Pharmacological Research 82 (2014) 40–50

Contents lists available at ScienceDirect

Pharmacological Research

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

α,β-Unsaturated aldehyde crotonaldehyde triggers cardiomyocyte contractile dysfunction: Role of TRPV1 and mitochondrial function

Zhaohui Pei[a,1], Zhiqiang Zhuang[b,1], Hanfei Sang[c,d,1], Zhenbiao Wu[e,1], Rongsen Meng[f], Emily Y. He[e,h], Glenda I. Scott[h], Jackie R. Maris[h], Ruiman Li[g,**], Jun Ren[h,*]

[a] Department of Cardiology, The Third Hospital of Nanchang, Nanchang, Jiangxi 330009, China
[b] Department of Rehabilitation Medicine, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, Guangdong 510080, China
[c] Department of Anesthesiology, Xijing Hospital, The Fourth Military Medical University, Xi'an, Shaanxi 710032, China
[d] Department of Clinical Pharmacology, Bethune International Peace Hospital of People's Liberation Army, Shijiazhuang, Hebei 050082, China
[e] Department of Clinical Immunology, Xijing Hospital, The Fourth Military Medical University, Xi'an, Shaanxi 710032, China
[f] Department of Cardiology, The Second People's Hospital of Guangdong Province, Guangzhou, Guangdong 511442, China
[g] Department of Gynecology and Obstetrics, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong 511442, China
[h] University of Wyoming College of Health Sciences, Laramie, WY 82071, USA

ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form 11 March 2014
Accepted 26 March 2014

Keywords:
Crotonaldehyde
Cardiomyocytes
Contractile function
TRPV1
Oxidative stress

ABSTRACT

Recent evidence has suggested that cigarette smoking is associated with an increased prevalence of heart diseases. Given that cigarette smoking triggers proinflammatory response *via* stimulation of the capsaicin-sensitive transient receptor potential cation channel TRPV1, this study was designed to evaluate the effect of an essential α,β-unsaturated aldehyde from cigarette smoke crotonaldehyde on myocardial function and the underlying mechanism with a focus on TRPV1 and mitochondria. Cardiomyocyte mechanical and intracellular Ca^{2+} properties were evaluated including peak shortening (PS), maximal velocity of shortening/relengthening ($\pm dL/dt$), time-to-PS (TPS), time-to-90% relengthening (TR_{90}), fura-2 fluorescence intensity (FFI), intracellular Ca^{2+} decay and SERCA activity. Apoptosis and TRPV1 were evaluated using Western blot analysis. Production of reactive oxygen species (ROS) and DNA damage were measured using the intracellular fluoroprobe 5-(6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate and 8-hydroxy-2′-deoxyguanosine (8-OHdG), respectively. Our data revealed that crotonaldehyde interrupted cardiomyocyte contractile and intracellular Ca^{2+} property including depressed PS, $\pm dL/dt$, ΔFFI and SERCA activity, as well as prolonged TR_{90} and intracellular Ca^{2+} decay. Crotonaldehyde exposure increased TRPV1 and NADPH oxidase levels, promoted apoptosis, mitochondrial injury (decreased aconitase activity, PGC-1α and UCP-2) as well as production of ROS and 8-OHdG. Interestingly, crotonaldehyde-induced cardiac defect was obliterated by the ROS scavenger glutathione and the TRPV1 inhibitor capsazepine. Capsazepine (not glutathione) ablated crotonaldehyde-induced mitochondrial damage. Capsazepine, glutathione and the NADPH inhibitor apocynin negated crotonaldehyde-induced ROS accumulation. Our data suggest a role of crotonaldehyde compromises cardiomyocyte mechanical function possibly through a TRPV1- and mitochondria-dependent oxidative stress mechanism.

1. Introduction

Chronic cigarette smoking is known to predispose the onset and progression of chronic obstructive pulmonary disease, cardiovascular diseases, infertility and cancer, representing a major health issue worldwide [1–4]. Epidemiologic evidence has demonstrated that cigarette smoking overtly increase the incidence of myocardial and coronary artery injuries through accumulation of oxidative stress [5,6]. Given the global health issue of cigarette smoking, a better understanding of cigarette smoking-related pathogenesis of cardiovascular and respiratory diseases is pivotal to manage smoking-related premature death [6–8]. Up-to-date, the genotoxic and mutagenic effects of cigarette smoke are well documented [6,8,9] although the potential contributions of various compounds present in cigarette smoke to organ damage are not well characterized. In particular, few studies were conducted to target on

* Corresponding author. Tel.: +1 307 766 6131; fax: +1 307 766 2953.
** Corresponding author.
E-mail addresses: hqyylrm@126.com (R. Li), jren@uwyo.edu (J. Ren).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.phrs.2014.03.010
1043-6618/

the underlying mechanisms of cigarette smoke components on myocardial structure and function [10–12]. Recent reports have suggested that acrolein, a major α,β-unsaturated aldehyde from cigarette smoke and a known environmental pollutant, directly compromises myocardial function [10,12–14]. However possible contribution from other cigarette smoke components to smoking-associated myocardial dysfunction remains less clear. In an effort to understand the mechanisms of action behind cigarette smoke-induced cardiac anomalies, we set out to investigate the role of crotonaldehyde, one of the major α,β-unsaturated aldehydes present in cigarette smoke and a lipid peroxidation endproduct, on cardiomyocyte contractile function and the underlying mechanisms involved. Cardiomyocyte contractile function, intracellular Ca^{2+} handling, sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) and mitochondrial function, oxidative stress and apoptosis were assessed in murine cardiomyocytes following short-term exposure to crotonaldehyde. Levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) were assessed as a sensitive marker for oxidative stress and DNA damage [15,16]. Recent evidence suggested a key role of transient receptor potential (TRP) vanilloid cation channels activated by various noxious chemicals including cigarette smoke in cigarette smoking-associated respiratory illnesses such as cough [17,18]. To this end, special focus was made toward understanding if and how TRPV1 cation channel plays a role in short-term crotonaldehyde exposure-induced cardiomyocyte mechanical dysfunction, if any.

2. Materials and methods

2.1. Isolation of murine cardiomyocytes and drug treatment

All experiment procedures were approved by our Institutional Animal Care and Use Committee at the University of Wyoming (Laramie, WY, USA). In brief, after ketamine/xylazine (80 and 12 mg/kg, respectively, i.p.) sedation, hearts were removed from adult male C57BL/6 mice and were perfused with KHB buffer containing (in mM): 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 10 HEPES and 11.1 glucose. Hearts were digested with Liberase for 20 min. Left ventricles were removed and minced before being filtered. Cardiomyocyte yield was ~75% [19]. Isolated murine cardiomyocytes were then incubated with crotonaldehyde at the given concentrations for 6 h prior to mechanical and biochemical assessment. Only rod-shaped cardiomyocytes with clear edges were used for mechanical evaluation. To examine the potential role of TRPV1 and oxidative stress in crotonaldehyde-induced cardiomyocyte contractile responses, if any, cohorts of murine cardiomyocytes were incubated with crotonaldehyde (25 μM) at 25 °C for 6 h in the absence or presence of the TRPV1 inhibitor capsazepine (1 μM) [20], the reactive oxygen species (ROS) scavenger glutathione (10 mM) [21] or the NADPH oxidase inhibitor apocynin (5 μM) [22] prior to the mechanical or biochemical assessment.

2.2. Cell shortening/relengthening

Mechanical properties of cardiomyocytes were assessed using a SoftEdge MyoCam® system (IonOptix Corporation, Milton, MA, USA). In brief, cells were placed in a Warner chamber mounted on the stage of an inverted microscope (Olympus, IX-70) and superfused (~1 ml/min at 25 °C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES at pH 7.4. The cells were field stimulated with supra-threshold voltage at a frequency of 0.5 Hz, 3 ms duration, using a pair of platinum wires placed on opposite sides of the chamber connected to a FHC stimulator (Brunswick, NE, USA). The myocyte being studied was displayed on the computer monitor using an IonOptix MyoCam camera. An IonOptix SoftEdge software was used to capture changes in cell length during shortening and relengthening. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS) – indicative of ventricular contractility, time-to-PS (TPS) – indicative of contraction duration, and time-to-90% relengthening (TR_{90}) – represents relaxation duration, maximal velocities of shortening ($+dL/dt$) and relengthening ($-dL/dt$) – indicatives of maximal velocities of ventricular pressure rise/fall [23].

2.3. Intracellular Ca^{2+} transient measurement

Myocytes were loaded with fura-2/AM (0.5 μM) for 10 min and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system (IonOptix). Cardiomyocytes were placed on an Olympus IX-70 inverted microscope and imaged through a Fluor × 40 oil objective. Cells were exposed to light emitted by a 75 W lamp and passed through either a 360 or a 380 nm filter, while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480 and 520 nm by a photomultiplier tube after first illuminating the cells at 360 nm for 0.5 s then at 380 nm for the duration of the recording protocol (333 Hz sampling rate). The 360 nm excitation scan was repeated at the end of the protocol and qualitative changes in intracellular Ca^{2+} concentration were inferred from the ratio of fura-2 fluorescence intensity (FFI) at two wavelengths (360/380). Fluorescence decay time was measured as an indication of the intracellular Ca^{2+} clearing rate. A single exponential curve fit program was applied to calculate the intracellular Ca^{2+} decay constant [24].

2.4. SERCA activity measured by $^{45}Ca^{2+}$ uptake

Following crotonaldehyde treatment for 6 h, cardiomyocytes were sonicated and solubilized in a Tris–sucrose homogenization buffer consisting of 30 mM Tris–HCl, 8% sucrose, 1 mM PMSF and 2 mM dithiothreitol. To determine SERCA-dependent Ca^{2+} uptake, cardiomyocytes were incubated with or without the SERCA inhibitor thapsigargin (10 μM) for 15 min. The difference between the two readings was deemed the thapsigargin-sensitive uptake through SERCA. Uptake was initiated by the addition of an aliquot of supernatant to a solution consisting of (in mM) 100 KCl, 5 NaN_3, 6 $MgCl_2$, 0.15 EGTA, 0.12 $CaCl_2$, 30 Tris–HCl pH 7.0, 10 oxalate, 2 ATP and 1 μCi $^{45}CaCl_2$ at 37 °C. Aliquots of samples were injected onto glass filters on a suction manifold and washed 3 times. Filters were then removed from the manifold, placed in scintillation fluid and counted. SERCA activity was expressed as cpm/mg protein [25].

2.5. Cell viability

The [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay is based on transformation of the tetrazolium salt MTT by active mitochondria to an insoluble formazan salt. Cardiomyocytes were incubated with crotonaldehyde at the given concentrations in the absence or presence of capsazepine or glutathione for 6 h. Cardiomyocytes were then plated in microtiter plate at a density of 3×10^5 cells/ml prior to the addition of MTT to each well with a final concentration of 0.5 mg/ml. The plates were then incubated for 2 h at 37 °C. The formazan crystals were dissolved in dimethyl sulfoxide (150 μl/well). Formazan was quantified spectroscopically at 560 nm using a SpectraMax® 190 spectrophotometer [26].

2.6. Caspase-3 assay

Caspase-3 is an enzyme activated during induction of apoptosis. In brief, 1 ml of PBS was added to flasks containing adult mouse cardiomyocytes and the monolayer was scraped and collected in a