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Full paper

Phosphatidylinositol4-phosphate 5-kinase prevents the decrease in the HERG potassium current induced by Gq protein-coupled receptor stimulation

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ABSTRACT

The human ether-a-go-go-related gene (HERG) potassium current (I_{HERG}) has been shown to decrease in amplitude following stimulation with Gq protein-coupled receptors (GqRs), such as α_1-adrenergic and M_1-muscarinic receptors (α_1R and M_1R, respectively), at least partly via the reduction of membrane phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$). The present study was designed to investigate the modulation of HERG channels by $PI(4,5)P_2$ and phosphatidylinositol4-phosphate 5-kinase (PI(4)P5-K), a synthetic enzyme of $PI(4,5)P_2$. Whole-cell patch-clamp recordings were used to examine the activity of HERG channels expressed heterologously in Chinese Hamster Ovary cells. The stimulation of α_1R with phenylephrine or M_1R with acetylcholine decreased the amplitude of I_{HERG} accompanied by a significant acceleration of deactivation kinetics and the effects on I_{HERG} were significantly attenuated in cells expressing PI(4)P5-K. The density of I_{HERG} in cells expressing GqRs alone was significantly increased by the coexpression of PI(4)P5-K without significant differences in the voltage dependence of activation and deactivation kinetics. The kinase-deficient substitution mutant, PI(4)P5-K-K138A did not have these counteracting effects on the change in I_{HERG} by M_1R stimulation. These results suggest that the current density of I_{HERG} is closely dependent on the membrane $PI(4,5)P_2$ level, which is regulated by PI(4)P5-K and GqRs and that replenishing $PI(4,5)P_2$ by PI(4)P5-K recovers I_{HERG}.

Introduction

The human ether-a-go-go-related gene (HERG) is known to encode a K^+ channel with electrophysiological properties similar to those of the rapid component of the delayed rectifier K^+ current (I_{Kr}) in the human heart (1, 2). I_{Kr} is essential for the proper repolarization of cardiac myocytes near the end of each action potential. Therefore, the HERG channel activity contributes to cardiac repolarization and is an important determinant of the QT interval on electrocardiograms. It is well known that excessive blockade of HERG channels by antiarrhythmic or non-antiarrhythmic drugs can lead to acquired long QT syndrome (LQTS) and torsade de pointes (TdP) (3, 4) and the accidental combination of genetic mutation of HERG channels and drugs appears to due to TdP (5)

The heart rate and contractility are continually altered in response to changing cardiovascular demands and stress induced by autonomic stimulation. The stimulation of autonomic receptors coupled to Gq proteins, such as the muscarinic M_1 receptor (M_1R) and α_1-adrenergic receptor (α_1R), activates phosphoinositide-specific phospholipase C (PLC), leading to the hydrolysis of the

Abbreviations: α_1R, α_1-adrenergic receptors; CHO, Chinese hamster ovary; I_{Kr}, delayed rectifier K^+ current; DAG, diacylglycerol; GqRs, Gq protein-coupled receptors; HERG, human ether-a-go-go-related gene; LQTS, long QT syndrome; M_1R, M_1-muscarinic receptors; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; PI(4)P5-K, phosphatidylinositol4-phosphate 5-kinase; PLC, phospholipase C; IP_3, inositol 1,4,5-trisphosphate; PKC, protein kinase C; TdP, torsade de pointes.

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membrane phospholipid phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) to form inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (6). It is well known that IP_3 stimulates receptors on intracellular Ca^{2+} stores to release Ca^{2+} and that DAG activates protein kinase C (PKC).

In addition to the role of the precursors of these second messengers, $PI(4,5)P_2$ itself plays a critical role in various cellular processes, including regulation of many ion channels and transporters (7, 8). A recent study reported that $PI(4,5)P_2$ have dual effect on Shaker potassium channels, namely, a gain-of-function effect on the maximal current amplitude and a loss-of-function effect by positive-shifting the activation voltage dependence (9). Evidence has also been presented to show that $PI(4,5)P_2$ controls both the movement of the voltage sensor and the stability of the open pore in HERG channels (10).

Phosphatidylinositol4-phosphate 5-kinase (PI(4)P5-K) is the enzyme that catalyzes the phosphorylation of phosphatidylinositol 4-phosphate (PI(4)P) to $PI(4,5)P_2$, thereby facilitating the replenishment of $PI(4,5)P_2$ following PLC-coupled receptor-mediated depletion (8). Previous reports have shown that an increased association between PI(4)P5-K and the cytoskeleton results in the enhanced formation of $PI(4,5)P_2$ and that the spatially and temporally organized regulation of $PI(4,5)P_2$ synthesis by PI(4)P5-K enables dynamic and versatile $PI(4,5)P_2$ signaling (11).

The present study was undertaken to investigate the function of PI(4)P5-K in the regulation of HERG channels under basal conditions and during GqRs stimulation based on the heterologous expression of wild type of PI(4)P5-K and a kinase-deficient substitution mutant, PI(4)P5-K-K138A (12).

Methods

Cell culture and transfection

Chinese hamster ovary (CHO) cells were maintained in DMEM/Ham's F-12 supplemented with 10% fetal calf serum (GIBCO) and antibiotics (100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin) in a humidified incubator gassed with 5% CO_2 and 95% air at 37 °C. The cells were seeded onto 35-mm plastic culture dishes with sterile glass coverslips (5 mm × 3 mm) on the bottom and incubated for 24–48 h. The CHO cells were transiently transfected with the following cDNAs using Lipofectamine (Invitrogen): HERG (kindly provided by Dr. M. Sanguinetti, University of Utah, Salt Lake City, UT, USA), the type Iα isoform of PI(4)P5-K (tagged with the hemagglutinin (HA) epitope, kindly provided by Dr. Oka, University of Tokyo, Japan), human M_1R (UMR cDNA Resource Center), human α_1AR (UMR cDNA Resource Center) and GFP (pEGFP, Clonetech). The substitution mutant PI(4)P5-K-K138A cDNA was constructed via oligonucleotide-directed mutagenesis using the QuikChange Site-Directed Mutagenesis Kit (Agilent Technologies). The amount of each cDNA used for transfection was as follows (μg $dish^{-1}$): 1.5 HERG, 1.0 PI(4)P5-K, 1.0 M_1R, 1.0 α_1R and 0.5 GFP. The cells were grown on glass coverslips for 48 to 72 h after transfection, and only GFP-positive cells were used for the electrophysiological studies.

Solutions and chemicals

The normal Tyrode solution contained (in mM); 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 0.33 NaH_2PO_4, 5.5 glucose and 5.0 HEPES (pH adjusted to 7.4 with NaOH) and was used as a standard external solution to measure I_{HERG}. The control pipette solution consisted of (in mM) 70 potassium aspartate, 50 KCl, 10 KH_2PO_4, 1 $MgCl_2$, 3 Na_2ATP (Sigma), 0.1 Li_2-GTP (Roche Diagnostics), 5 EGTA and 5 HEPES (pH adjusted to 7.2 with KOH). The concentration of free Ca^{2+} and Mg^{2+} in the pipette solution was calculated to be approximately 1.5×10^{-10} M ($pCa = 9.8$) and 3.7×10^{-5} M ($pMg = 4.4$), respectively (13,14). Acetylcholine (ACh, Sigma) or L-phenylephrine (Phe, Sigma) was used at the desired final concentration in the normal Tyrode solution.

Whole-cell patch-clamp technique and data analysis

The cells were voltage-clamped using the whole-cell configuration of the patch-clamp technique (15) with an EPC-8 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany). The patch electrodes were fabricated from glass capillaries (1.5 mm outer diameter, 0.9 mm inner diameter; Narishige Scientific Instrument Laboratory, Tokyo, Japan) using a Sutter P-97 microelectrode puller (Novato, CA, USA), and the tips were fire polished using a microforge. The patch electrodes had a resistance of 2.0–4.0 MΩ when filled with the pipette solution. After gaining access to the cell interior (via rupture of the patch membrane), the membrane potential was held at −80 mV and repetitively (every 20 s) depolarized to a test potential of +10 mV for 2 s followed by a repolarizing step to −60 mV for 15 s before the I_{HERG} became stable. All experiments were performed at 34–36 °C. The PatchMaster (HEKA) and IGOR Pro (ver. 6.2, WaveMetrics) software programs were used for the data acquisition and analysis. The cell membrane capacitance (C_m) of each cell was calculated by fitting a single exponential function to the decay phase of the transient capacitive current resulting from 5-mV hyperpolarizing steps applied from a holding potential of −80 mV (16). The current amplitude was divided by C_m to obtain the current density (pA pF^{-1}). The concentration- response relationship for the inhibition of I_{HERG} by ACh was drawn according to the least-squares fit of the Hill equation: $R = R_{max}/[1 + (IC_{50}/[agonist])^{nH}]$, where R_{max} represents the maximal degree of inhibition expressed as a percentage, IC_{50} is the concentration giving a half-maximal inhibition and n_H is the Hill coefficient. The voltage dependence of I_{HERG} activation was determined by fitting the normalized I–V relationship of the tail currents to a Boltzmann equation: $I_{HERG,\,tail} = 1/\{1 + \exp[(V_{1/2} - V_m)/k]\}$, where $I_{HERG,\,tail}$ is the tail current amplitude normalized with reference to the maximum value measured at 10 mV, $V_{1/2}$ is the voltage at the half-maximal membrane voltage of activation or inactivation, V_m is the test potential and k is the slope factor. The time course for decay of the I_{HERG} tail current was fitted with the sum of two exponential functions: $I_{HERG,\,decay} = A_{fast} \exp(-t/\tau_{fast}) + A_{slow} \exp(-t/\tau_{slow})$, where A_{fast} and A_{slow} represent the amplitudes of the fast and slow components, respectively, and τ_{fast} and τ_{slow} are time constants for the fast and slow components, respectively. The effects of the agonist on I_{HERG} were examined at least 3 min after application.

Immunocytochemical analysis

CHO cells cotransfected with M_1R and HERG with or without HA-tagged PI(4)P5-K or PI(4)P5-K-K138A were fixed with 4% paraformaldehyde for 30 min and permeabilized/blocked with 0.2% Triton X-100 and 2% BSA in PBS for 60 min at room temperature. The permeabilized cells were incubated with rabbit polyclonal anti-HA antibodies (1:500, MBL, Co.) overnight at 4 °C. The cells were then probed with an AlexaFluor® 546-conjugated secondary antibody (1:400, Molecular Probes-Invitrogen). Fluorescent signals were analyzed using a confocal laser scanning system Clsi (Nikon) on an Eclipse TE2000-E inverted microscope (Nikon).

Statistics

The data are presented as the mean ± S.E.M., and n indicates the number of cells studied. For single comparisons, Student's paired or unpaired t-test was used. For multiple group comparisons, an