Life Sciences 94 (2014) 99–105

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Protective effect of piperine on electrophysiology abnormalities of left atrial myocytes induced by hydrogen peroxide in rabbits

Yan Liu [a,b,1], Yu Zhang [a,c,1], Kun Lin [a], De-xian Zhang [a,c], Miao Tian [d], Hong-yang Guo [a], Yu-tang Wang [e], Yang Li [f,*], Zhao-liang Shan [a,**]

[a] Department of Cardiology, Chinese PLA General Hospital, Beijing 100853, China
[b] Department of Cardiology, Liao He Hospital, Liao Ning 111000, China
[c] Nankai University School of Medicine, Nankai University, Tianjin 300071, China
[d] Department of Cardiology, the First Affiliated Hospital of Chinese PLA General Hospital, Beijing 100048, China
[e] Department of Geriatric Cardiology, Chinese PLA General Hospital, Beijing 100853, China
[f] Institute of Geriatric Cardiology, Chinese PLA General Hospital, Beijing 100853, China

ARTICLE INFO

Article history:
Received 6 June 2013
Accepted 18 October 2013

Keywords:
Piperine
Hydrogen peroxide
Atrial myocytes
Action potential

ABSTRACT

Aims: Piperine had protective effects on oxidative stress damage of ventricular myocytes by hydrogen peroxide (H_2O_2). In this study we aimed to explore the protective effect of piperine on abnormalities of the cardiac action potential (AP) and several ion currents induced by hydrogen peroxide (H_2O_2) in single rabbit left atrial myocyte.
Main methods: Conventional microelectrodes were used to record action potential duration (APD), resting membrane potential (RMP) and some ion currents ($I_{Ca,L}$,I_{to},I_{K1} and I_{kur},ect.), before and after H_2O_2 administration with or without piperine.
Key findings: The piperine (7 μmol/L) had no significant effect on APD, $I_{Ca,L}$,I_{to},I_{K1} and I_{kur} and their channel dynamics. In the presence of 50 μmol/L H_2O_2, APD_{50} and APD_{90} shortened ($P < 0.01$), amplitude of RMP decreased ($P < 0.05$), the peak of $I_{Ca,L}$ reduced significantly ($P < 0.05$). Piperine (7 μmol/L) significantly alleviated the inhibiting effect of H_2O_2 on APD and $I_{Ca,L}$ ($P < 0.01$) and protected the changes of $I_{Ca,L}$ dynamics induced by H_2O_2. The peak current of I_{to} was reduced significantly ($P < 0.05$); Piperine (7 μmol/L) significantly alleviated the inhibiting effect of H_2O_2 on I_{to} ($P < 0.01$). In addition, piperine protected the changes of I_{to} dynamics induced by H_2O_2. The peak current of I_{K1} and I_{KUr} was significantly reduced ($P < 0.05$); Piperine (7 μmol/L) alleviated the inhibiting effect of H_2O_2 on I_{K1} and I_{KUr} significantly ($P < 0.01$). In addition, piperine protected the changes of I_{KUr} dynamics induced by H_2O_2.
Significance: These results suggest that piperine effectively protects atrial myocytes from oxidative stress injury in atrial electrophysiology.

Introduction

Piperine (PIP) widely exists in pepper plants and is a common drug in Chinese Medicine, Mongolian Medicine and Tibetan Medicine. It has protective effects on sulfydryl, antioxidant molecules and antioxidase in cells via the inhibition of free radicals, reactive oxygen species (ROS) and lipid peroxidation (Srinivasan, 2007). The study indicated that PIP had protective effects on oxidative stress damage of ventricular myocytes by hydrogen peroxide (H_2O_2) (Hu et al., 2009). Piperine exhibited antioxidant action in experimental conditions, both in vivo as well as in vitro, through its radical quenching effect and by preventing GSH depletion (Mittal and Gupta, 2000). Additionally, piperine also possesses antiinflammatory (Kumar et al., 2007), antidepressant (Li et al., 2007), antiplatelet (Park et al., 2007), antipyretic and analgesic (Ahmad et al., 2002) characteristics.

The present study was designed to investigate the effects of H_2O_2 on the cardiac action potential (AP); L-type calcium current ($I_{Ca,L}$), transient outward potassium current (I_{to}), inward rectifier potassium current (I_{k1}) and ultra rapid delayed rectifier potassium (I_{kur}) of rabbits' left atrial myocytes using a whole-cell configuration of the patch-clamp technique. Also studied, was the protective effect of PIP.

Material and methods

Experimental animals

Forty New Zealand rabbits (weighing 1–1.5 kg) were used for the study. The investigation conformed to the Guide for the Care and Use of Laboratory Animals issued by the National Committee of Science and Technology of China, and the study was approved by the Animal Investigation Committee of the Chinese PLA General Hospital.

* Corresponding author. Tel./fax: +86 10 66 93 6762.
** Corresponding author. Tel.: +86 10 5549 9210; fax: +86 10 5549 9209.
E-mail addresses: liyangbsh@163.com (Y. Li), shanzl301@sina.com (Z. Shan).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.lfs.2013.10.024

Drugs and solutions

Tyrode's solution (in mmol/L): NaCl 136, KCl 5.4, $MgCl_2$ 1.0, NaH_2PO4 0.33, $CaCl_2$ 1.8, hydroxyethyl PIPerazine ethanesulfonic acid (HEPES) 10, and glucose 10 (pH 7.4). Kreb's buffer solution (in mmol/L): KCl 40, KH_2PO_4 25, MgSO4 3.0, KOH 80, L-glutamine 50.3, taurine 20, HEPES 10, glucose 10, and EGTA 0.5 (pH 7.4). For AP recording, the internal pipette solution contained (in mmol/L) KCl 120, NaCl 10, $CaCl_2$ 1, MgATP 5, EGTA 11 and HEPES 10 (pH 7.4). For $I_{Ca\text{-}L}$ recording, the internal pipette solution contained (in mmol/L) CsCl 140, Na_2ATP 5.0, TEA-Cl 10, EGTA 10, $MgCl_2$ 3.0, and HEPES 10 (pH 7.4), while the bath solution contained (in mmol/L) choline chloride 137, CsCl 4.6, $CaCl_2$ 1.8, $MgCl_2$ 1.0, TEA-Cl 10, glucose 10, 4-AP 5.0, and HEPES 5.0 (pH 7.4). For potassium current recording, the internal PIPette solution contained (in mmol/L) KCl 45, K-aspartate 85, Na-pyruvate 5, MgATP 5.0, EGTA 10, HEPES 10, glucose 11 (pH 7.4), while the bath solution contained (in mmol/L) N-methyl-D-glucamine (NMG) 149,$MgCl_2$ 5,$CaCl_2$ 0.65,HEPES 5. For $I_{Ca,L}$ recording, 4-aminopyridine (4-AP) (50 μmol/L), Dofetilide (5 nmol/L) and tetraodontoxin (TTX) (100 μmol/L) were added to the superfusion to block $I_{KUr,}$ $I_{Kr,}$ and I_{Na}. For I_{to} and I_{Kur} recording, $BaCl_2$ (200 mM) and $CdCl_2$ (200 mM) were added to the superfusion to block I_{K1} and I_{Ca}. For I_{K1} recording, Dof (5 nmol/L), TTX (100 μmol/L) and $CdCl_2$ (200 mM) were added to the superfusion to block I_{Kr}, I_{Na} and I_{Ca}.

Single atrial myocyte preparation

Left atrial myocytes were isolated enzymatically from the atrium of New Zealand rabbits (weighing 1.0–1.5 kg) as previously described.[8–10] Briefly, the rabbit was anesthetized with an iv. injection of sodium pentobarbital (40 mg/kg), and then was heparinized (300 U/kg i.p.). The heart was excised immediately and mounted on a Langendorff apparatus. The heart was then perfused retrogradely via the aorta with oxygenated, calcium-free Tyrode's solution for 5 min, and with the Tyrode's solution containing 1.4 mg/mL of type II collagenase (Invitrogen, USA) and 0.24 mg/mL of trypsinase (Merck, Germany) for 15–20 min. Subsequently, the left atrial appendage was cut into small pieces in a dish containing Kreb's buffer solution and shaken gently to ensure the dispersion of dissociated cardiac myocytes. The cells were kept at 4 °C in Kreb's buffer solution. All the solutions were continuously gassed with 95% O_2 and 5% CO_2 and were maintained at 37 °C.

Pharmacology

Cells were divided into 4 groups: in the control group, cells were cultured without treatment for 1.5 h ($n = 15$); in the PIP group, cells were cultured with PIP (7 μmol/L) for 1.5 h ($n = 15$); in the H_2O_2 group, cells were cultured without treatment for 1 h, then with H_2O_2 for 0.5 h ($n = 15$); in the PIP + H_2O_2 group, cells were cultured with PIP (7 μmol/L) for 1 h and then cultured with H_2O_2 for 0.5 h ($n = 15$).

Electrophysiological recording

Membrane currents were measured using a whole-cell configuration of the patch-clamp technique with Axonpatch 700B amplifiers (Axon Instruments, USA). To stabilize the current, experiments were performed 5 min after entering the whole-cell configuration. All the measurements were obtained at a temperature of 22 °C. Parameters of the AP were measured with 2-Hz electrical stimuli before and after drug administration in the LA without spontaneous activity, which was measured after 10 min of superfusion. The AP amplitude (APA) was obtained from the resting membrane potential (RMP) or maximum diastolic potential to the peak of AP depolarization. The AP durations at a repolarization of 90%, and 50% of the APA were measured as APD_{90}, and APD_{50}, respectively. The maximum upstroke velocity was acquired using the maximum positive value of the first derivative of the AP. Spontaneous activity was defined as the constant occurrence of spontaneous APs in the absence of any electrical stimuli. Burst firing was defined as the occurrence of accelerated spontaneous potential (faster than the basal rate) with sudden onset and termination. The resistance of Pipettes in the bath solution ranged from 2 M to 5 M. No leak subtraction was applied during recording. The current densities were calculated by dividing the current amplitudes by the cell capacitance. Voltage dependence of channel activation or inactivation was obtained by fitting normalized curves with the Boltzmann function. Time course of recovery from inactivation (τ) was quantified by fitting measured data with a 1-exponential function.

Statistical analysis

Statistical analysis was performed with SPSS version 13.0 (Chicago, IL, USA). Data are presented as mean ± standard deviation. ANOVA was used for multiple-group comparisons, followed by a Bonferroni-corrected *t*-test. A 2-tailed value of $P < 0.05$ was taken as statistically significant.

Results

The effects of H_2O_2 and PIP on AP

H_2O_2 (50 μmol/L) could shorten atrial myocytes' APD_{50} and APD_{90} and decrease the amplitude of the resting membrane potential (RMP). PIP mitigate the changes partly. The AP amplitude (APA) of 4 groups changed little (Fig. 1 and Table 1).

The effects of H_2O_2 and PIP on $I_{Ca,L}$

Fig. 2**A, B** shows H_2O_2 (50 μmol/L) reduced the peak of $I_{Ca,L}$ density from (−18.3 ± 1.2) pA/pF to (−9.3 ± 0.9) pA/pF at the test potential of 0 mV ($P < 0.01$). PIP alleviated the changes (−11.9 ± 1.2 pA/pF). H_2O_2 (50 μmol/L) made the I–V curve shift upward, but PIP alleviated the changes (Fig. 2**C**). In the presence of 50 μmol/L H_2O_2, the steady-state activation curve shifted to a more positive potential, the half activation potentials ($V_{1/2}$) at which 50% of channels activated were from (−34.64 ± 2.15) mV to (−20.34 ± 2.20) mV ($P < 0.01$), but PIP made the $V_{1/2}$ change to (−28.81 ± 3.95) mV, and the slope factors (k) of 4 groups were not changed, these may reveal that H_2O_2 reduced the current via decreasing the channels activation (Fig. 2**D**). In the presence of 50 μmol/L H_2O_2, the steady-state inactivation curve was shifted to a more negative potential, the half inactivation potentials ($V_{1/2}$) at which 50% of channels inactivated were from (−19.12 ± 0.67) mV to (−29.47 ± 1.82) mV ($P < 0.01$), but PIP made the $V_{1/2}$ change to (−25.94 ± 1.74) mV, and the slope factors (k) of 4 groups were not changed, these may reveal that H_2O_2 reduced the current via increasing the channels inactivation (Fig. 2**E**). However, as in the examples shown in Fig. 2**F**, the time course of recovery from inactivation did not change. PIP alone did not influence these parameters of $I_{Ca,L}$ significantly (Fig. 2).

The effects of H_2O_2 and PIP on I_{to}

Fig. 3**A, B** shows H_2O_2 (50 μmol/L) reduced the peak of I_{to} density from (42.6.3 ± 3.0) pA/pF to (20.8 ± 2.0) pA/pF ($P < 0.05$, $n = 15$). PIP alleviated the changes (31.8 ± 2.7 pA/pF). H_2O_2 (50 μmol/L) made the I–V curve shifted downward, but PIP alleviated the changes (Fig. 3**C**). In the presence of 50 μmol/L H_2O_2, the steady-state activation curve was shifted to a more positive potential (Fig. 3**D**), the half activation potentials ($V_{1/2}$) at which 50% of channels activated were from (−31.51 ± 2.27) mV to (−19.62 ± 1.53) mV ($P < 0.01$), but PIP made the $V_{1/2}$ change to (−26.86 ± 0.53) mV (Fig. 3E), and the slope factors (k) of 4 groups were not changed (Fig. 3**F**), these may