EDITORIAL COMMENT

The Failing Human Heart

Another Battlefield for the NAD(P)H Oxidase?

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Chronic congestive heart failure (CHF) is a clinical syndrome characterized by progressive left ventricular (LV) systolic and/or diastolic dysfunction. Recent studies point to a crucial role of oxidative stress as the underlying mechanism being at least in part responsible for ventricular dysfunction (1). Excess production of reactive oxygen species (ROS) may alter the activity and/or expression of proteins involved in the excitation–contraction coupling, such as sarcolemmal ion channels (2), ion exchangers (3), sarcoplasmic reticulum calcium release channels (4), and myofilament proteins (5), all of which may contribute to myocardial contractile dysfunction. These in vitro findings were supported by a recent in vivo study by Ide et al. (6). In an animal model of CHF, the authors found that rapid ventricular pacing for a four-week period markedly increased ROS levels such as superoxide anions, hydroxyl radicals, and hydrogen peroxide in canine myocardial tissue homogenates. There was a significant positive correlation between myocardial ROS levels and LV contractile dysfunction (6). In patients with heart failure, pericardial levels of isoprostanes, a marker for ROS production (7), correlate closely with the functional severity of heart failure (5). Increased ROS production within the myocardium of hearts from experimental models of CHF may also be due to impaired antioxidant defense capacity (8), as demonstrated by reduced activity of Cu/Zn superoxide dismutase (9,10) and catalase (11), respectively.

In the setting of CHF, increased ROS production may arise from different enzymatic sources, including the xanthine oxidase (12), the mitochondria (13), the cyclooxygenase, the nitric oxide synthase, and the non-phagocytic NAD(P)H oxidase. During the past decade it has become evident that the NAD(P)H-driven oxidase plays an important pathogenetic role in vascular tissue. In the presence of cardiovascular risk factors for the development of atherosclerosis, such as hypercholesterolemia (14), hypertension (15), and diabetes mellitus (16), endothelial dysfunction in animal models as well as in human vascular tissue from human patients (17,18) was consistently shown to be associated with increased NAD(P)H oxidase-mediated vascular superoxide production. Vascular endothelial and adventitial cells, as well as inflammatory cells such as neutrophils and macrophages, express the NAD(P)H oxidase, which consists of the flavocytochrome b558 subunits gp91phox and p22phox as well as the cytosolic factors p47phox and p67phox and the small GTPase rac1 (19,20). In contrast to endothelial, adventitial, and inflammatory cells, smooth muscle cells of large arteries lack gp91phox, but recent studies identified the existence of two gp91phox homologues, namely nox-1 and nox-4 (21,22). Activity and expression of the vascular non-phagocytic enzyme have been found to increase upon stimulation with angiotensin II, aldosterone, endothelin-1, and cytokines such as tumor necrosis factor alpha (TNF-α). Because plasma levels of these neurohormones and cytokines are increased in the setting of CHF, it is tempting to speculate that a similar phenomenon may also apply to cardiomyocytes of the failing heart. Indeed, incubation of cardiomyocytes with angiotensin II and TNF-α increases ROS production and causes cell enlargement, which was effectively suppressed by antioxidants and catalase (23). In contrast to vascular tissue (15), catecholamine-stimulated hypertrophic signaling in cultured cardiomyocytes involves NAD(P)H oxidase-mediated superoxide production (24). More recently, Bendall et al. (25) provided evidence that the hypertrophic response of the myocardium to subpressor doses of angiotensin II is pivotally regulated by the myocardial NAD(P)H oxidase, because this phenomenon was completely prevented in gp91phox-/- mice. Using a guinea pig model of progressive LV hypertrophy, the same group revealed increased expression of the NAD(P)H oxidase subunits p22phox, gp91phox, p67phox, and p47phox as well as an activation of the oxidase during progression of cardiac hypertrophy to failure (26). However, up to now, the evidence of whether the enzyme exists in human myocardium and whether it is regulated in the setting of HF was still lacking.

In this issue of the Journal, Heymes et al. (27) for the first time provide evidence of the activation and expression of the NAD(P)H oxidase in human cardiomyocytes. They were able to detect the expression of NAD(P)H oxidase subunits p22phox, gp91phox, p67phox, and p47phox both at the messenger ribonucleic acid and protein level in non-failing and failing human myocardium. Immunohistochemical staining for the catalytic subunit gp91phox and the regulatory subunit p47phox revealed co-localization with cardiomyocytes. In cardiomyocytes of non-failing hearts, gp91phox was primarily co-localized with sarcolemmal membranes, and p47phox was localized in the cytosol. Even though the overall level of

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expression was unaltered in failing compared with non-failing myocardium, the rate of superoxide production was significantly increased in total tissue homogenates of failing hearts (27). Activation of the NAD(P)H oxidase requires the translocation of the p47phox subunit to the myocyte membrane. Accordingly, Heymes et al. (27) detected significantly enhanced p47phox staining in sarcosomal membranes in failing myocardium, indicating translocation of p47phox from the cytosol to cardiomyocyte membranes. These findings are not surprising but provide support for the hypothesis that the NAD(P)H oxidase plays a significant role in myocardial superoxide production in the failing human heart and therefore opens an area of further research required to determine the functional relevance of an activated NAD(P)H oxidase for the pathogenesis of HF.

Although the authors provide evidence for an increase in NAD(P)H oxidase-mediated superoxide production in the failing human heart, no information concerning functional consequences was provided. It would be of particular interest to investigate, for example, the effect of targeted NAD(P)H subunit disruption (gp91phox or p47phox knock-out) on myocardial contractility, remodeling, and apoptosis in HF models. It remains to be elucidated whether the NAD(P)H oxidase of cardiomyocytes is composed exclusively of phagocytic type subunits or whether there is evidence for an expression of non-phagocytic gp91phox (nox-2) isoforms, nox-1, -3, -4, or -5. Because the activity of the enzyme was measured in total tissue homogenates, it remains to be established to what extent cardiomyocytes versus vascular tissue versus fibroblasts contribute to the observed increases in NAD(P)H-driven superoxide production. Further questions may include to what extent the activity and expression of the myocardial NAD(P)H oxidase is inhibited by in vivo treatment with angiotensin-convertase enzyme inhibitors, AT1- receptor antagonists, anti-TNFα antibodies, and β-receptor blockers, all of which have been shown to improve myocardial function as well as prognosis (except for TNF-α antibodies) in patients with CHF.

Taken together, the important and new information provided by Heymes et al. (27) points to a significant role of the NAD(P)H oxidase in myocardial ROS production. Increased myocardial ROS production may decrease myocardial nitric oxide bioavailability, which could lead to increased myocardial oxygen consumption (28) and to an impairment of diastolic function (29). The reaction product of nitric oxide and superoxide, peroxynitrite, may cause an inactivation of the sarcoplasmatic Ca2+-ATPase and subsequently a dysregulation of Ca2+-homeostasis within the myocardium, as recently demonstrated for cytokine-induced myocardial contractile failure (30,31). Thus, therapeutic strategies to modulate this detrimental response should be a target of future research. Finally, studies like those of Heymes et al. shed light on mechanisms whereby proven therapies benefit myocardial dysfunction in HF.

REFERENCES


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