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Review Article

# Role of oxidants on calcium and sodium movement in healthy and diseased cardiac myocytes

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ABSTRACT

In this review article we give an overview of current knowledge with respect to redox-sensitive alterations in $Na^+$ and $Ca^{2+}$ handling in the heart. In particular, we focus on redox-activated protein kinases including cAMP-dependent protein kinase A (PKA), protein kinase C (PKC), and Ca/calmodulin-dependent protein kinase II (CaMKII), as well as on redox-regulated downstream targets such as $Na^+$ and $Ca^{2+}$ transporters and channels. We highlight the pathological and physiological relevance of reactive oxygen species and some of its sources (such as NADPH oxidases, NOXes) for excitation—contraction coupling (ECC). A short outlook with respect to the clinical relevance of redox-dependent $Na^+$ and $Ca^{2+}$ imbalance will be given.



**Contents**



*Abbreviations:* AKAP, A-kinase anchor protein; Ang II, angiotensin II; AP, action potential; APD, action potential duration; BH4, tetrahydrobiopterin; cAMP, cyclic adenosine monophosphate; CaM, calmodulin; CaMKII, Ca/calmodulin-dependent protein kinase II; CaMKII$\delta_C$, cytosolic splice variant of Ca/calmodulin-dependent protein kinase II; DAD, delayed afterdepolarization; DAG, diacylglycerol; DCM, dilated cardiomyopathy; DOX, doxorubicin; EAD, early afterdepolarizations; ECC, excitation-contraction coupling; ER, endoplasmic reticulum; ETC, electron transport chain in the mitochondria; FKBP12.6, calstabin; GSH, gluthathione; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; $I_{Ca}$, inward $Ca^{2+}$ current; $I_{Na}$, inward $Na^+$ current; $I_{Na,\,late}$, late $Na^+$ current; $I_{Ti}$, transient inward current; $I_{to}$, outward rectifying $K^+$ currents; LTCC, L-type $Ca^{2+}$ channels; MPT, mitochondrial permeability transition; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; NaV1.5, $Na^+$ channel protein; NCX1, sarcolemmal $Na^+/Ca^{2+}$ exchanger; NHE, $Na^+/H^+$ exchanger; NKA, sarcolemmal $Na^+/K^+$ ATPase; NO, nitric oxide; NOS, nitric oxide synthase; NOS1, neuronal nitric oxide synthase (also nNOS); NOS2, inducible nitric oxide synthase (also iNOS); NOS3, endothelial nitric oxide synthase (also eNOS); NOX, NADPH oxidase; $ONOO^-$, peroxynitrite; PKA, cAMP-dependent protein kinase A; PKC, protein kinase C; PLB, phospholamban; PLM, phospholemman; PPI-1, protein phosphatase inhibitor 1; ROS, reactive oxygen species; RyR2, ryanodine receptors; SERCA2a, sarcoplasmic reticulum $Ca^{2+}$-ATPase; SOD, superoxide dismutase; SR, sarcoplasmic reticulum; Trx, thioredoxin; XOR, xanthine oxidase.

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## Introduction

Heart failure (HF) is a highly prevalent disease syndrome that is characterized by an inability of the heart to provide sufficient blood flow to meet the body's needs. Various disease states such as (i) vascular dysfunction, (ii) diseased heart valves, (iii) hypertension, (iv) infection/inflammation, (v) primary cardiomyopathies (such as dilated cardiomyopathy, DCM), or even (vi) toxic factors such as (radio-)chemotherapy can cause HF. Failing hearts are usually characterized by a progressively deteriorated contractile function (HF with reduced ejection fraction, HFrEF) except for the situation when contractility is normal despite symptoms (HF with preserved ejection fraction, HFpEF).

On a cellular level, cardiac myocytes that represent the functional core element of the heart reveal dramatically impaired *functional* properties. In that regard, it is now accepted that an impaired intracellular $Ca^{2+}$ handling can causally contribute to contractile dysfunction [1]. A typical example of a relevant defect in $Ca^{2+}$ handling that can contribute to impaired contractility is diastolic $Ca^{2+}$ leakage from the sarcoplasmic reticulum (SR). The SR represents the intracellular $Ca^{2+}$ store of the cardiac myocyte. *Diastolic* SR $Ca^{2+}$ leakage leads to a progressive SR $Ca^{2+}$ depletion. In turn, less $Ca^{2+}$ can be released from the SR during *systole.* This results in a diminished increase in cytosolic $Ca^{2+}$ during systole (i.e., decreased systolic $Ca^{2+}$ transient). As a consequence, insufficient activation of myofilaments results in an impaired myocyte contraction. SR $Ca^{2+}$ leak is a result of "leaky" $Ca^{2+}$ release channels (ryanodine receptors, RyR2). Insufficient sealing of the SR is a typical feature in human and animal failing cardiac myocytes [2]. The underlying mechanisms are complex and may involve increased RyR2 phosphorylation by activated serine/threonine protein kinases [3,4]. Moreover, the concentration of reactive oxygen species (ROS) is elevated in failing hearts and might further amplify redox-regulated protein kinase's activity at the RyR2 [5,6]. In addition, it is becoming increasingly clear that ROS themselves can alter broad aspects of $Na^+$ and $Ca^{2+}$ handling in healthy and diseased cardiac myocytes [7–11]. For instance, RyR2s are rich in cystein residues and are ready targets to redox modifications. In fact, ROS were shown to directly induce pathological SR $Ca^{2+}$ leak in failing myocytes by redox modification [12]. Myocytes from failing hearts reveal a pattern of impaired $Na^+$ and $Ca^{2+}$ handling in the face of increased ROS generation. It is therefore tempting to speculate that hampered $Na^+$ and $Ca^{2+}$ handling is due to disturbed ROS homeostasis. In turn, redox-modified $Ca^{2+}$ handling may contribute to the disease progression, which would render it a novel and promising therapeutical target.

In that regard, this review aims to give a comprehensive overview of current knowledge with respect to redox-sensitive alterations in cardiac $Na^+$ and $Ca^{2+}$ handling. It particularly focuses on redox-activated protein kinases including cAMP-dependent protein kinase A (PKA), protein kinase C (PKC), and Ca/calmodulin-dependent protein kinase II (CaMKII), as well as on redox-regulated downstream targets such as $Na^+$ and $Ca^{2+}$ transporters and channels. We will try to highlight the physiological and pathological relevance of reactive oxygen species and some of its chosen sources (such as NADPH oxidases, NOXes) for excitation—contraction coupling (ECC). Finally, a short outlook with respect to the clinical relevance of redox-dependent $Na^+$ and $Ca^{2+}$ imbalance will be given.

## Role of oxidants on calcium and sodium movement in healthy and diseased cardiac myocytes

### *Principles of regular excitation—contraction coupling*

Physiological cardiac function requires the coordinated temporal and spatial activation of the heart. Excitation—contraction coupling describes the transformation of an electrical stimulus (i.e., an action potential, AP) into a mechanical response in a single cardiac myocyte. In that finely tuned process, intracellular $Ca^{2+}$ and $Na^+$ play key roles [13]. When the cell becomes excited (i.e., the membrane depolarizes), voltage-dependent $Na^+$ channels open, which initiate a large inward $Na^+$ current ($I_{Na}$, $>10$ nA). This transitory $Na^+$ influx that lasts for $\sim$10 ms results in the fast upstroke of the AP. The following plateau phase of the AP is maintained by activation of L-type $Ca^{2+}$ channels (LTCC) mainly located in the transverse tubule. The resulting inward $Ca^{2+}$ current ($I_{Ca}$) counterbalances the partial membrane repolarization as it is induced by (i) fast $Na^+$ current ($I_{Na}$) inactivation and (ii) opening of transient outward rectifying $K^+$ currents ($I_{to}$). $I_{Ca}$ leads to a localized increase of the $Ca^{2+}$ concentration in the "dyadic cleft" in close neighborhood to RyR2 of the SR. This transsarcolemmal $Ca^{2+}$ influx activates the RyR2 and results in the so called "$Ca^{2+}$-induced $Ca^{2+}$ release" from the SR, which provides the main component of the temporary (or "transient") increase in cytosolic $Ca^{2+}$ during systole ($Ca^{2+}$ transient). The systolic $Ca^{2+}$ transient causes myofilament activation (via the binding of $Ca^{2+}$ to troponin C) and contraction. Repolarization is introduced by inactivation of $I_{Ca}$ and activation of delayed rectifying $K^+$ currents. $Ca^{2+}$ is removed from the cytosol via two major pathways: while (i) the SR $Ca^{2+}$ ATPase (SERCA2a) that is located in the membrane of the SR pumps $Ca^{2+}$ back into the SR lumen, (ii) sarcolemmal $Na^+/Ca^{2+}$ exchanger (NCX1) transfers $Ca^{2+}$ into the extracellular space. Regular $Na^+$ concentrations are mainly maintained by the activation of the sarcolemmal $Na^+/K^+$ (NKA) exchanger that extrudes $Na^+$ for $K^+$ ions.

### *$Na^+$ and $Ca^+$ handling is impaired in failing cardiac myocytes*

Based on this simplified scheme of normal ECC, it is easy to understand that any factor that hampers this delicately balanced ion homeostasis will be of great pathophysiological relevance to the cell and, hence, to the heart. There are numerous examples of cardiac disease states, in which an imbalanced $Na^+$ and $Ca^{2+}$ handling is known to contribute to the resulting heart disease. In HF, functionally and structurally disturbed $Na^+$ and $Ca^{2+}$ handling causes contractile dysfunction. Failing myocytes display a pattern of diminished $Ca^{2+}$ content in the SR, which leads to decreased systolic $Ca^{2+}$ transients and weakened myocyte contraction [3,14]. Mechanistically, the *balanced* relationship between $Ca^{2+}$ release from and reuptake into the SR is severely disturbed. Failing cardiomyocytes show increased diastolic SR $Ca^{2+}$ release (i.e., SR $Ca^{2+}$ leak) together with impaired $Ca^{2+}$ reuptake into the SR.