Review

NADPH oxidase-dependent redox signalling in cardiac hypertrophy, remodelling and failure

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Abstract

Markers of increased oxidative stress are known to be elevated following acute myocardial infarction and in the context of chronic left ventricular hypertrophy or heart failure, and their levels may correlate with the degree of contractile dysfunction or cardiac deficit. An obvious pathological mechanism that may account for this correlation is the potential deleterious effects of increased oxidative stress through the induction of cellular dysfunction, energetic deficit or cell death. However, reactive oxygen species have several much more subtle effects in the remodelling or failing heart that involve specific redox-regulated modulation of signalling pathways and gene expression. Such redox-sensitive regulation appears to play important roles in the development of several components of the phenotype of the failing heart, for example cardiomyocyte hypertrophy, interstitial fibrosis and chamber remodelling. In this article, we review the evidence supporting the involvement of reactive oxygen species and redox signalling pathways in the development of cardiac hypertrophy and heart failure, with a particular focus on the NADPH oxidase family of superoxide-generating enzymes which appear to be especially important in redox signalling.

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1. Introduction

Chronic heart failure (CHF) is associated with substantial morbidity and mortality despite medical treatment and affects up to 2% of the adult population in the western world, notably the elderly. The main precursors to CHF are typically either myocardial infarction (MI) or chronic pressure overload due to systemic hypertension [1]. Following MI, the heart usually adapts through a process known as cardiac remodelling which involves changes in the structure and function of cardiomyocytes in the non-infarcted myocardium as well as a profound remodelling of the extracellular matrix. These changes collectively lead to substantial alterations in the shape and volume of the heart and progressive ventricular dilatation and impairment of function—i.e., adverse cardiac remodelling. In the setting of chronic pressure overload, the heart initially adapts through the development of left ventricular hypertrophy (LVH), which involves alterations in cardiomyocyte, extracellular matrix, and coronary vessel structure and function that are driven largely by changes in gene expression. The increase in LV wall thickness and mass and changes in contractile properties that occur with LVH are usually considered to initially be adaptive by normalizing wall stress. However, progressive LVH leads eventually to contractile depression, ventricular dilatation, significant interstitial cardiac fibrosis and the development of CHF.

The mechanisms responsible for the gradual development of CHF in response to either MI or sustained pressure overload are undoubtedly multifactorial and are the subject of intense investigation [2]. Increased oxidative stress has been recognized to be an important contributory mechanism for many years. It is well established that patients with CHF have evidence of increased oxidative stress (e.g., elevated...
plasma markers of oxidative stress), which correlates with myocardial dysfunction and the overall severity of heart failure [3–5]. In addition to increased production of reactive oxygen species (ROS), the levels and activity of antioxidants may be reduced in CHF, potentially reflecting both a primary abnormality and the consequence of increased ROS production. Not only CHF but its precursors are also associated with evidence of increased ROS production which appears to contribute to disease pathophysiology. For example, the development of experimental pressure overload LVH or the transition of compensated LVH to failure in rodents are inhibited by antioxidants, supporting a role for ROS production in cardiac hypertrophy in vivo [6,7]. Similarly, increased ROS production is implicated in the development of adverse LV remodelling following experimental MI [8,9].

2. Effects and sources of ROS in the heart

Reactive oxygen species (ROS) may in principle have several different effects in the heart. The most widely recognized effect of increased oxidative stress is the oxidation and damage of macromolecules, membranes, DNA and enzymes involved in energy production, thereby contributing to cellular damage, energetic deficit and the acceleration of cell death through apoptosis and necrosis [10]. Such effects may be especially important in advanced heart failure or in the context of acute myocardial ischaemia–reperfusion. However, it is now appreciated that oxidative stress may exert other more subtle modulatory effects. In particular, the tightly regulated production of relatively low levels of ROS is involved in modulating the activity of diverse intracellular molecules and signalling pathways (a mechanism commonly termed “redox signalling”), with the potential to induce highly specific acute and chronic changes in cell phenotype [11]. Secondly, some of the effects of the ROS, superoxide (O$_2^-$), may involve inactivation of the signalling molecule nitric oxide (NO), thereby leading to reduced NO bioavailability, an important contributor to disease pathophysiology [12]. In particular, endothelial dysfunction contributes to systemic vasoconstriction and increased cardiac loading in CHF. Furthermore, the reaction of O$_2^-$ with NO results in the formation of peroxynitrite (ONOO$^-$), a species that is also capable of involvement in redox signalling.

Growing evidence supports important pathophysiological roles for redox-sensitive signalling pathways in the processes underlying LVH, adverse LV remodelling and CHF. For example, the hypertrophy of isolated cardiomyocytes induced by $\alpha$-adrenergic agonists, angiotensin II (AngII), endothelin-1, tumor necrosis factor-$\alpha$ (TNF$\alpha$) or cyclic stretch have all been shown to involve increased ROS production [13–15]. Similarly, the inhibition of cellular Cu–Zn superoxide dismutase (SOD) activity, which leads to increased intracellular ROS levels, induces hypertrophy of isolated cardiomyocytes [15]. Increased ROS production promotes the development of interstitial and perivascular fibrosis as well as promoting increased extracellular matrix turnover, at least in part through the activation of matrix metalloprotease enzymes (MMPs) [16–18]. ROS may also induce specific changes in the function of proteins involved in myocardial excitation–contraction coupling, e.g., the sarcoplasmic reticulum Ca$^{2+}$ ATPase pump (SERCA2a), ryanodine receptor and contractile proteins [19,20].

While there are several potential ROS sources in the heart, for example mitochondria, xanthine oxidase, uncoupled nitric oxide synthases (NOSs) and infiltrating inflammatory cells, studies over the last decade have indicated that a family of NADPH oxidases is especially important in redox signalling. Detailed consideration of the roles of ROS derived from mitochondria, xanthine oxidase and uncoupled NOSs is beyond the scope of this article, and the interested reader is directed to several recent reviews covering these sources [10,12,21].

Excessive mitochondrial ROS has been reported in cardiomyocytes from experimental models of rapid pacing-induced heart failure or MI [22,23]. The ROS species in these studies appeared to be mainly hydroxyl (OH) radicals and the amount of ROS was proportional to the degree of LV contractile dysfunction [22]. In a model of adverse remodeling after experimental murine MI, the elevated ROS were associated with mitochondrial damage and dysfunction [23]. The same group reported that cardiomyocyte mitochondrial ROS production could be enhanced by TNF-$\alpha$ [24], levels of which are known to be increased in CHF following MI [25].

An elevation of xanthine oxidase expression and activity has been documented in both end-stage human heart failure [26] and canine rapid pacing-induced heart failure [27,28]. Furthermore, the latter study found that LV contractile function and myocardial efficiency were improved by treatment with the xanthine oxidase inhibitor, allopurinol. These detrimental effects of xanthine oxidase may involve inactivation of NO since the latter is known to reduce myocardial O$_2$ consumption and improve cardiac efficiency. Recently, chronic treatment with allopurinol was reported to significantly reduce adverse LV remodelling following experimental MI in mice [29] or rats [30], implying an involvement of xanthine oxidase in this process. In chronic pressure overload, it was reported that xanthine oxidase activity was elevated only at the stage of CHF but not during compensated hypertrophy, suggesting that xanthine oxidase may be more important in the advanced stages of cardiac remodelling [31]. Increased xanthine oxidase activity is also implicated in the pathogenesis of vascular endothelial dysfunction associated with human CHF [32]. Indeed, human patients with decompensated CHF usually have elevated serum uric acid concentrations, consistent with the notion that there may be systemically increased xanthine oxidase activity [33]. Nevertheless, clinical evidence to substantiate the suggestion that inhibition of
xanthine oxidase activity may be beneficial in human CHF remains lacking.

Any setting in which there is significant oxidative stress can potentially lead to further ROS production by uncoupled NOs, secondary to the oxidation of the essential NO co-factor BH4 [34]. Recently, it was suggested that uncoupled endothelial NOs (eNOS) contributes to LV remodelling in response to chronic pressure overload in mice, at least in part by promoting the activation of MMPs which degrade the extracellular matrix and facilitate LV dilatation [35]. These authors found that wild-type animals subjected to transverse thoracic aortic constriction developed a significant reduction in BH4 levels and an uncoupling of eNOS in association with LV dilatation and contractile dysfunction. The abnormal LV structure and function could be partially inhibited by chronic BH4 treatment whereas eNOS knock-out mice subjected to similar surgery demonstrated reduced LV remodelling, less hypertrophy and less interstitial fibrosis compared to wild-type animals. In contrast to these results, Ruetten et al. [36] reported that eNOS−/− mice subjected to aortic constriction developed greater hypertrophy and more interstitial fibrosis than wild-type animals together with worse contractile function. In line with the latter results, Scherrer-Crosbie et al. [37] reported that LV remodelling post-MI was greater in eNOS−/− mice than wild-type. The reasons for these divergent results remain unclear but it is possible that they reflect opposing effects of NO (anti-hypertrophic and anti-fibrotic) and ROS derived from uncleaved NOs (pro-hypertrophic and pro-fibrotic), with the balance between NO and ROS production perhaps influenced by the nature and/or intensity of the overload stimulus [38]. Indeed, NO is known to inhibit agonist-induced hypertrophy of isolated cardiomyocytes [39–41], through both cGMP-dependent and -independent pathways. Likewise, NO is established as having inhibitory effects on cardiac fibroblast proliferation [40].

3. NADPH oxidases as sources of ROS

The NADPH oxidase family of enzymes are major sources of ROS in the cardiovascular system [42–44]. Indeed, NADPH oxidases are the only enzymes discussed so far whose primary function appears to be ROS production [45]. Each member of the NADPH oxidase family contains a catalytic unit termed Nox that forms a heterodimer with a lower molecular weight subunit called p22phox; this heterodimeric cytochrome is the site of electron transfer from NADPH to molecular O3 resulting in the formation of O2−. Five Nox isoforms (Nox1–5) have been identified to date, each encoded by separate genes and forming the basis of different NADPH oxidases [45]. Interestingly, the biochemical regulation of different Noxs varies significantly. Notably, Nox1 and Nox2 both require the association of cytosolic regulatory subunits (namely p47phox, p67phox, p40phox and Rac, or isoforms thereof) with the cytochrome in order to activate O2− production [45]. In marked contrast, Nox4 activation does not seem to require these cytosolic subunits [46–48].

Cardiovascular cells exhibit specific patterns of Nox expression, with several cell types expressing more than one isoform. Nox1 is highly expressed in cultured vascular smooth muscle but is not significantly expressed in cardiomyocytes or endothelial cells [44,45,49]. Nox2 is abundantly expressed in cardiomyocytes [50–52], endothelial cells (reviewed in [42]) and fibroblasts [53–55]. Nox4 appears to be the most widely expressed isoform, being found in endothelial cells [56], cardiomyocytes [50] and fibroblasts [53,57]. Of particular relevance to cardiac pathophysiology, NADPH oxidase activity is significantly enhanced by several stimuli that are relevant to LVH and heart failure, e.g., cyclic stretch [58,59], angiotensin II [60–62], α-adrenergic agonists [52], endothelin-1 [63] and tumor necrosis factor-α [64,65], which act both through acute post-translational modification of oxidase regulatory subunits and transcriptional pathways. Interestingly, several studies have shown that ROS produced by NADPH oxidases can serve to promote further ROS generation by other sources. For example, O2− from NADPH oxidase may oxidize and degrade BH4, thereby leading to NO uncoupling, a mechanism that has been demonstrated in ApoE-deficient mice and experimental hypertension [34,66]. Similarly, NADPH oxidase-derived ROS may also activate xanthine oxidase [67]. Therefore, NADPH oxidases may be pivotal even in settings where part of the oxidative stress emanates from other enzymes.

An increasing body of data indicates important roles for NADPH oxidases in LVH and CHF, analogous to now well established evidence that these enzymes are involved in the pathophysiology of VSM proliferation, angiotensin II-dependent and low renin hypertension, atherosclerosis and angiogenesis, largely through the modulation of redox-sensitive signaling pathways [42,44,68]. This evidence extends to human CHF patients where our group and others recently showed that end-stage failing myocardium exhibits increased NADPH oxidase subunit expression and activity [69,70]. Recently, polymorphisms in components of NADPH oxidase were reported to be associated with an increased risk of CHF occurring in response to cytotoxic therapy [71].

4. NADPH oxidase and cardiac hypertrophy

NADPH oxidase subunit expression and activity were found to be increased in experimental pressure overload LVH in guinea-pigs, in parallel with activation of mitogen activated protein kinases (MAPKs) [51]. Oxidase expression was documented in both cardiomyocytes and endothelial cells in this study. ROS derived from the oxidase contributed to the inactivation of endothelium-derived NO and consequent LV diastolic dysfunction in this model [72].
Similar NADPH oxidase activation has also been documented in pressure overload LVH in mice [50,73]. The role of NADPH oxidases has recently been investigated more directly by studies employing mice deficient in the Nox2-containing NADPH oxidase (Nox2<sup>−/−</sup> mice). In a model of in vivo cardiac hypertrophy induced by short-term (7–14-day) subpressor infusion of angiotensin II, Bendall et al. [74] found that increases in heart/body weight ratio, myocyte area and mRNA expression of ANF and βMHC were all markedly inhibited in Nox2<sup>−/−</sup> mice compared to wild-type controls. This was associated with an absence of angiotensin II-induced increases in LV NADPH oxidase activity in Nox2<sup>−/−</sup> mice. These data suggest an essential role for the Nox2 oxidase in short-term angiotensin II-induced cardiac hypertrophy. In keeping with this, angiotensin II-induced signalling and hypertrophy of isolated cardiomyocytes were found to be dependent upon Nox2 [75,76] while the small GTP-binding protein Rac1 (which is involved in NADPH oxidase activation) was reported to be involved in isolated myocyte hypertrophy induced by endothelin1, angiotensin II or phenylephrine [77–79]. NADPH oxidases are also implicated in norepinephrine-induced hypertrophy in isolated cardiomyocytes [80].

In order to investigate the possible role of Nox2 oxidase in LVH occurring in response to the more complex stimulus of in vivo pressure overload, Byrne et al. [50] studied the effects of aortic constriction on wild-type and Nox2<sup>−/−</sup> mice. This study found that, in contrast to angiotensin II infusion, both morphological LVH and the associated rises in mRNA expression of molecular markers such as ANF were similar in Nox2<sup>−/−</sup> and wild-type mice. Similar data were also reported independently by Maytin et al. [73]. Interestingly, however, Byrne et al. [50] found that LV NADPH oxidase activity was significantly increased by aortic banding not only in wild-type but also in Nox2<sup>−/−</sup> mice, which was attributed to an increased expression of the Nox4 isoform in banded Nox2<sup>−/−</sup> animals. Furthermore, chronic oral treatment of banded Nox2<sup>−/−</sup> mice with the antioxidant N-acetyl-cysteine (NAC) significantly reduced the extent of LVH, consistent with ROS generation contributing to in vivo LVH [50]. These data suggest that Nox4-derived ROS could contribute to the development of pressure overload-induced LVH whereas Nox2 appears indispensable for the response to short-term angiotensin II infusion. In keeping with this idea, a previous study reported that chronic statin treatment could partially inhibit pressure overload-induced LVH in mice through a Rac-dependent antioxidant action [78,81], although whether an NADPH oxidase isoform was involved was not addressed. Also consistent with a non-essential role of Nox2 in the response to pressure overload, in vivo LVH occurring in response to pressor infusion of angiotensin II is not inhibited in Nox2<sup>−/−</sup> mice to the same extent as with subpressor infusion [82]. Similarly, LVH in a model of chronic RAAS activation of the renin–angiotensin–aldosterone system was found to be independent of Nox2 [83]. Nevertheless, further studies suggest that Nox2 has an important role in the development of other aspects of the cardiac phenotype in response to pressure overload (Fig. 1). Detailed analyses of LV contractile function by echocardiography and LV conductance pressure–volume measurements in wild-type and Nox2<sup>−/−</sup> mice subjected to aortic banding showed that Nox2<sup>−/−</sup> mice were significantly protected against the LV systolic and diastolic dysfunction observed in wild-type mice.

Fig. 1. Schematic illustrating involvement of Nox2 NADPH oxidase in the cardiac response to activation of the renin angiotensin aldosterone system (RAAS) or to chronic pressure overload. Hypertrophy in response to short-term RAAS activation is dependent upon Nox2, whereas the hypertrophic response to pressure overload is not. However, Nox2 is essential for the development of interstitial fibrosis in response to either stimulus.
animals [84]. This protection against contractile dysfunction was also evident at the level of isolated cardiomyocytes.

The signal transduction pathways through which the above effects of NADPH oxidase may be mediated remain to be fully elucidated. Potential redox-sensitive downstream targets that have been shown to be activated by NADPH oxidase-derived ROS in other tissues include RAS, c-src, the MAPKs, the PI3 kinase (PI3K)/Akt pathway, NF-κB, AP-1, HIF-1 and others [80,85–90]. The small GTPase stretch or angiotensin II [91]. Activation of these pathways is thought to play a role in the development of cardiac fibrosis in vivo has recently been addressed in studies with Nox2−/− mice. Interstitial cardiac fibrosis occurring in response to a 2-week infusion of angiotensin II was found to be almost completely abolished in these animals, both with subpressor and pressor doses of angiotensin II [74,82]. The basis of this effect was an inhibition of increases in the mRNA expression of procollagen I, III and connective tissue growth factor (CTGF), the activation of NF-κB and the activation of MMP-2 in Nox2−/− mice [82]. Similar results were also obtained in a model of aldosterone-induced interstitial fibrosis, which was also inhibited in Nox2−/− mice [82]. In line with the latter results, Sun et al. [94] previously reported that aldosterone-driven interstitial cardiac fibrosis in rats was associated with evidence of increased oxidative stress together with increased ventricular Nox2 expression although a cause-effect relation-}

6. Redox signalling in post-MI remodelling

An important role for increased oxidative stress in adverse LV remodelling post-MI is well recognized. Markers of oxidative stress are elevated post-MI [96] and, in experimental models, various antioxidant approaches (e.g., probucol, dimethylthiourea or genetic manipulation) have been found to ameliorate the adverse remodelling. For example, the chronic treatment of mice with the ROS scavenger dimethylthiourea after MI inhibited adverse LV remodelling by attenuating increases in collagen volume fraction, MMP activity and myocyte size [8]. Likewise, treatment with probucol prevented LV dilation and wall thinning and reduced cardiac fibrosis after experimental MI in rats [9]. Post-MI remodelling was also prevented in mice overexpressing glutathione peroxidase [97]. The benefits of these antioxidant approaches extended to an improvement in contractile function and lower mortality.

Recently, a role for Nox2 oxidase as a specific source of ROS involved in the above effects has been suggested. The expression of the NADPH oxidase subunits, Nox2 and p22phox, was found to be increased after experimental MI in rats and it was also suggested that the increases correlated with the inflammatory phase of tissue repair [98]. Human myocardium from patients who had died of acute MI was also found to exhibit increases in the expression of Nox2 and p22phox, which were localized to cardiomyocytes [99]. Recent studies from our laboratory found that that adverse LV remodelling after MI was significantly attenuated in Nox2−/− mice, providing more direct evidence of an involvement of Nox2 in this response [100]. Interestingly, however, Nox2 may have other effects in the context of acute myocardial ischaemia. Thus, Bell et al. [101] found that Nox2 was required for the ischaemic myocardial preconditioning of isolated hearts in response to transient global ischaemia, indicating a beneficial effect of Nox2 in this context. On the
other hand, infarct size after permanent in vivo coronary ligation was no different between NADPH oxidase-deficient mice and wild-type controls [102].

7. Conclusions

A large body of data now suggests an important role for increased ROS production in the pathophysiology of CHF and its antecedent conditions, LVH and post-MI remodelling. Several different sources of ROS are implicated but the NADPH oxidases appear to be especially important in modulating redox-sensitive signalling pathways that underlie the development of cardiac myocyte hypertrophy, interstitial fibrosis and adverse LV remodelling. Recent studies in gene-deficient mice lacking the NADPH oxidase isoform Nox2 indicate an important role for this isoform but other Nox isoforms, in particular Nox4, are also likely to have distinct effects. A better understanding of the specific roles of different ROS sources in the redox signalling processes involved in the development of CHF may inform new therapeutic strategies for this condition.

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