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Research Paper

Suppression of hERG K^+ current and cardiac action potential prolongation by 4-hydroxynonenal via dual mechanisms

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ABSTRACT

Oxidative stress under pathological conditions, such as ischemia/reperfusion and inflammation, results in the production of various reactive chemicals. Of these chemicals, 4-hydroxynonenal (4-HNE), a peroxidation product of ω6-polyunsaturated fatty acid, has garnered significant attention. However, the effect of 4-HNE on cardiac electrophysiology has not yet been reported. In the present study, we investigated the effects of 4-HNE on several cardiac ion channels, including human ether-a-go-go-related (hERG) channels, using the whole-cell patch clamp technique. Short-term exposure to 100 μM 4-HNE (4-HNE_{100S}), which mimics local levels under oxidative stress, decreased the amplitudes of rapidly activating delayed rectifier K^+ current (I_{Kr}) in guinea pig ventricular myocytes (GPVMs) and HEK293T cells overexpressing hERG (I_{hERG}). MS analysis revealed the formation of 4-HNE-hERG adduct on specific amino acid residues, including C276, K595, H70, and H687. Long-term treatment (1–3 h) with 10 μM 4-HNE (4-HNE_{10L}), suppressed I_{Kr} and I_{hERG}, but not I_{Ks} and $I_{Ca,L}$. Action potential duration (APD) of GPVMs was prolonged by 37% and 64% by 4-HNE_{100S} and 4-HNE_{10L}, respectively. Western blot analysis using surface biotinylation revealed a reduction in mature membrane hERG protein after treatment with 4-HNE_{10L}. Proteasomal degradation inhibitors, such as bortezomib, prevented the 4-HNE_{10L}-induced decrease in mature hERG, suggesting a retrograde degradation of membrane hERG due to 4-HNE. Taken together, 4-HNE_{100S} and 4-HNE_{10L} suppressed I_{hERG} via functional inhibition and downregulation of membrane expression of hERG, respectively. The exposure of 4-HNE under pathological oxidative stress may increase the risk of proarrhythmic events via APD prolongation.

1. Introduction

Human *ether-a-go-go*-related gene (*KCNH2*) encodes hERG protein (Kv11.1), which is responsible for the rapidly activating delayed rectifier potassium current (I_{Kr}) in cardiomyocytes. I_{Kr} is critical for determining the cardiac action potential duration (APD) and QT interval in electrocardiograms [1]. Functional impairment of I_{Kr} due to genetic mutations in hERG or exposure to various cardiotoxic drugs can induce inherited or acquired long QT syndrome (LQTS), respectively [2]. It has been suggested that dysregulation of hERG channels by excessive reactive oxygen species production under oxidative stress increases the risk of arrhythmia [3–5]. A recent study demonstrated that application of hydrogen peroxide (H_2O_2) decreases the hERG current (I_{hERG}) by directly interacting with Cys^{723} in the cytoplasmic C-terminal [6]. However, it is not known whether alternative intrinsic oxidative agents, such as lipid peroxidants, affect hERG via a similar mechanism, especially during the long-term exposure.

The compound 4-hydroxynonenal (4-HNE) is a highly reactive end-product of polyunsaturated fatty acid oxidation that forms protein adducts with nucleophilic residues, such as cysteine, histidine and lysine. Under physiological conditions, 4-HNE is conjugated to glutathione, or metabolized by aldehyde reductase and aldehyde dehydrogenase [7,8]. However, under conditions of severe oxidative stress with decreased metabolization, 4-HNE accumulates and becomes a significant pathophysiolgical factor [9,10] and can act as a toxic mediator of free radicals [11]. The physiological concentrations of 4-HNE in interstitial

Abbreviations: I_{Kr}, rapidly activating delayed rectifier potassium current in cardiomyocyte; I_{Ks}, slowly activating delayed rectifier potassium current in cardiomyocyte; $I_{Ca,L}$, , L-type calcium current in cardiomyocyte; I_{hERG}, outward K^+ current in hERG overexpressed conditions; APD, action potential duration; V_{max}, maximal upstroke velocity; RMP, resting membrane potential; TA, total amplitude; LQTS, long QT syndrome; GPVM, guinea pig ventricular myocyte

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fluids range from 0.3 to 5 μM and increase by 10–100 fold under pathological conditions [12,13]. Although 4-HNE concentrations are lower in plasma than in tissues, elevated plasma 4-HNE concentrations (1–10 μM) have been observed in patients experiencing ischemic brain hemorrhage and stroke [14,15].

Previous studies have suggested pathophysiological roles of 4-HNE accumulation in cardiovascular diseases, such as ischemia-reperfusion injury, dilated heart dysfunction, heart failure, and atherosclerosis [16–19]. However, the influence of 4-HNE on cardiac ion channels and action potential (AP) is poorly understood. An early study of rat ventricular myocytes demonstrated that a treatment with 400 μM of 4-HNE increased APD, depolarized the resting membrane potential (RMP), increased the window current of Na^+ channels, and decreased the inward rectifier K^+ current [20]. However, it is not known whether 4-HNE regulates I_{Kr} and I_{Ks} (slowly activating delayed rectifier potassium current), which are essential for determining APD and are frequently intrinsic to pharmacological arrhythmogenesis. Moreover, the effects of physiologically or pathophygiolgically meaningful concentrations (5–100 μM) of 4-HNE on the cardiac ion channels have yet to be characterized. Considering that the relatively lower concentration (ca. 10 μM) of 4-HNE can be maintained in plasma for a prolonged period in the brain hemorrhage and stroke [14,15], it is requested to examine the effects of both short- and long-term effects on the cardiac ion channels.

On these backgrounds, we investigated the effect of short-term (< 10 min) and long-term (0.5–12 h) 4-HNE exposure on cardiac ion channels and APD in primary guinea pig ventricular myocytes (GPVMs) and HEK293 cells overexpressing hERG. To clarify the adduct formation between hERG and 4-HNE, mass spectrometry post-translational modification (PTM) analysis was performed. Furthermore, membrane expression of the mature form of hERG was measured to characterize the effects of long-term exposure to 4-HNE on the lifetime of functional hERG.

2. Methods and materials

2.1. Cell culture and plasmid transfection

Basic electrophysiological studies of I_{hERG} and hERG protein expression were performed using HEK293 cells stably expressing hERG1a (hERG-HEK cells) kindly donated by Dr. Han Choe (University of Ulsan, Seoul, Korea). The hERG-HEK cells were maintained in minimum essential medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Serana Europe, Pessin, Germany). For electrophysiological studies using transient expression systems, human isoform of KCNH2 (hERG1a), KCNQ1, and KCNE1 channels were used. Except for wild-type hERG1a (hERG-HEK cells), all complementary DNA was transiently transfected into HEK293 cells using FuGENE 6 kit (Roche, Penzberg, Germany). Detailed cell culture and transfection methods have been published previously [21].

2.2. Isolation of GPVMs

Male guinea pigs (weighing ~ 350 g) were purchased from Koatech (Seoul, Korea). The guinea pigs were housed in standard cages and light conditions and fed a standard diet with ad libitum access to drinking water. All animal experiments were approved by the Institutional Animal Care and Use Committee of Seoul National University (Approval number: SNU-141125-3-1). Guinea-pigs were anesthetized using an intraperitoneal injection of a mixture of pentobarbital sodium (50 mg/kg) and heparin (300 U/ml). The heart was transferred to a Langendorff apparatus and then retrogradely perfused with Ca^{2+}-free Tyrode's solution (135 mM NaCl, 5.4 mM KCl, 3.5 mM $MgCl_2$, 5 mM glucose, 5 mM HEPES, 0.4 mM Na_2HPO_4, and 20 mM taurine adjusted to a pH 7.4 using NaOH) containing collagenase (1 mg/ml, Worthington Biochemical Co., NJ, USA), protease (0.1 mg/ml, Sigma), and 1% bovine serum albumin (Sigma, St. Louis, MO, USA). After 10 min of enzyme perfusion, the left ventricle was isolated and digested again with fresh collagenase-containing Tyrode's solution for 8 min. Isolated GPVMs were kept in Kraft-Bruhe (K-B) solution (70 mM KOH, 55 mM KCl, 20 mM KH_2PO_4, 10 mM HEPES, 20 mM glucose, 50 mM L-glutamate, 20 mM taurine, 0.5 mM EGTA, and 3 mM $MgCl_2$ adjusted to a pH 7.3 with KOH) at 4 °C.

2.3. Electrophysiological recording

Conventional whole-cell voltage and current clamps were performed at room temperature. The signals were amplified and digitized with an Axopatch 200B amplifier (Axon Instruments, Foster, CA, USA) and Digidata 1440B AD-DA converter (Axon Instruments). For electrophysiological recording and analysis of currents and AP, pClamp 10.1 (Axon Instruments) and Origin 8.0 (Microcal, Northampton, MA, USA) were used. Microglass pipettes (World Precision Instruments, Sarasota, FL, USA) were pulled to resistances of 2–3 MΩ with a PP-830 puller (Narishige, Tokyo, Japan). A 3 M KCl agar bridge was used to prevent junction potential artifacts when reducing agents were used [22]. I_{Kr} was recorded in either hERG-HEK cells or GPVMs. I_{Ks} was recorded in either HEK293 cells transiently transfected with KCNQ1/KCNE1 (KCNQ1/E1-HEK cells) or GPVMs. $I_{Ca,L}$ and AP were recorded in GPVMs.

The extracellular bath solution for I_{Kr} and I_{Ks} recordings in hERG-HEK cells contained 145 mM NaCl, 3.6 mM KCl, 10 mM HEPES, 1 mM $MgCl_2$, 1.3 mM $CaCl_2$, and 5 mM glucose adjusted to a pH 7.4 with NaOH. The intracellular pipette solution for I_{Kr} and I_{Ks} recording contained 100 mM K-aspartate, 25 mM KCl, 5 mM NaCl, 10 mM HEPES, 1 mM $MgCl_2$, 4 mM MgATP, and 10 mM BAPTA adjusted to pH 7.25 with KOH. Extracellular bath solution for recording I_{Kr} and I_{Ks} from GPVMs contained 145 mM NaCl, 5.4 mM KCl, 10 mM HEPES, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM glucose, and 0.001 mM nifedipine adjusted to pH 7.4 with NaOH. I_{Kr} and I_{Ks} from GPVMs were isolated using the selective inhibitors E4031 (1 μM) and chromanol293B (Ch293B; 10 μM), respectively. The intracellular solution contained 120 mM K-aspartate, 20 mM KCl, 5 mM NaCl, 2 mM $CaCl_2$, 5 mM EGTA, 10 mM HEPES, and 5 mM MgATP adjusted to pH 7.25 with KOH. The compositions of the extracellular and intracellular solutions used for AP recording were same as for the I_{Kr} and I_{Ks} recording solutions except for the absence of nifedipine. The extracellular bath solution for $I_{Ca,L}$ contained 145 mM CsCl, 10 mM HEPES, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM glucose adjusted to pH 7.4 with CsOH. The intracellular solution for $I_{Ca,L}$ contained 106 mM CsCl, 20 mM TEA-Cl, 5 mM NaCl, 10 mM HEPES, 5 mM MgATP, and 10 mM EGTA adjusted to pH 7.25 with CsOH.

2.4. Surface biotinylation and Western blot analysis

For surface biotinylation, 4-HNE-treated hERG-HEK cells were washed twice with ice-cold PBS and then incubated with PBS containing 1 mg/ml of Sulfo-NHE-SS-Biotin (Thermo Fisher Scientific, Waltham, MA, USA) for 1 h at 4 °C. Cells were then incubated with 50 mM tris (pH 7.4) for 20 min at 4 °C to quench unreacted biotin. Following cell lysis, biotinylated proteins were recovered by incubating the cell lysates with NeutrAvidin-coated agarose Beads (Thermo Fisher Scientific) in PBS buffer for 1 h at room temperature. Beads were then washed 5 times with PBS plus 0.1% SDS. Biotinylated proteins were eluted from the beads in 2X Laemmli sample buffer + β-mercaptoethanol at 37 °C for 30 min. Eluted proteins were separated on 4–12% gradient gels (Koma Biotech, Seoul, Korea) and analyzed by Western blot.

Whole cell lysates from hERG-HEK cells were used for Western blot analysis. For mature (155 kDa) and immature (135 kDa) hERG detection, proteins were separated on 6% tris-glycine gels and electroblotted on polyvinylidene difluoride membranes. The membranes were immunoblotted with a rabbit anti-KCNH2 primary antibody (ab81160, Abcam, Cambridge, UK). Detection of hERG signals was performed