differs significantly from α_1-AR activation alone-induced inhibition of I_{kr} tail current. These data suggests that pre-activation of β-ARs suppresses the effect of α_1-AR activation on I_{kr} tail current, and pre-activation of α_1-ARs inhibits the effect of β-AR activation on I_{kr} tail current. Thus, pre-activation of one subtype adrenoceptor restrains the effect of another adrenoceptor subtype on I_{kr} tail current. That is, crosstalk exists in regulating I_{kr} current via acute adrenergic stimulation in physical circumstances. We propose that this is a protective regulatory mechanism against severe inhibition of I_{kr} tail current that occurs during excessive release of catecholamines in pathological circumstances.

Conclusions

I_{kr} in guinea pig cardiomyocyte can be regulated by both the α_1 adrenergic system through the α_{1A} subtype and the β adrenergic system through the β_1 subtype. The absence of an inhibitory effect on I_{kr} by ISO was noted in cells pretreated with PE. The PE effect on I_{kr} was also attenuated in cells

pretreated with ISO. Our results suggest a negative feedback loop intrinsic to norepinephrine stimulation in the heart and signaling crosstalk between α_{1A}- and β_1-adrenergic cascades, which might be involved in I_{kr} regulation in guinea pig ventricular myocytes. Signaling crosstalk may have a crucial role when plasma levels of both endogenous regulators are elevated.

Limitations

Our study found that crosstalk between α_{1A}-ARs and β_1-ARs in I_{kr} regulation exists. However, the precise adrenergic effects require further study to elucidate the molecular mechanisms that underlie the functional crosstalk as well as to identify putative intermediate proteins in the signal transduction pathways involved in modulation of I_{kr} current via adrenergic activation.

Acknowledgements

We thank Xiang-Jian Chen, Yang-Di, and Dao-Wu Wang for their assistance, and the Research Institute of Cardiovascular Disease of the First Affiliated Hospital of Nanjing Medical University.
Funding: This study was supported by the National Scientific Foundation of China (NSFC No. 81100123).
Disclosure: The authors declare no conflict of interest.

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Cite this article as: Wang S, Min XY, Pang SS, Qian J, Xu D, Guo Y. Adrenergic regulation of the rapid component of delayed rectifier K+ currents in guinea pig cardiomyocytes. J Thorac Dis 2014;6(12):1778-1784. doi: 10.3978/j.issn.2072-1439.2014.12.27

