Journal of Molecular and Cellular Cardiology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Review article

Reactive oxygen species and excitation–contraction coupling in the context of cardiac pathology

Anne C. Köhler [a,b], Can M. Sag [a], Lars S. Maier [a,*]

[a] Department of Internal Medicine II, University Hospital Regensburg, Germany
[b] University of Leipzig, Leipzig, Germany

ARTICLE INFO

Article history:
Received 5 December 2013
Received in revised form 5 February 2014
Accepted 1 March 2014
Available online xxxx

Keywords:
Excitation–contraction coupling
Calcium
Sodium
Free radicals
CaMKII

ABSTRACT

Reactive oxygen species (ROS) are highly reactive oxygen-derived chemical compounds that are by-products of aerobic cellular metabolism as well as crucial second messengers in numerous signaling pathways. In excitation–contraction-coupling (ECC), which links electrical signaling and coordinated cardiac contraction, ROS have a severe impact on several key ion handling proteins such as ion channels and transporters, but also on regulating proteins such as protein kinases (e.g. CaMKII, PKA or PKC), thereby pivotally influencing the delicate balance of this finely tuned system. While essential as second messengers, ROS may be deleterious when excessively produced due to a disturbed balance in Na^+ and Ca^{2+} handling, resulting in Na^+ and Ca^{2+} overload, SR Ca^{2+} loss and contractile dysfunction. This may, in the end, result in systolic and diastolic dysfunction and arrhythmias. This review aims to provide an overview of the single targets of ROS in ECC and to outline the role of ROS in major cardiac pathologies, such as heart failure and arrhythmogenesis.

Contents

Abbreviations: CaMKII, Ca^{2+}/calmodulin dependent protein kinase II; cAMP, cyclic adenosine monophosphate; cTn, cardiac Troponin; DAD, delayed afterdepolarization; EAD, early afterdepolarization; ECC, excitation–contraction coupling; H_2O_2, hydrogen peroxide; HNO, Nitroxyl; I_{Ca}, inward Ca^{2+} current; I_{Na}, inward Na^+ current; IP_3R, Inositol-1,4,5-Trisphosphate Receptors; LTCC, L-type voltage-gated cardiac calcium channels; Na_v (1.5), voltage-gated cardiac sodium channels; NCX, sodium/calcium exchanger; NHE, sodium/proton exchanger; NKA, Na^+–K^+-ATPase; O_2^-, superoxide anion; OH, hydroxyl radical; PKA, cAMP dependent protein kinase A; PKC, protein kinase C; PLB, phospholamban; PMCA, plasma membrane Ca^{2+}-ATPase; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; ROS, reactive oxygen species; RyR2, cardiac ryanodine receptor (ryanodine receptor 2); SERCA2a, sarco/endoplasmic reticulum calcium ATPase.

* Corresponding author at: Department of Internal Medicine II, University Hospital Regensburg, 93042 Regensburg, Germany. Tel: +49 941 944 7201; fax: +49 941 944 7202.
E-mail address: lars.maier@ukr.de (L.S. Maier).

http://dx.doi.org/10.1016/j.yjmcc.2014.03.001
0022-2828/

1. Introduction

Reactive oxygen species (ROS) are highly reactive metabolites of molecular oxygen (O_2). In aerobic organisms, they are an inevitable by-product of the reduction of O_2 in the mitochondrial respiratory chain [1,2]. Depending on the number of transferred electrons, several intermediates may be generated, such as superoxidanion (O_2^-) and hydrogen peroxide (H_2O_2). These can be further reduced to water (H_2O), but in the presence of divalent metal ions, the highly reactive hydroxyl radical (OH•) can be generated from molecular oxygen and hydroxygen peroxide in the Haber-Weiss-reaction. In an aerobic cellular setting, ROS are constantly produced endogenously, not only in mitochondria, but also by enzymatic systems such as NADPH oxidases, xanthine oxidase or uncoupled nitric oxide synthases [3–5]. To prevent cellular oxidative damage, there are numerous enzymatic scavenging systems such as superoxide dismutase, catalase and glutathione peroxidase or non-enzymatic scavenging systems, e.g. thioredoxin, vitamins or flavenoids [6]. Under physiological conditions, ROS production and scavenging capacity are balanced. A moderate increase of ROS alters protein expression and function due to oxidative modification and serves as an important second messenger [6–8]. This has been shown to occur in several pathologies, e.g. the induction of adaptive processes in hypoxia and reperfusion [9]. When the equilibrium is largely shifted due to *exogenous* ROS infliction (e.g. due to administration of chemotherapy) or increased *endogenous* ROS production (e.g. in several cardiac pathologies such as heart failure), antioxidant systems can no longer prevent severe oxidative damage, which affects proteins, lipids and DNA and may induce apoptosis [6,10,11]. This shift toward a pro-oxidative milieu is referred to as oxidative stress.

In cardiomyocytes, there are many deleterious consequences of oxidative stress, such as hypertrophy, fibrosis, apoptosis, inflammation and structural cardiac remodeling [12–16]. In addition, cardiac excitation–contraction coupling (ECC), which transduces electrical excitation into a coordinated contraction, is also affected by oxidative stress. Upon depolarization, voltage-gated sodium channels (Na_v 1.5) open, leading to the so-called up-stroke of the cardiac action potential, a fast depolarization of the membrane potential due to a large Na^+ inward current [17,18]. This current lasts for only ~10 ms, until Na_v closes again as a result of open-state inactivation. Subsequently, voltage-gated Ca^{2+} channels (LTCC) open and initiate a Ca^{2+} inward current, which in turn leads to Ca^{2+}-induced opening of ryanodine receptors (RyR2), the Ca^{2+} release channels of the sarcoplasmic reticulum [19]. This Ca^{2+}-induced Ca^{2+} release is responsible for a 10-fold increase of cytosolic Ca^{2+} during systole and activates the myofilaments via troponin C-binding. Repolarization occurs as a result of inactivation of LTCC and activation of delayed rectifying K^+ currents. This finely tuned process is regulated by a number of protein kinases and phosphatases, including Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), cAMP-dependent protein kinase A (PKA) and protein kinase C (PKC) as well as protein phosphatase 1 (PP1) and phosphatase 2A (PP2A) [20–22]. Many of these proteins are sensitive to redox-modifications and their functions have been reported to be altered in a pro-oxidant milieu. ROS-induced cardiac damage, e.g. contractile dysfunction in heart failure and cardiac arrhythmogenesis, are suspected to be conveyed by oxidation of key proteins in ECC on a posttranslational level. This review highlights physiologically relevant effects of ROS on key (regulating) proteins in ECC and aims to discuss their significance in cardiac pathology.

This subject has recently been reviewed [23,24]. For a comprehensive overview, we refer to these articles as well.

2. Modification of ion channels and transporters

2.1. Cardiac ryanodine receptor

The cardiac ryanodine receptor (RyR2), a sarcoplasmic Ca^{2+} channel, is crucially involved in ECC as the main effector of Ca^{2+}-induced Ca^{2+} release. RyR2 is co-localized with the LTCC in the t-tubule region (see Fig. 2), and is activated by Ca^{2+} entering the cell through LTCC. RyR2 activation in turn induces Ca^{2+} release from the SR, leading to a large increase in cytosolic Ca^{2+} concentration, which activates the contractile system.

The RyR2 complex is a tetrameric formation of four RyR2 monomers paired with four stabilizing proteins FKBP12.6. It is also co-localized with protein kinases such as CaMKII and PKA and several other regulatory proteins (e.g. a cAMP-specific type 4 phosphodiesterase or triadin) [25]. There are many known inhibitors of RyR2, e.g. Mg^{2+}, as well as activators, e.g. ATP [25,26]. These factors, as well as local $[Ca^{2+}]_i$ and $[Ca^{2+}]_{SR}$, have an impact on RyR2-mediated Ca^{2+} release and consequently on systolic Ca^{2+} transient amplitude and cardiac contractile force. RyR2 function is subject to redox-regulation, which is of physiological and pathophysiological relevance (see Fig. 1). Oxidative stress and ROS in particular have been shown to activate RyR2, presumably due to formation of disulfide bonds [27]. In one RyR2 monomer, there are 89 cysteine residues, approximately 21 of which are free [28], and have effectively been shown to be subject to oxidative modification [29]. Indeed it could be shown that under oxidative conditions (e.g. upon 2,2′-dithiodipyridine, DTDP or H_2O_2 exposure or glutathione deprivation), RyR2 open probability (P_o) is increased, resulting in an increased Ca^{2+} loss from the SR, especially during diastole. Consistently, reducing agents, such as DTT or β-mercaptoethanol, lead to decreased RyR2 activation [30–33]. Interestingly, application of scavengers

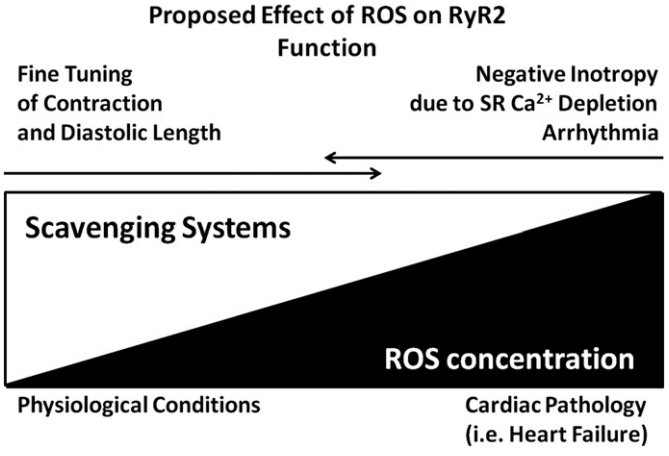

Fig. 1. When present in a low to moderate concentration, ROS play a decisive role as second messengers in cardiac homeostasis, here shown for cardiac RyR2. RyR2 oxidative activation can be important in terms of fine tuning of cardiac contraction as well as relaxation, since RyR2 activation sensitively influences local cytosolic Ca^{2+} concentration. However, when excessively produced, ROS can induce arrhythmia by delayed afterdepolarization due to Ca^{2+} sparks as a consequence of RyR2 activation and cause diastolic Ca^{2+} overload and SR Ca^{2+} depletion, thereby impairing contractile force.

Please cite this article as: Köhler AC, et al, Reactive oxygen species and excitation–contraction coupling in the context of cardiac pathology, J Mol Cell Cardiol (2014), http://dx.doi.org/10.1016/j.yjmcc.2014.03.001