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COMMENTARY

β_2-Adrenoceptors, NADPH oxidase, ROS and p38 MAPK: another 'radical' road to heart failure?

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Persistent activation of the cardiac β-adrenergic system may contribute to the pathogenesis of congestive heart failure. Both β_1- and β_2-adrenoceptors are known to mediate these noxious effects, yet the β_1-adrenoceptor-PKA axis has received greater attention with less information available on β_2-adrenoceptor driven pathways. In the present issue, Xu and colleagues provide new evidence, showing that β_2-adrenoceptor over-expression leads to increased reactive oxygen species (ROS) emission, mainly caused by up-regulation of reduced nicotinamide adenine dinucleotide phosphate oxidase (Nox) 2 and 4. Increase in ROS levels is accompanied by p38 mitogen-activated protein kinase activation, fibrosis, apoptosis and cardiac dysfunction. Both Nox inhibition and administration of the antioxidant N-acetyl cysteine prevent these adverse effects. Interestingly, antioxidant treatment also prevents the increase in Nox expression, suggesting that β_2-adrenoceptor stimulation triggers a vicious cycle eventually amplified by both Nox isoforms. The possible existence of a circuitry to enhance ROS signalling and detrimental consequences on myocardial remodelling are also discussed, in light of the recent description of intracellular localization of Nox4.

LINKED ARTICLE

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Abbreviations

CHF, congestive heart failure; LV, left ventricle; MAO, monoamine oxidase; MAPK, mitogen-activated protein kinase; NAC, N-acetyl-L-cysteine; Nox, NADPH oxidase; ROS, reactive oxygen species; β_2-TG, β_2-adrenoceptor over-expressing mice

In failing hearts, elevated sympathetic activity initially compensates for decreased cardiac contractility. However, persistent pressure/volume-overload leads to a progressive decay in cardiac reserve, turning compensatory hypertrophy into maladaptive remodelling (Diwan and Dorn, 2007). β-Adrenoceptor-mediated signalling is markedly attenuated in congestive heart failure (CHF) subjects, owing to the down-regulation and desensitization of the receptors and their uncoupling from adenylyl cyclase (Rockman *et al.*, 2002). While this new level of β-adrenoceptor-mediated activity can reflect an attempt by the heart to evade the possible deleterious consequences of over-stimulation of β_1-adrenoceptors, the loss in β_1-adrenoceptor-mediated support may however further jeopardize cardiac contractility, thus contributing to the worsening of function in CHF patients. In contrast to β_1-adrenoceptors, the levels of β_2-adrenoceptors remain unchanged in the failing myocardium, although the receptor is markedly uncoupled from G proteins (Rockman *et al.*, 2002). β_2-Adrenoceptor signalling can also grant protection against programmed cell death in myocytes, countering the pro-apoptotic action of β_1-adrenoceptor stimulation via a Gi-mediated process (Rockman *et al.*, 2002). However, the transgenic approach indicates that the functional outcome of over-expressing β_2-adrenoceptors is dose-dependent. The 60-fold line of over-expressors exhibits augmented basal cardiac function with no signs of pathological consequences (up to 1 year of observation), the 100-fold ones develop a delayed fibrotic cardiomyopathy and CHF, whereas the 350-fold over-expressors are rapidly progressing towards left ventricular (LV) remodelling and decompensation (Liggett *et al.*,

2000). Moreover, when transgenic mice with a ≅200-fold increase in β_2-adrenoceptor density are subjected to aortic constriction, they display higher mortality and are more seriously ill compared with their littermates (Du *et al.*, 2000).

In the current issue of *The British Journal of Pharmacology*, Xu *et al.* (2010) provided us with new key pieces in the complex mosaic of β_2-adrenoceptor activation in myocardial biology and function. They used mice over-expressing cardiac-specific β_2-adrenoceptors that have ≅200-fold increase in β_2-adrenoceptor density in the heart (β_2-adrenoceptor over-expressing mice, β_2-TG). These mice have elevated adenosine 3′,5′ cyclic monophosphate levels and enhanced ventricular contractility and heart rate in the absence of agonist stimulation (Milano *et al.*, 1994). Here, the authors confirmed the existence of an early time point of functional hypercompensation (5 months) that in the long-term gives way to structural and functional LV disarrangement (15 months). This transition (around 7 months of age) was accompanied by increased reactive oxygen species (ROS) production, mostly superoxide generation, as revealed by electron spin resonance. The authors identified both reduced nicotinamide adenine dinucleotide phosphate oxidase (Nox) 2 and 4 as the major source of this oxidative burden, because their gene expression and activity were markedly up-regulated. Consistently, in isolated myocytes, β-adrenoceptor stimulation led to augmented superoxide formation, an effect fully offset by Nox inhibitors such as apocynin and diphenyleneiodonium. The authors went on to report that in β_2-TG mice, the activation of p38 mitogen-activated protein kinase (MAPK) is mediated at least in part by the rise in Nox activity as its inhibition suppressed the increase in phosphorylation of p38 MAPK and its downstream target heat-shock protein 27. This set of data shows for the first time that the axis – β_2-adrenoceptor/Nox/ROS/p38 MAPK – exists and operates in ventricular myocytes. Thus, this study both reiterates the central role exerted by ROS in triggering or sustaining LV remodelling, and reaffirms Nox activity as one of the most prominent cardiac sources of ROS (Kuroda and Sadoshima, 2010). However, it also reveals how incomplete our understanding of how and when 'exactly' ROS-based mechanisms of injury fit into the frame of the complex signalling cascades involved in cardiac remodelling and dysfunction still is. In fact, not only the inhibition of Nox activity but also the use of the thiol-donating compound N-acetyl-L-cysteine (NAC) was very effective in preventing oxidative stress, activation of p38 and all the functional sequelae reported in the β_2-TG mice. More importantly, NAC pretreatment attenuated Nox2 and Nox4 up-regulation in gene expression and activity. Hence, an additional ROS source should sit upstream of Nox activation, while both Nox isoforms probably act as amplifiers of the initial igniting species in a rather complex ROS circuitry. This superfluous formation of ROS dictates that there is a need to understand where ROS are originally generated in order to enhance our possibilities to effectively stop this phenomenon at its outset. Mitochondria are considered the major site for ROS generation in cardiac myocytes. Nox is usually associated with the plasma membrane localization; therefore, the present study highlighting a central role for Nox might suggest that either ROS formation occurs mostly in non-myocyte cells or mitochondria are not involved. However, recent evidence shows that Nox4 is localized mostly in those organelles that could play a role in generating the initial oxidative stress required for Nox over-expression (Kuroda and Sadoshima, 2010). In fact, as well as Nox4, mitochondria contain at least three additional systems for generating ROS, namely the respiratory chain, $p66^{Shc}$ and monoamine oxidases (MAO), that were also proven to contribute to different cardiac pathologies (Kaludercic *et al.*, 2010a). The mechanisms controlling the cross-talk between these various systems have not been elucidated, yet they might be linked in a vicious cycle whereby the inhibition of one component hampers ROS formation by the other enzymes as shown in the case of MAO and $p66^{Shc}$. In addition, MAO-A are emerging as a relevant source of H_2O_2 in isolated myocytes and failing hearts, where its inhibition prevents the development of maladaptive hypertrophy and its transition to heart failure (Kaludercic *et al.*, 2010b). Thus, it is plausible that mitochondrial MAO are part of a ROS circuitry triggering or enhancing intracellular Nox activity. However, the deleterious consequences of NOX4 activity have recently been challenged by a study demonstrating the occurrence of adverse remodelling when NOX4 is deleted (Zhang *et al.*, 2010).

Oxidative stress generates a wide array of deleterious processes that synergize in contributing to adverse cardiac remodelling. Besides hampering cell viability due to alterations in energy metabolism and ionic homeostasis, ROS-induced oxidation of myofibrillar proteins has been shown to correlate with contractile impairments in both experimental models and clinical settings (Canton *et al.*, 2010), in a process that might be mediated by p38 MAPK activation (Heusch *et al.*, 2010). On the other hand, ROS also mediate interactions between cardiomyocytes and extracellular matrix components such as fibroblasts, widening the effects produced by the activation of the β_2-adrenoceptor/Nox/ROS/p38 MAPK axis. This concept is supported by present evidence showing that NAC treatment for 3 months suppressed both latent and active metalloproteinase 2, reducing extracellular matrix remodelling (Xu *et al.*, 2010).

Further delineating the reciprocal β_2-adrenoceptor-oxidative stress influence is the fact that upon NAC treatment, β_2-TG mice displayed reduced oxidative burden, increased β_2-adrenoceptor and α-myosin heavy chain gene expression and fully preserved *in vivo* structural and functional cardiac assets (Xu *et al.*, 2010). This finding suggests that possible support provided by β_2-adrenoceptor stimulation to a deteriorated myocardial function comes with some 'hazardous' fee, that is increased ROS emission that may ultimately delete the beneficial effects of β_2-adrenoceptors. Indeed, prolonged use of β_2-adrenoceptor agonists is structurally and functionally detrimental in both animals and humans. In the former, it induces ventricular hypertrophy and impairment of cardiac relaxation, among others, reducing expression/activity of sarcoplasmic reticulum Ca^{2+} ATPase (Ryall *et al.*, 2008). In patients with LV dysfunction, despite an improvement in cardiac function in the short-term (Matera *et al.*, 2010), β_2-adrenoceptor agonists given within 3 months of hospitalization cause an increased risk of CHF hospitalization (Au *et al.*, 2003). Yet, treating rats with myocardial infarction for 12 months with a combined β_1-adrenoceptor blocker and β_2-adrenoceptor agonist confers more protection than the 'classical' combination of β_1-adrenoceptor and angiotensin-converting enzyme inhibi-

tor (Ahmet *et al.*, 2009). Given the substantial failure exhibited by treatment with antioxidants alone in CHF patients, one may entertain the intriguing possibility of conducting studies in which β_2-adrenoceptor agonist administration is combined with 'targeted' anti-ROS therapy, perhaps centred on different isoforms of Nox. To the best of our knowledge, such studies, either at the experimental level or in clinical trials, are not currently available.

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