Involvement of NADPH Oxidases in Cardiac Remodelling and Heart Failure

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Abstract
Cardiac remodelling occurs in response to stress, such as chronic hypertension or myocardial infarction, and forms the substrate for subsequent development of heart failure. Key pathophysiological features include ventricular hypertrophy, interstitial fibrosis, contractile dysfunction, and chamber dilatation. Although the molecular mechanisms are complex and not fully defined, substantial evidence now implicates increased oxidative stress as being important. The NADPH oxidase (‘Nox’) enzymes are a particularly important source of reactive oxygen species that are implicated in redox signalling. This article reviews the evidence for an involvement of NADPH oxidases in different aspects of adverse cardiac remodelling. A better understanding of the roles of this complex enzyme family may define novel therapeutic targets for the prevention of heart failure.

Introduction
Cardiac remodelling is a term used to denote structural and functional alterations in the heart that develop in response to various pathogenic stimuli, including chronic pressure overload (e.g. systemic arterial hypertension), chronic volume overload (e.g. valvular regurgitation), and myocardial infarction (MI) [1]. Chronic pressure overload initially results in myocyte hypertrophy (with increased width relative to length) and ventricular wall thickening without chamber dilatation, a response that may be compensatory by normalizing wall stress. However, ongoing pressure overload leads to increased interstitial fibrosis, reduced contractile function, ventricular dilatation, and eventually overt heart failure. Similarly, there is usually localized myocyte hypertrophy in non-infarcted parts of the ventricle post-MI which may be adaptive but which becomes associated with increased interstitial fibrosis, extracellular matrix remodelling, contractile dysfunction, and altered ventricular shape, size, and coordination [2]. The development of overt chronic heart failure is associated with major morbidity and mortality. Elucidation of the mechanisms underlying the adverse remodelling process that leads to heart failure is a major research goal with substantial potential for the development of new therapies for this debilitating condition [1, 3–5]. While multiple mechanisms are undoubtedly involved, in this article we consider the role of oxida-
Oxidative Stress and Heart Failure

Oxidative stress refers to situations where there is an imbalance between the production of ROS and antioxidant defences, such that there is increased propensity for oxidative modification of numerous biological substrates. ROS encompass free radicals such as superoxide (\(\text{O}_2^\cdot\)) and hydroxyl (OH) as well as non-radicals such as hydrogen peroxide (\(\text{H}_2\text{O}_2\)), while antioxidant systems include a host of enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase), vitamins (e.g. C and E), and other molecules (e.g. peroxiredoxin, thioredoxin/thioredoxin reductase, etc.) [6]. At high concentrations, ROS are well known to exert damaging effects by non-specific interactions with proteins, lipid membranes, mitochondria, and DNA. Such deleterious effects are relevant in advanced heart failure and in reperfusion after myocardial ischaemia [8]. However, at lower levels, the specific production of ROS (in particular \(\text{H}_2\text{O}_2\) which is relatively stable and diffusible) may mediate reversible modulation of the activities of numerous enzymes, proteins, ion channels, and transcription factors and thereby have important pathophysiological signalling actions – broadly termed ‘redox signalling’ [9, 10]. In addition, superoxide reacts avidly with the signalling molecule nitric oxide (NO), thereby both inactivating the latter agent and creating the reactive peroxynitrite (ONOO–) species.

Substantial clinical and experimental evidence supports an important role for oxidative stress and ROS in cardiac remodelling and failure [8]. For example, markers of oxidative stress are elevated in patients with heart failure and correlate with the severity of contractile dysfunction and heart failure [11–13]. Enhanced ROS generation has been directly documented in the failing heart using highly specific methods such as electron spin resonance spectroscopy [14, 15]. Redox-sensitive signalling pathways are implicated in the development of cardiac hypertrophy, fibrosis, matrix remodelling, and apoptosis [1, 5, 16, 17], and several experimental studies indicate that various antioxidant approaches can attenuate these conditions [18–20].

Potential sources of increased ROS generation in the remodelling and failing heart include the mitochondria [21] and enzymes, such as xanthine oxidase [22], cytochrome P450 oxidases, uncoupled NO synthases (which generate superoxide, instead of NO, when they are deficient in tetrahydrobiopterin) [23], and NADPH oxidases [24], from resident cells and infiltrating inflammatory cells. Recent studies have demonstrated NADPH oxidases to be important in the pathogenesis of several aspects of cardiac remodelling and its antecedent conditions, largely through actions on redox-sensitive signal transduction. Furthermore, expression and activity of NADPH oxidase have been confirmed to be increased in the myocardium of patients with ischaemic and non-ischaemic heart failure [25–27].

NADPH Oxidase Structure and Regulation

NADPH oxidase was first identified in neutrophils, where it is involved in non-specific host defence against microbes during the process of phagocytosis [6, 28]. The enzyme catalyzes electron transfer from NADPH (reduced nicotinamide adenine dinucleotide phosphate) to molecular oxygen, producing superoxide. In the neutrophil, the oxidase is normally quiescent but becomes activated during phagocytosis, when it generates high levels of ROS and protons that are involved in the microbicidal process. In the last 8 years, several homologues of this enzyme have been discovered in numerous other cell types, where they are involved in many different non-phagocytic functions [6, 29]. Unlike other sources of ROS, the NADPH oxidases clearly appear to have ROS generation as their primary function. With regard to redox signalling, it is notable that NADPH oxidases are specifically activated by diverse agonists that evoke the activation of cellular signal transduction pathways (fig. 1).

Agonists and stimuli relevant to myocardial remodelling and heart failure that are also known to activate NADPH oxidases include: (1) G-protein-coupled receptor agonists, e.g. angiotensin II, endothelin-1, and \(\alpha\)-adrenergic agonists [30–32]; (2) growth factors, e.g. vascular endothelial growth factor [33] and insulin [34]; (3) cytokines, e.g. tumour necrosis factor alpha [35, 36]; (4) mechanical forces [37], and (5) hypoxia-reoxygenation [38].

Five different isoforms of NADPH oxidase (or Noxs) and two isoforms of the related Duox enzymes, each encoded by separate genes and with distinct tissue distributions, have been identified [6]. Each NADPH oxidase isoform contains a catalytic Nox subunit (Nox1–Nox5 which facilitate electron transfer) and a smaller p22phox subunit that associate to form a heterodimeric cytochrome. Some
of the isoforms, such as Nox1 and Nox2, also require additional protein subunits for activation of the enzyme. Nox2 (also known as gp91phox) is the isoform that forms the core of the classical phagocyte NADPH oxidase, but is now known to also be expressed in several other cell types, e.g. endothelial cells [30, 35, 39, 40], cardiomyocytes [31, 41, 42], and fibroblasts [43]. The other main isoforms that appear to be important in the cardiovascular system are Nox1 which is expressed in vascular smooth muscle cells [44] and Nox4 which is expressed in endothelial cells [45], cardiomyocytes [46, 47], vascular smooth muscle cells [48], and fibroblasts [49].

Each Nox isoform is predicted to have five to six transmembrane domains, a haem moiety that can undergo cyclical reduction and oxidation, and a carboxy-terminal portion that is cytoplasmic and contains binding sites for NADPH and FAD (flavin adenine dinucleotide) [6]. Association of the p22phox subunit with the Nox subunit seems to be important for stability of the complex as well as for interactions with other protein-binding partners.

In the case of Nox2, activation of the oxidase requires the association of at least four cytosolic proteins (p67phox, p47phox, p40phox, and the small guanosine triphosphatase Rac) with the p22phox-Nox2 heterodimer to form an active enzyme complex (fig. 2). These cytosolic subunits translocate to the membrane to associate with the p22phox-Nox2 heterodimer in a highly regulated process that involves specific posttranslational modifications of these subunits. Notably, this involves (1) the multiple phosphorylation of the p47phox subunit by several protein kinases (including protein kinases B and C) which induces a conformational change that facilitates its binding to p22phox, (2) the activation of Rac [50], and (3) the binding of p67phox to an activation site on Nox2 [51]. The purpose of this highly complex system of activation is presumably to allow tight regulation of ROS generation and thereby to prevent the inappropriate production of potentially harmful species.
Interestingly, since activation of Rac is inhibited by the HMG-CoA reductase inhibitors (i.e., statins), inhibition of Rac-requiring NADPH oxidases may be one of the many pleiotropic actions of these drugs.

The activation of Nox1-containing oxidase is highly similar to that of Nox2, although it is believed to involve homologues of p67phox and p47phox, known as NOXA1 and NOXO1, respectively [6]. In contrast to Nox1 and Nox2, the activation of Nox4 is quite distinct in that it does not appear to require any of the conventional regulatory subunits [52, 53]. The mechanisms responsible for regulating the Nox4 activity remain poorly understood, but it is thought that the enzyme may be constitutively active and that increased activity may be related to increased expression of Nox4 (table 1).

The ways in which NADPH-oxidase-derived ROS exert downstream cellular effects have been extensively studied and are reviewed in detail elsewhere [7, 54]. In brief, these include (1) alterations in the activity of redox-sensitive protein kinases, such as mitogen-activated protein kinases (MAPKs) and protein kinases B, C, and D, which may occur indirectly following inactivation of tyrosine phosphatases or through direct activation; (2) altered activity of transcription factors, including nuclear factor-kappa B (NF-κB), activating protein-1, hypoxia-inducible factor-1, and STATs, and (3) direct effects on enzymes, e.g., matrix metalloproteinases (MMPs), receptors, or ion channels.

**NADPH Oxidases in Cardiac Hypertrophy**

Hypertrophy of isolated cardiomyocyte preparations in response to α-adrenergic agonists [31], angiotensin II [55], endothelin 1 [56], tumour necrosis factor alpha [55], or cyclic stretch [57] has been shown to involve an increased production of ROS. Work from our laboratory [58] sought to identify the sources of ROS involved in cardiac hypertrophy in vivo. In an in vivo model of pressure overload induced by aortic banding in the guinea pig, we found that there was a gradual increase in Nox2 oxidase subunit expression (in both cardiomyocytes and endothelial cells) and in ROS generation with progression of hypertrophy [58]. This was paralleled by the activation of MAPKs, suggesting a pathophysiological role for NADPH oxidase. More definitive data have derived from studies using genetically modified mice. In mice with a global deficiency of Nox2 [59], it was found that the in vivo cardiac hypertrophic response to short-term subpressor angiotensin II infusion was markedly inhibited together with reduced expression of molecular markers of myocardial hypertrophy [41]. In line with these results, work done by Nakagami et al. [60] showed that angiotensin-II-induced hypertrophy was inhibited in cardiomyocytes. Recent studies from the same group [42] investigated the effects of conditional cardiomyocyte-specific deletion of Rac1 on the in vivo response to angiotensin II infusion. This work demonstrated that the hypertrophic response to angiotensin II was inhibited in the Rac1 knockout mice and, furthermore, that this was accompanied by a reduction in the Nox2 oxidase activity. These results are in accordance with those reported in prior studies [42, 61], showing that transfection of cultured neonatal cardiomyocytes with constitutively active Rac1 induces hypertrophy, whereas a dominant-negative Rac1 mutant inhibits the hypertrophic response to phenylephrine.

In contrast to angiotensin-II-induced hypertrophy, Byrne et al. [46] found that the hypertrophic response to pressure overload induced by aortic banding was similar
in Nox2−/− mice and wild-type controls. Likewise, there were similar increases in molecular markers of hypertrophy, such as atrial natriuretic factor mRNA, in the two groups. Maytin et al. [62] also reported similar data in Nox2−/− mice. Interestingly, however, Byrne et al. [46] found that the NADPH oxidase activity increased to a similar level after aortic banding in both knockout and wild-type animals and that this was attributable to an increased expression of Nox4 mRNA and protein after aortic banding. Furthermore, the extent of hypertrophy could be attenuated in Nox2−/− mice by chronic treatment with the antioxidant N-acetylcysteine, suggesting that other sources of ROS – possibly Nox4 oxidase – may contribute to the development of pressure overload hypertrophy in this setting. This study suggests that Nox2 and Nox4 may be differentially activated in angiotensin II versus pressure overload hypertrophy.

The downstream pathways through which superoxide generated by NADPH oxidase leads to cardiac hypertrophy remain to be fully defined (fig. 3). In isolated cardiomyocytes induced to undergo hypertrophy in response to α-adrenergic agonists, NADPH-oxidase-dependent activation of ERK-1/2 appears to be important [31, 63]. The small guanosine triphosphatase Ras is also activated upon α-adrenoreceptor agonism of ventricular myocytes, downstream of NADPH oxidase [64]. In conditional Rac1 knockout mice, Satoh et al. [42] showed that the activations of apoptosis-signal-related kinase 1 (ASK-1) and NF-κB (both known to be highly redox sensitive) were essential in the in vivo hypertrophic response to angiotensin II. Consistent with these results, it was previously reported [56, 65] that knockout of ASK-1 suppressed the in vivo cardiac hypertrophic response to angiotensin II, whilst expression of a dominant-negative mutant of ASK-1 inhibited the hypertrophic response of cultured cardiomyocytes to G-protein-coupled agonists. ASK-1 sits upstream of the MAPKs p38MAPK and JNK, both of which have been shown to be activated downstream of NADPH oxidases [66].

### Interstitial Fibrosis

Pathological cardiac hypertrophy is generally accompanied by an increase in the amount of fibrous tissue between cardiomyocytes – or interstitial cardiac fibrosis – which increases the diastolic chamber stiffness and contributes to impaired cardiac filling and also plays a role in the increased propensity to arrhythmias by altering the electrical conduction within the myocardium. An increase in perivascular fibrosis contributes to the mismatch between coronary perfusion and increased muscle mass that is another hallmark of pathological hypertrophy. The mechanisms underlying an increase in interstitial and perivascular fibrosis have been extensively investigated [67–69] and include the proliferation of fibroblasts, their transformation into myofibroblasts [70] which are characterized by the upregulation of smooth muscle antigens such as α-smooth muscle actin and by the copious production of extracellular matrix components, and increased inflammatory cell infiltration. Increased oxidative stress is associated with increased fi-

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**Fig. 3.** NADPH-dependent redox signalling in cardiac hypertrophy following stimulation by angiotensin II (Ang II) or other G-protein-coupled receptor (GPCR) agonists. ET-1 = Endothelin-1; ROS = reactive oxygen species; ERK-1/2 = extracellular-signal-regulated kinases 1/2; ASK-1 = apoptosis-signalling kinase-1; NFAT = nuclear factor of activated T cells; NF-κB = nuclear factor kappa B.
brosis in many in vitro [70, 71] and in vivo settings in different organs, including lungs, liver, and kidneys [72–75]. Increased NADPH oxidase activation has also been implicated in vascular fibrosis [76, 77].

Studies from our group examined how Nox2 oxidase may be involved in interstitial cardiac fibrosis. In the study by Bendall et al. [41], using subpressor angiotensin II infusion referred to earlier, it was observed that interstitial fibrosis was significantly reduced in Nox2 knockout mice as compared with wild-type mice. These differences persisted when experiments were repeated with a pressor infusion of angiotensin II [78]. Similarly, angiotensin-II-induced interstitial fibrosis was inhibited in conditional Rac1 knockout mice [42] and ASK-1 knock- out mice [65]. Johar et al. [78] found that administration of the mineralocorticoid receptor blocker spironolactone inhibited angiotensin-II-driven increases in NADPH oxidase activity and interstitial fibrosis, suggesting that mineralocorticoid receptor activation may be involved in the response to angiotensin II. In the latter study, the response to chronic aldosterone infusion in uninephrectomized mice fed a 1% NaCl diet (a model of mineralocorticoid-receptor-dependent hypertension) was also studied. Wild-type animals subjected to this intervention demonstrated increased cardiac NADPH oxidase activity and substantial interstitial cardiac fibrosis, both of which were significantly attenuated in Nox2 knockout mice [78]. Mineralocorticoid-receptor-dependent interstitial cardiac fibrosis in rats has also been reported to be associated with increased oxidative stress and Nox2 expression, while chronic treatment with a Nox2 oxidase inhibitor, apocynin, inhibited cardiac fibrosis in this setting [79, 80].

We have also examined the involvement of NADPH oxidase in the cardiac fibrotic response to aortic banding. We found that banded Nox2 knockouts had substantially less interstitial fibrosis as compared with wild-type mice despite a similar degree of hypertrophy [81]. These results are in keeping with prior findings that interstitial fibrosis and hypertrophy may be subject to independent regulation and clearly indicate that the requirement of Nox2 for different components of the response to chronic pressure overload varies significantly.

The mechanisms underlying the above-mentioned Nox2-oxidase-dependent increases in interstitial cardiac fibrosis were recently addressed by Johar et al. [78] (fig. 4). A key profibrotic cytokine that was induced in a Nox2-dependent fashion in the context of angiotensin II infusion was connective tissue growth factor which was substantially upregulated in wild-type mice, but not in Nox2 knockout animals. In addition, the upregulation of profibrotic genes, such as procollagen-1, procollagen-3, and fibronectin, was found to be Nox2 dependent. These may be driven by an increase in the activity of the transcription factor NF-κB which was found to be also Nox2 dependent. In addition, angiotensin II infusion was accompanied by a significant activation of MMP2 in wild-type mice, an effect that was blunted in Nox2−/− animals. The latter result is in keeping with other data that found involvement of NADPH oxidase in MMP activation in response to vascular smooth muscle stretch or angiotensin II [82, 83]. Taken together, these data indicate that Nox2-dependent induction of fibrosis involves the activation of several key targets, including connective tissue growth factor, NF-κB, profibrotic genes, and MMPs.

Fig. 4. Nox2-oxidase-dependent redox signalling in cardiac interstitial fibrosis induced by angiotensin II (Ang II). GPCR = G-protein-coupled receptor; ROS = reactive oxygen species; NF-κB = nuclear factor-kappa B; CTGF = connective tissue growth factor; MMP = matrix metalloproteinase.
Adverse Remodelling following MI

Ventricular remodelling and dysfunction following an acute MI is the commonest cause of heart failure in developed countries. The process of adverse remodelling includes several of the changes already described in the context of chronic pressure overload (e.g. hypertrophy, fibrosis), but also involves a significant inflammatory component – especially in the early stages after MI – and a major remodelling of the extracellular matrix [84, 85]. A significant body of evidence supports an important role for oxidative stress in the genesis of post-MI remodelling. An increase in oxidative stress after MI has been consistently described in both animal and human models [86]. Furthermore, lowering the levels of ROS either with pharmacological agents or by genetic overexpression of endogenous antioxidant enzymes can attenuate adverse ventricular remodelling after experimental MI [18–20].

Data from experimental rat models of MI and from human subjects who had died of MI indicate that there is an increase in cardiac Nox2 expression after MI, not only in inflammatory cells but also in cardiomyocytes [87, 88]. Recent studies performed in gene-modified mice have provided more direct evidence for an involvement of Nox2 oxidase in adverse remodelling following MI. In our laboratory, Nox2 knockout mice and wild-type controls were subjected to permanent left anterior descending coronary artery ligation to assess the remodelling response to MI [89]. Whereas infarct sizes were similar in wild-type and Nox2 knockouts (at both 24 h and 4 weeks after MI), the knockout animals were found to display significantly reduced left ventricular dilatation and a higher fractional shortening and left ventricular ejection fraction than wild-type mice. These echocardiographic findings were corroborated by invasive in vivo cardiac catheterization which confirmed better preserved left ventricular systolic and diastolic functions in Nox2 knockouts as compared with wild-type mice. At a microscopic level, Nox2 knockout mice were found to exhibit less myocyte hypertrophy and reduced interstitial fibrosis in the non-infarcted myocardium than the wild-type group. In keeping with these observations, Nox2 knockout mice also had significantly blunted increases in mRNA expression of atrial natriuretic factor, connective tissue growth factor, procollagen-1, and fibronectin as well as significantly attenuated increases in MMP activity. Strong independent evidence for an important role of Nox2 oxidase is provided by Doerries et al. [90] who undertook similar studies in p47\textsuperscript{phox} knockout mice and controls. These authors found that there was no difference in infarct size between wild-type and knockout animals, but that ventricular dilatation and global contractile dysfunction were markedly attenuated and that the overall survival was significantly increased in p47\textsuperscript{phox} knockout mice. This group also reported that p47\textsuperscript{phox} knockout mice had reduced levels of myocyte apoptosis after MI.

The above studies provide the first direct evidence for an important role of Nox2 NADPH oxidase in post-MI remodelling and suggest that therapeutic targeting of this system could be beneficial. In this regard, it is of interest that a number of studies have reported improvement in experimental post-MI remodelling after treatment of animals with statins [91–93], although it should be acknowledged that statins may have multiple actions in addition to NADPH oxidase inhibition.

Contractile Dysfunction

The development of myocardial contractile depression is a landmark in the transition from a ‘compensated’ or ‘adaptive’ cardiac remodelling response to a decompensated phase which subsequently progresses to overt heart failure. Multiple mechanisms contribute to contractile dysfunction, including abnormalities of cardiomyocyte excitation-contraction coupling, mitochondrial dysfunction and energetic deficit, loss of myocytes from the ventricular walls, alterations in the extracellular matrix, and changes in chamber shape and synchrony. Evidence exists in the literature to support a potential effect of oxidative stress through each of these mechanisms.

The possible involvement of NADPH oxidases in cardiac contractile dysfunction has been addressed in a few studies. In our laboratory, Grieve et al. [81] studied wild-type and Nox2 knockout mice subjected to aortic banding and found that the Nox2-deficient animals had a significantly better contractile function than wild-type mice, as assessed either by echocardiography or by detailed pressure-volume analyses in isolated ejecting hearts, despite having a similar extent of morphological hypertrophy. Furthermore, ventricular myocytes isolated from these animals and studied ex vivo also showed a better contractile function, indicating that at least part of this effect was mediated at the level of the myocyte. This demonstration of divergence between contractile dysfunction and hypertrophy per se may be of considerable importance, since it implies that identifying the different underlying mechanism for the two effects could lead to therapeutic targeting of maladaptive pathways indepen-
dent of adaptive responses. Nox2-oxidase-mediated actions also contribute to the impairment of the contractile function in the context of adverse remodelling after MI, as discussed in the previous section.

The downstream effects of NADPH-oxidase-derived ROS that may contribute to contractile dysfunction at the level of the cardiomyocyte have not been addressed in detail in the context of adverse cardiac remodelling. In the study by Grieve et al. [81] referred to earlier, it was notable that short-term (24–36 h) treatment with the antioxidant N-acetylcysteine significantly improved contractile dysfunction in wild-type banded mice, suggesting that acute effects of ROS may be important. Activity and function of several proteins involved in myocardial excitation-contraction are known to be modulated by ROS – e.g. sarcolemmal ion channels, sarcoplasmic reticulum calcium pump (SERCA 2a), and the sarcoplasmic reticulum calcium release channel (the ryanodine receptor), which are implicated in calcium cycling modulating the calcium sensitivity to contractile proteins themselves [94–99]. ROS can also impair cellular energetics through effects on mitochondrial respiration and enzyme activities [100]. Although such effects of NADPH oxidase have not been addressed in cardiac remodelling, there is supportive evidence from other settings [101]. NADPH oxidase in cardiomyocytes was reported to mediate the myocardial depression found in an experimental model of Gram-negative sepsis [102] and was implicated in that seen with streptozotocin-induced diabetes [103]. Additionally, the depressant action of leptin on ventricular myocyte function appears to involve the endothelin-1 receptor and NADPH oxidase downstream of this [104].

Furthermore, NADPH-oxidase-derived ROS generated in other cell types within the heart may also influence the contractile function. For example, in a guinea pig model of in vivo aortic banding, MacCarthy et al. [105] found that NADPH-oxidase-derived ROS contributed to impaired left ventricular relaxation by inactivating endothelium-derived NO which has important relaxant effects on the myocardium.

Apoptosis

The loss of cardiomyocytes through programmed cell death (i.e. apoptosis) is believed to be an important factor that contributes to the decompensation of the contractile function in the remodelling heart, whether after MI or during chronic pressure overload [106, 107]. ROS and redox-sensitive pathways are well recognized to be involved in apoptosis [17]. These include (1) direct effects of ROS on mitochondria, leading to cytochrome c release; (2) activation of pro-apoptotic pathways and altered activity of enzymes involved in apoptosis signalling (e.g. ASK-1, JNK, and p38 MAPK) [66], and (3) effects on the cellular anti-apoptotic/survival machinery (e.g. Akt, ERK-1/2, and heat shock proteins). Alternative cell fates (i.e., apoptosis or survival) may be determined by the number of different aspects of the redox signal, such as ROS type involved, its amounts, and whether its presence is transient or sustained [66, 108].

The involvement of NADPH oxidase as a specific source of ROS in cardiomyocyte apoptosis has recently been investigated. The reduction in apoptosis observed in p47phox knockout mice after MI was referred to earlier, but could clearly be a secondary effect. In vitro work on rat embryonal cardiomyocytes demonstrated that the use of the NADPH inhibitor apocynin blunted increases in the NADPH oxidase activity in response to angiotensin II [109]. The apoptotic response to angiotensin II was abolished by apocynin pretreatment, suggesting that this pathway is NADPH oxidase dependent. Subsequent in vivo work by the same group in a rabbit MI model [110] demonstrated that apocynin was able to reduce the rise in NADPH oxidase activity after MI and likewise to reduce the levels of apoptosis and of the apoptosis-related protein Bax. Bcl-2 protein, which has anti-apoptotic actions, showed a smaller reduction following MI in animals treated with apocynin.

Supporting evidence for a role of NADPH oxidase in apoptosis is found outside the cardiovascular system. Using various inhibitors of this enzyme and/or mice with genetic modifications of the enzyme’s subunits, NADPH oxidase has been implicated in mediating the apoptosis of (1) pancreatic acinar cells in experimental models of acute pancreatitis [111, 112]; (2) hepatocytes in response to hyperosmolarity [113] or exposure to bile salts [114], and (3) sympathetic neurons deprived of nerve growth factor in an in vitro model of normal neuronal loss during nervous system maturation [115]. Collectively, these findings suggest that this enzyme may have widespread actions in the redox modulation of apoptosis, in both physiological and pathological settings.

Conclusions

Adverse ventricular remodelling is the underlying basis for the development of chronic heart failure and is, therefore, a prime target for therapeutic intervention. The key to the development of new therapies is a better
understanding of the mechanisms responsible for adverse remodelling. There has been increased recognition in recent years of the importance of oxidative stress, redox signalling, and the NADPH oxidases in modulating some of the key processes involved in adverse cardiac remodelling. An important finding emerging from the recent studies on the role of NADPH oxidases is the potential to selectively target detrimental aspects of the phenotype of the failing heart, independent of other aspects that may be adaptive. While much further work is necessary in order to accurately delineate the pathways that involve NADPH oxidases, the remarkable complexity of regulation of these enzymes may paradoxically allow the development of quite targeted manipulation of their activity. Their complexity and multiplicity of actions may also explain why blunt non-specific approaches such as the use of antioxidant vitamins have not been successful in clinical trials to date.

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NADPH Oxidases and Cardiac Remodelling


