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Reactive Oxygen Species at High Altitude (Hypobaric Hypoxia) on the Cardiovascular System

Patricia Siques, Julio Brito and Eduardo Pena

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Abstract

Reactive oxygen species (ROSs) play important physiological and physiopathological roles in the cardiovascular system. An imbalance between ROS and antioxidants, termed oxidative stress, can contribute to endothelial dysfunction and cardiovascular remodeling. ROSs have been demonstrated to be increased and to regulate the following main pulmonary vasculature changes that occur at high altitude (hypobaric hypoxia): hypoxic pulmonary vasoconstriction (HPV), pulmonary hypertension, right ventricular hypertrophy (RVH), and ultimately, cardiac failure. Thus, ROS increases are a public health concern for the increasing number of people living or working at high altitudes. ROSs trigger the activation of different metabolic signaling pathways that alter the activity of redox-sensitive transcription factors and translational signals. Consequently, we provide a comprehensive review of the literature on the main factors, sources, and mechanisms of action of ROS and their effects on the cardiovascular system under hypobaric hypoxic conditions. Although ROS generation is a normal physiological activity, under hypobaric hypoxia (high altitude) conditions, ROS levels are elevated. The principal sources of ROS are nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-4 (NOX4) in the vascular system and NOX2 in cardiac tissue. Thus, the information presented in this review provides a broad view of the relationship between ROS and hypoxia.

Keywords: reactive oxygen species, altitude, hypoxia, cardiovascular system, pulmonary hypertension, right ventricle hypertrophy

1. Introduction

In the cardiovascular system, reactive oxygen species (ROSs) and reactive nitrogen species (RNSs) play important physiological roles in the control of endothelial functions, vascular tone, and cardiac functions, as well as a pathophysiological role in inflammation, hypertrophy, fibrosis,

angiogenesis, cell proliferation, apoptosis, and migration. The regulation of this biological activity is the result of a balance between oxidants and the buffering action of antioxidants, such that an imbalance between ROS or RNS and antioxidants (called "oxidative stress"), wherein ROS or RNS is increased, contributes to cellular signaling that leads to endothelial dysfunction and cardiovascular remodeling. On the one hand, ROS triggers the activation of different cellular pathways by activating specific proteins (e.g., Akt1/2: serine/threonine protein kinase; PKC: protein kinase C; PDK: 3-phosphoinositide-dependent kinase; Erk1/2: extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinase; PI3K: phosphatidylinositol-3-kinase; and JAK: Janus kinase) in different tissues. On the other hand, ROSs alter the activity of redox-sensitive transcription factors (i.e., AP-1: activator protein 1; NF-κB: nuclear factor-κB; HIF-1α: hypoxia-inducible factor-1α; and STAT: signal transducer and activator of transcription) to induce direct effects on enzymes, receptors, or ion channels and different cellular responses [1]. Oxidative stress is generated by external factors, such as a decrease in the partial pressure of oxygen (PO_2) in hypobaric hypoxia due to high-altitude exposure. Over 100 million people live in hypoxic conditions worldwide [2, 3], the number of people exposed to hypoxic conditions is higher if we include people traveling to high altitudes for either leisure or work. Human beings, except Tibetans, are not naturally adapted or genetically equipped to live at high altitudes. Therefore, depending upon its degree and duration or the altitude, hypoxia generates several physiological or pathological effects on the human body [4].

The main effects of exposure to hypobaric hypoxia are excessive erythrocytosis and high-altitude pulmonary hypertension (HAPH). Features of the latter include high pulmonary artery pressure, vascular remodeling of pulmonary arteries, right ventricle hypertrophy (RVH), and cardiac failure. In addition to the mechanical explanation usually considered for this phenomenon, new data suggest other, mechanical-independent mechanisms. We attempt to provide a comprehensive review of the principal factors, sources, and mechanism of action of ROS in the development of cardiovascular diseases under hypobaric hypoxia and/or similar stressors, with a specific focus on the cardiovascular system.

2. High altitude (hypobaric hypoxia) and oxidative stress considerations

2.1. ROS and RNS

ROSs are small molecules that derive from O_2 and include the superoxide anion ($O_2^{\bullet-}$), hydroxyl ion (OH), peroxyl (RO_2), and alkoxy agents ($RO^{\bullet}$), as well as certain nonradicals that are either oxidizing or easily converted into radicals, such as hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2). There are other types of molecules that are oxidizing agents but contain nitrogen; these radicals are called RNS. One example is peroxynitrite (NOO^-), which is derived from nitric oxide (NO) when oxidized by $O_2^{\bullet-}$ [5]. These molecules are highly reactive due to the presence of an unpaired valence electron layer [6, 7], and through this electronic condition, ROSs avidly interact with a large number of molecules, including the plasma membrane and organic macromolecules such as proteins, lipids, carbohydrates, and

nucleic acids, to achieve electron stability. Through such interactions, ROS can irreversibly alter or destroy the function of specific molecules in the cell; for this reason, ROSs are recognized as important players in many cellular signaling and physiological processes [8].

Based on the above, ROSs are considered harmful molecules that promote cellular aging in biological organisms. However, to date, at least one beneficial function has been described: ROSs produced by leukocytes, neutrophils, and macrophages were found to play a major role in the defense against host molecules or foreign agents [9]. Additionally, ROSs were recently proposed to participate not only in cellular damage and the destruction of pathogens but also in several reversible regulatory processes in all cells and tissues [9]. In other words, in a healthy organism, the cell normally produces low levels of ROS, which activates specific signaling pathways that contribute to normal responses to various stimuli [10]; however, the inability to adequately compensate for an increase in ROS by the antioxidant system of the tissue or organism (known as "oxidative stress") can result in the development of several pathologies [11].

Therefore, under oxidative stress, high levels of ROS produce changes in the cell through the following mechanisms: (1) activating redox-sensitive protein kinases, such as JAKs, PKC, PI3K, and PDK; (2) activating mitogen-activated protein kinase (MAPK) family members, such as Akt, JNKs, Erk1/2, and p38, which are involved in angiogenesis and cell proliferation, differentiation, migration, growth, motility, survival, and apoptosis; (3) altering the activity of redox-sensitive transcription factors, such as AP-1, NF-κB, HIF-1α, and STAT; (4) inhibiting protein tyrosine phosphatase (PTP), which produces high levels of phosphorylated proteins; (5) producing an increase in the concentration of intracellular calcium $[Ca^{2+}]_I$; (6) producing direct effects on cellular structures, such as enzymes, receptors, and ion channels, or generating indirect effects on these structures through polyunsaturated fatty acids (PUFAs), which are highly susceptible to ROS, such that the oxidative breakdown of n-3 PUFAs may compromise membrane lipid matrix dynamics and, hence, the structure and function of membrane-associated proteins, such as enzymes, receptors, and transporters; and (7) stimulating the activity and expression of pro-inflammatory molecules and pro-oncogenes [1, 7, 8, 12–14].

For these reasons, regulating ROS production modulates the activity of various intracellular molecules and various cell signaling pathways, thereby inducing specific acute and chronic changes in the phenotype and function of a cell (commonly referred to as "redox signaling"). Thus, with a specific focus on the cardiovascular system, ROSs play an important physiological role in the control of endothelial functions, vascular tone, and cardiac functions, as well as a pathophysiological role in inflammation, hypertrophy, fibrosis, angiogenesis, cell proliferation, apoptosis, and migration, whereby all these processes synergistically contribute to endothelial dysfunction and cardiovascular remodeling [7], as we demonstrate later in this chapter.

2.2. High-altitude exposure: types of exposure and principal cardiovascular responses

As a result of decreased barometric pressure and oxygen partial pressure (PaO$_2$), exposure to high altitudes generates an important effect on the cardiovascular system known as *hypobaric hypoxia*, where reduced uptake of oxygen leads to a decrease in O$_2$ transported by the blood

to all the cells in the organism [15, 16]. The important physiological effects in living beings are derived from acclimatization or adaptability to high altitude, and these effects fundamentally depend on the level of altitude and the duration of exposure [3].

Acute hypoxia (AH) occurs when a person (e.g., a tourist or alpinist) is exposed to high altitudes for short periods of time (days or hours), whereas *chronic hypoxia* (CH) occurs when a person is permanently exposed to hypoxic conditions (i.e., living at high altitude). A new and distinct form of exposure has recently been shown to be different from all types of hypobaric hypoxia described to date and is related to mining exploitation, thus termed "Chilean mining model of chronic intermittent exposure to high altitude" [17]. This type of hypobaric hypoxia involves working over 3000 m above sea level in shifts (days of work at high altitude and days of rest at sea level) and maintaining this condition for years. It has been estimated that over 200,000 people work under these conditions [18]. This biological condition is classified as chronic intermittent hypobaric hypoxia (CIHH).

There are many effects of high altitude that could ultimately lead to pathologies. However, the principal effects are an increase in hematocrit levels by accumulative red cell production or excessive erythrocytosis (chronic mountain sickness) and the development of acute mountain sickness (AMS), which can begin as mild to severe (as cerebral edema or lung edema). Another effect is the development of hypoxic pulmonary vasoconstriction (HPV), which leads to HAPH, with a prevalence of up to 15% in individuals exposed to high altitude [4].

The latter is of utmost interest, since its consequences are the clinical development of pulmonary hypertension and RVH or cor pulmonale [19–21]. Nevertheless, it must be noted that these effects appear to be less severe in CIHH exposure than in chronic exposure (CH) [16, 22].

3. ROS, hypoxia, and the cardiovascular system

3.1. The cardiovascular system and hypoxia-induced ROS

Previously, it was suggested that exposure to high altitude limits O_2 supplementation in the organism in general and thus reduces the generation of free radicals (ROS), which are derived from this important gas [23]. However, this concept was later disputed with data suggesting that exposure to high altitude (>3000 m) leads to an increase in ROS production in many cell lines, thus generating an O_2 supplementation paradox [24–26]. Finally, the high-altitude-induced increase in ROS products was confirmed by human studies, in which the concentrations of specific biomarkers of oxidative stress (plasmatic lipid peroxidation and iso-8-prostaglandin F-2α level in urine) were found to be increased after acute or chronic exposure to high altitude (4300 m) and without exercise [6]. Therefore, these findings suggest that exposure to hypoxia produces oxidative stress, thus causing all the aforementioned effects on both physiological and pathological cell signaling responses [9, 27].

Studies have evaluated the main sources of ROS in several cell lines under hypoxic conditions and concluded that the predominant source of ROS in the cardiovascular system is the enzymatic

complex *nicotinamide adenine dinucleotide phosphate* (NADPH) *oxidase* (NOX), which prevails over other ROS-generating systems, such as mitochondria and xanthine oxidase [5, 28]. NADPH oxidases comprise a complex multicomponent family of transmembrane and cytosolic proteins that use NADPH as an electron donor to reduce molecular oxygen to the superoxide anion and hydrogen peroxide. The prototype NADPH oxidase was formerly known as gp91phox and was first described in leukocytes [1]. However, it is important to highlight that subsequent studies characterized seven members of the NOX family (NOX1 to 5 and dual oxidases 1 and 2) with diverse distributions among specific tissues and organs [10]; these NOX family members have since been described in nonphagocytic cells, including neurons, skeletal muscle, myocytes, hepatocytes, endothelial cells, hematopoietic cells, stem cells, and cardiomyocytes [28]. For example, previous studies found that stimulating rat cardiomyocytes with angiotensin II (Ang II) directly activated the NADPH oxidase complex, specifically the NOX2 isoform. This NOX2 complex can be activated in healthy organisms by several factors, including a G-protein receptor agonist (Ang II) and endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), and mechanical shear stress from blood flow. However, the pathological activation of NOX2 (e.g., cytokines such as tumor necrosis factor-alpha) can result in the generation of much higher concentrations of ROS that appear to contribute to pathological states, including endothelial dysfunction, myocardial hypertrophy, fibrosis, heart failure, inflammation, atherosclerosis, coronary artery disease, stroke, and renal and pulmonary fibrosis [10].

Studies of the vascular system have shown that the predominant isoform of the NADPH complex is NOX4 [27, 29], and previous investigations revealed that NOX4 is involved in oxygen sensing, vasomotor control, angiogenesis, fibrosis, cell proliferation, differentiation, migration, apoptosis, and senescence. Elevated expression of NOX4 has been reported in a number of cardiovascular diseases, including atherosclerosis, pulmonary fibrosis, cardiac failure, and ischemic stroke [30].

Notably, previous studies have demonstrated that a single mutation in NOX4 disrupts $O_2^{\bullet-}$ production; these studies showed that although $O_2^{\bullet-}$ production was undetectable in NOX4-transfected cells, there was robust production of H_2O_2, in contrast to the mixture of $O_2^{\bullet-}$ and H_2O_2 production following transfection with NOX1-NOX3 and NOX5 [31].

This effect of NOX4 was found due to the mutation of a highly conserved histidine residue in the E-loop of the NOX4 structure that promotes the rapid dismutation of $O_2^{\bullet-}$ before it leaves the enzyme [32], highlighting that higher concentrations of NOX4-produced H_2O_2 also elicit multiple effects. These effects are smooth muscle cell hypertrophy, activation of metalloproteases, and a low concentration of NOX4, which has been proposed as a cardiac protector [33]. Preliminary data from DNA microarray screens indicate that H_2O_2 causes a more than two-fold induction in the expression of nearly 100 genes, with a more than two-fold reduction in the expression of many more. Further, many transcription factors have been shown to be activated by H_2O_2. For example, as mentioned above, nuclear factor-κB (NF-κB) usually resides in the cytoplasm in association with an inhibitor protein (IκB) but is dissociated from IκB in the presence of H_2O_2. This process generates the nuclear translocation of NF-κB, and other transcription factors directly affected by exogenous H_2O_2, such as activator protein 1 (AP-1) (a complex composed of the *jun* and *fos* gene products) [1].

3.2. HAPH and NOX4-produced ROS

As mentioned previously, HAPH is one of the principal pathologies involved in hypoxic exposure and arises from the narrowing of pulmonary arteries, which elevates pulmonary vascular resistance and, consequently, pulmonary artery pressure. HAPH is characterized by excessive proliferation and hypertrophy of pulmonary arterial medial smooth muscle and adventitial remodeling. ROS may serve as important regulators of pulmonary vascular remodeling, and some evidence supports a prominent role of NOX4 in the pathogenesis of HAPH [27]. For example, NOX4 is the major NADPH oxidase homolog expressed in human pulmonary artery smooth muscle cells, and its expression at both the mRNA and protein levels is significantly increased in lungs from patients with idiopathic pulmonary arterial hypertension (IPAH) compared to that in healthy lungs [34], which may suggest a strong correlation between NOX4 and the onset of HAPH.

In addition, NOX4 expression was found to be increased in a CH-induced pulmonary artery hypertension (PAH) experimental mouse model. Therefore, NOX4 may also mediate hypoxia-induced growth of human pulmonary smooth muscle cells [35]. Indeed, this was corroborated in studies that silenced NOX4 expression by RNA interference; the results demonstrated a decrease in the growth of human pulmonary arterial smooth muscle cells and fibroblast proliferation [30].

Furthermore, if we focus on the HPV response to high altitude (hypobaric hypoxia), this effect is explained by smooth muscle cell contraction. Studies have shown that one of the main pathways involves an increase in intracellular calcium $[Ca^{2+}]_I$ from the extracellular space and intracellular stores through voltage-activated potassium channels (KV) and nonspecific cation channels (NSCC) [36]. Nevertheless, further studies in lung cells found an increase in hypoxia-induced ROS that produced the activation of a calcium sensor (SMIT1) in the endoplasmic reticulum (ER), where this protein activates CRAC channels that contribute to the increase in intracellular Ca^{2+} [37].

In the nitric oxide (NO) pathway, studies have reported that intermittent hypobaric hypoxia exposure reduces the bioavailability of NO in lung parenchyma and vasculature [27, 38]. NO is an endogenous vasodilator that activates cyclic GMP, which in turn activates protein kinase G (PKG) and ultimately causes reuptake of Ca^{2+} and the opening of calcium-activated potassium channels, leading to the relaxation of vascular smooth muscle cells (VSMCs). The decrease in NO bioavailability observed following exposure to intermittent hypobaric hypoxia may be due to the destruction of NO by hypobaric hypoxia-induced ROS, such as superoxide anion (O^{2-}), which is produced by the enzymatic complex NAPH oxidase, specifically the NOX4 subunit [27, 29]. In agreement with these findings, studies silencing NOX4 and p22phox, another subunit involved in the activation of NADPH oxidase-NOX4, showed attenuation of ROS formation and proliferation in human and rat pulmonary artery smooth muscle cells (PASMCs) [39]. Therefore, all these ROS-activated cellular mechanisms may contribute to pulmonary artery remodeling, pulmonary hypertension, and finally, cardiac failure due to RVH.

Recently, other studies noted that NOX4 does not contribute to the development of hypoxia-induced pathologies, such as HPV or pulmonary hypertension. However, these studies found increases in superoxide anion ($O_2^{\bullet-}$) levels in SMCs of NOX2- and NOX1-overexpressing mice and that NOX4 overexpression increased H_2O_2 levels. Therefore, NOX4 may be incapable of

destroying NO; this contrasts with NOX1- and NOX2-derived $O_2^{\bullet-}$, which destroys NO and contributes to the formation of $ONOO^-$, thus leading to vascular dysfunction [40].

NOX4 has received considerable attention because it differs from NOX1 and NOX2 in several aspects: (1) NOX4 mRNA expression is higher than that of the other NOX homologs (>1000-fold higher copy number than NOX1 and NOX2) and different from NOX1 and NOX2, which are induced by Ang II in VSMCs. This is supported by studies in cultured cells showing the expression of NOX4 mRNA at copy numbers greater than 10- to 100-fold that of NOX2 and greater than 100-fold that of NOX1 [41]. Therefore, NOX4 is the most abundant NOX isoform in the vasculature. However, one must be mindful that mRNA levels may not accurately reflect protein expression levels of the various NOX isoforms [42]. (2) NOX4 expression increases over the course of differentiation and is required for the maintenance of the differentiated phenotype in cultured cells. (3) NOX4, unlike NOX1 and NOX2, is independent of cytosolic activator subunits and thus is potentially constitutively active. This is supported by overexpression studies conducted mostly in HEK293 cells, which have suggested that NOX4-dependent ROS production is controlled by the abundance of the enzyme. This aspect does not exclude the possibility that other interacting proteins, such as Poldip2 or protein disulfide isomerase, alter the activity of NOX4. Conversely, NOX4 predominantly releases H_2O_2, which cannot alter NO. NOX4 overexpression in the presence of NO does not lead to $ONOO^-$ formation, which strongly argues against significant $O_2^{\bullet-}$ formation by the enzyme [33].

Therefore, eNOS uncoupling is an important mechanism that leads to endothelial dysfunction. It is becoming progressively clear that the presence of low concentrations of H_2O_2 not only acts as a vasodilator by activating kinase G $I\alpha^3$ but also may activate and induce eNOS by several mechanisms [33]. Thus, NOX4 might have an antagonistic function to NOX1 and NOX2, since it differs from these NADPH oxidases. NOX4 is a special NOX because it is highly constitutively active and is highly expressed in many cardiovascular cells. However, studies using both anti-NOX4 antibodies and *in situ* hybridization showed that NOX4 is primarily expressed in the middle layer of pulmonary blood vessels in both mice and humans [34].

Numerous studies have shown that NOX4 is robustly upregulated in response to transforming growth factor-beta (TGF-β) stimulation in various cell types, including aortic and pulmonary smooth muscle cells, pulmonary and cardiac fibroblasts, and endothelial and embryonic kidney cells [43]. However, tumor necrosis factor-alpha is less specific and can increase NOX1, NOX2, and NOX4 activity and/or the expression of these oxidases in various vascular cells. Other stimuli that induce NOX4 expression are ER stress, shear stress, hypoxia, and ischemia, as well as the activation of PKCα, NF-κB, HIF-1α, and Nrf2. These pathways are also likely dependent on the stimulus and cell type [44], and as mentioned above, Ang II has been shown to potently activate NOX1 and NOX2, but its effect on NOX4 expression is much less pronounced [30].

3.3. Cardiac myocytes and HIF-1α

HIF-1α is a heterodimeric subunit of the transcription factor HIF-1, which regulates the transcription of genes involved in adaptive responses to hypoxia. Therefore, HIF-1 induces and promotes the expression of several genes containing hypoxia-responsive elements (HREs) in their

regulatory region, such as proangiogenic factors (VEGF) or stromal-cell derived factor-1α (SDF-1α, CXCL12), vasoconstrictors (endothelin-1), and inflammation-associated genes (iNOS—inducible nitric oxide synthase and COX2—cyclooxygenase). Many of these factors promote angiogenesis and wound healing and are thus critical for the response to local hypoxia and injury. This HIF-1 system is also used to measure the systemic oxygen supply and to control the formation of red blood cells [12] through the glycoprotein erythropoietin (EPO). EPO has strong organ-protective effects in the heart, brain, and kidney, promotes re-endothelialization, and induces the mobilization of endothelial progenitor cells (EPCs), where ROS-NOX2 production is fundamental for EPO-induced mobilization of EPCs and vascular repair in hypoxic conditions [12].

However, the role of HIF-1α in the development of cardiac hypertrophy has been sparsely documented [45]. More interestingly, carvedilol, a β-receptor blocker, has emerged as a beneficial treatment for cardiac hypertrophy, as it inhibits the overexpression of HIF-1α during pressure overload in the rat heart [46]. Subsequent studies in cardiomyocytes under mild hypoxic conditions showed that HIF-1α controls the process of cardiac hypertrophy through the activation of transient receptor potential canonical 3 (TRPC3) and 6 (TRPC6), producing an increase in the levels of $[Ca^{2+}]_i$ and calcineurin [47].

TRPC channels are nonselective cation channels that mediate Ca^{2+} influx into several cell types, including cardiac myocytes [48]. TRPC expression in cardiac hypertrophy has been studied by several laboratories, with somewhat variable results. For example, previous studies have shown that TRPC3 promotes cardiomyocyte hypertrophy in several animal models, including abdominal aortic banding (AAB) rats and spontaneous hypertensive heart failure rats [47]. Other studies have demonstrated that TRPC6 sequentially initiates a calcineurin signaling circuit during pathological cardiac hypertrophy. However, Ohba et al. [49] demonstrated that TRPC1, TRPC3, TRPC5, and TRPC6 are constitutively expressed, but only TRPC1 expression is significantly increased in hypertrophic hearts from AAB rats. However, these studies regarding the role of HIF-1α in cardiac hypertrophy were based on pathological situations, and their conclusions were controversial. Therefore, the potential role of HIF-1α in adaptive cardiac hypertrophy needs to be clarified.

Further, previous studies showed that the HIF-1 pathway is involved in hypoxia-induced autophagy in cardiomyocytes and that HIF-1-induced autophagy may, therefore, help cardiomyocytes to overcome hypoxic injury and increase survival [50]. In other words, HIF-1α upregulation can increase autophagy and ameliorate the hypoxia-induced reduction in cell viability. Regarding survival and cardiac viability in hypoxic conditions, cardiac muscle cell survival plays a critical role in maintaining the correct function of the heart and, possibly, in cardiac embryogenic development. In contrast, adult cardiomyocytes are thought to be terminally differentiated and therefore have lost their proliferative capacity. One of the mechanisms that cardiomyocytes employ to protect themselves from deleterious stimuli is the release of survival cytokines capable of promoting cytoprotection in an autocrine/paracrine manner [51, 52]. One of these cytokines is cardiotrophin-1 (CT-1). CT-1 is a member of the interleukin-6 family with hypertrophic properties in neonatal and adult cardiomyocytes [53]. In adult cardiomyocytes, CT-1 exerts a protective function in response to death stimuli (apoptosis and necrosis), such as Ang II, H_2O_2, and ischemia-reperfusion. The cardioprotective properties of CT-1 under stress conditions suggest that it may be upregulated during cardiac diseases that are characterized by

an environment of reduced oxygen availability, inflammation, and oxidative stress. Indeed, circulatory levels of CT-1 are elevated in pathological conditions associated with ischemia, including unstable angina pectoris, acute myocardial infarction, hypertensive heart disease, and heart failure. Importantly, studies have shown that hypoxia increased CT-1 in cardiac cells (*in vitro* and *in vivo*) through direct regulation of the *CTF1* promoter by HIF-1α, and this CT-1 activation may protect cells from apoptosis, thus supporting a protective role of CT-1 as a survival factor for cardiomyocytes [52].

3.4. Myocardium, myocytes, and hypoxia-induced ROS

HAPH-induced RVH or end-stage cor pulmonale [19–21] is primarily explained as a compensatory effect of right ventricular afterload. However, numerous investigations have established new avenues for the development of cardiac hypertrophy that highlight oxidative stress as the main mediator [5, 54].

To support the involvement of oxidative stress, a study evaluating both smooth muscle cells and endothelial cells in the development of pulmonary artery remodeling in CH was conducted. This study found that such arterial remodeling occurs via a mitochondrial factor, which requires the Rieske iron-sulfur protein (RISP), a mitochondrial complex III protein required for ROS generation. RISP depletion in endothelial cells and smooth muscle cells prevented CH-induced pulmonary hypertension, but it did not prevent RVH, suggesting that right ventricle remodeling in CH occurs through a mechanism independent of the increase in pulmonary artery pressure [55]. Thus, RVH could be directly produced by hypoxia-induced ROS, such that some *in vitro* experiments showed increased ROS levels in chicken cardiomyocytes and Hep3B cells cultured under AH [56].

Therefore, acute and chronic hypoxic exposure could generate oxidative stress [6] and may activate a large variety of protein kinases, such as MAPK, tyrosine kinases, and Rho kinases, and transcription factors (NF-κB, AP-1, and HIF-1α) that are derived from cellular hypertrophy [57] may also inactivate PTP. Both combined and separate effects induce an increase in the phosphorylation cascade or produce an increase in the concentration of intracellular calcium $[Ca^{2+}]_I$ and stimulate the activity and expression of pro-inflammatory genes and proto-oncogenes [7].

Regarding cardiomyocytes, NOX2-mediated $O_2^{\bullet-}$ formation has been found to activate the protein kinase B or serine/threonine kinase (Akt) signaling pathways through PI3K, JNK, ERK1/2, and p38-MAPK. Thus, activation of these signaling pathways may play a central role in Ang II-stimulated cardiomyocyte hypertrophy [5, 58, 59]. Consequently, oxidative stress could play a fundamental role in cardiac hypertrophy, specifically RVH (possibly independent of the mechanical explanation) as a result of exposure to hypoxia. This is congruent with other studies demonstrated that NOX2 knockout attenuated Ang II and myocardial infarct-induced myocardial fibrosis and cardiomyocyte hypertrophy in mice. Hence, it could be surmised that NOX2 may play an important role in the development of cardiac hypertrophy in either hypoxic or other conditions, and this role may be independent of changes in blood pressure [60].

In addition to NOX2, several studies have reported a relative abundance of NOX4 expression in human and mouse cardiac myocytes [61, 62] and in pulmonary arteries under hypoxia [27].

NOX4 is induced in experimental models of heart failure and in humans [61]. Recent studies using cardiac-specific NOX4 knockout mice revealed decreased levels of ROS and improved performance along with reduced hypertrophy, fibrosis, and apoptosis. Conversely, an experiment using a transgenic cardiac-specific NOX4-overexpressing mouse showed deleterious effects, such as promoting dysfunction, fibrosis, and apoptosis, in response to pressure overload [62]. While these results suggest that NOX4 is a major source of oxidative stress involved in the failing heart, there are reports showing opposite effects using a global NOX4 knockout and cardiac-specific NOX4 transgenic model [63]. These contradictory findings could be explained by differences in the methodology used to induce heart failure.

Supporting a more active role of NOX4, studies have revealed that NOX4 induces positive endothelial effects by producing H_2O_2, which in turn activates protein kinase G Iα by thiol oxidation and subsequent dimerization. Moreover, H_2O_2 also activates endothelial NOS (eNOS). Therefore, it is necessary to determine how NOX4 may mediate such contradictory roles [30].

Another important source of ROS in cardiomyocytes is the mitochondrial complex (electron transport chain). Previous studies have found that mitochondria in cardiomyocytes increase their generation of ROS during hypoxia (1–5% O_2), with the increased ROS generation originating from the proximal region of the electron transport chain, most likely complex III. These observations suggest that ROS generated by mitochondria may trigger p38 phosphorylation (activation) during hypoxia and thus highlight that the role of p38 phosphorylation in cardiomyocytes is highly dependent on PO_2. Moreover, this ROS-induced p38 activation has been shown through another source independent of the electron transport chain, cobalt chloride [64]. However, hemoglobin (Hb), which is increased in hypobaric hypoxia exposure, depending on the type and duration of exposure, has intrinsic heme-oxidase activity that leads to the production of superoxide and thus contributes to oxidative stress. Therefore, the release of superoxide by Hb is favored in the T structure. Thus, sustained or excessive desaturation of Hb (T structure) may increase ROS production [65], and the phosphorylation of p38 MAPK during hypoxia may involve several ROS sources.

Although ROS generation is a normal physiological process, its counterbalance seems to be impaired under hypobaric hypoxia. The resulting imbalance leads to changes with potential pathological consequences for the cardiovascular system.

4. Conclusion

Under hypobaric hypoxia, ROS levels are elevated, resulting in a subsequent unbalanced oxidative status. The principal sources of hypobaric hypoxia are NOX4 in the vascular system and NOX2 in cardiac tissue. The main effects of this oxidative increase include cellular damage, impaired NO pathway signaling, and the activation of calcium channels, transcription factors, pro-inflammatory molecules, and kinase proteins, all of which have deleterious effects on the cardiovascular system.

Therefore, this exaggerated or unbalanced ROS activity is closely related to the development of specific changes in the cardiovascular system under hypoxia, such as HPV, altitude pulmonary

hypertension, pulmonary artery remodeling, and RVH. Notably, although most of the sources in this review described results from nonhypobaric hypoxia conditions, the information gathered reveals a broad view of the relationship between ROS and hypoxia. However, it is still necessary to further elucidate the undefined aspects of this association and the controversies concerning the poor characterization of hypobaric hypoxia.

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