Editorial

iNOS — Another cardiac target of calcineurin

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See article by Obasanji-Blackshire et al. [3] (pages 672–683) in this issue.

1. Calcineurin action in the heart

The serine–threonine kinase calcineurin was first detected in the nervous system in 1979 [1]. Twenty years later the first report about a role of calcineurin in cardiac hypertrophy appeared [2]. Since then, research on calcineurin function in the heart has strongly intensified. During these years of research, multifunctional roles of calcineurin in cardiac hypertrophy, apoptosis, contractile function, and, as now shown in this issue of Cardiovascular Research [3], preconditioning, have been revealed. These findings point to calcineurin/NFAT signaling as being a central factor in cardiac pathophysiology. However, it is still a matter of debate whether the final result of the signaling cascade is good or bad for the heart.

Several approaches have been used to characterize the role of calcineurin in cardiac hypertrophy. What became evident from these studies is that overexpression of calcineurin or its well-known downstream effector, the transcription factor NFAT, promotes hypertrophic growth of the heart in transgenic mice [2]. However, depending on the hypertrophic stimuli and the developmental stage of the myocytes, calcineurin/NFAT are not always activated and therefore not necessarily involved in hypertrophic growth: under conditions of physiological hypertrophy, as it is induced by exercise training of rats or by growth hormone/insulin-like growth factor 1 treatment of hearts or neonatal cardiomyocytes, the calcineurin/NFAT pathway is not activated [4]. In contrast, under pathological conditions of cardiac hypertrophy induced by pressure overload or after myocardial infarction with progression to heart failure, activation of calcineurin and NFAT occurs [4]. This indicates that calcineurin/NFAT is related to pathological conditions of heart failure. However, the expression of calcineurin/NFAT does not necessarily result in hypertrophy: in skeletal muscle, calcineurin expression was essential for myogenesis but not for hypertrophy [5], and in adult rat cardiomyocytes inhibition of NFAT by decoy oligonucleotides could not block hypertrophic growth induced by α-adrenoceptor stimulation with phenylephrine [6]. After aortic constriction, inhibition of calcineurin by cyclosporine A often [7,8] but not always [9] resulted in reduction of hypertrophic growth of the heart. Thus, it can be assumed that calcineurin/NFAT are important mediators of cardiac hypertrophy under certain pathological conditions.

Interestingly, calcineurin/NFAT signaling is not only related to a negative outcome of heart failure progression. There are also studies that describe protective roles of this pathway in the heart. NFAT4 overexpression in neonatal cardiomyocytes has been shown to prevent apoptosis induced by hydrogen peroxide or staurosporine [10]. And enhanced apoptosis after stimulation of cardiomyocytes with phenylephrine was found under conditions where NFAT was inhibited by VIVIT, a selective peptide inhibitor [10].

Another positive effect of NFAT during phenylephrine stimulation is the upregulation of the sarcoplasmic reticulum Ca^{2+} pump (SERCA) expression. Only under this condition are cardiomyocytes able to maintain normal cell shortening during hypertrophic growth stimulation [6]. Induction of SERCA as well as the anti-apoptotic effects are early responses of cardiomyocytes to stimulation with phenylephrine and both may therefore contribute to the adaptive character of α-adrenergic growth stimulation in the heart.
2. iNOS effects in the heart

Besides SERCA, several other target genes exist that have NFAT binding sites in their promoters and are consequently targets of calcineurin/NFAT signalling. To this group of genes Obansanjo-Blackshire and co-workers now add iNOS as another target gene of NFAT [3]. They demonstrate in this issue of the Journal that overexpression of calcineurin enhances iNOS expression in cardiomyocytes via NFAT activation and induces iNOS-dependent protection against simulated ischemia.

iNOS is one of three NO synthases that are expressed in diverse cell types of the heart. eNOS (endothelial NO synthase, NOS3) and nNOS (neuronal NO synthase, NOS1) are the constitutive, calcium-dependent isoforms that produce small amounts of NO. iNOS (inducible NO synthase, NOS2) produces in a calcium-independent manner a relatively large amount of NO, which in cardiac myocytes is known to induce apoptosis [11]. iNOS is usually undetectable in healthy tissue but can be induced by biological stress (e.g., ischemia and reperfusion) [12] or by several cytokines including TNF-α, interferon-γ, interleukin-1β, and interleukin 6 under inflammatory conditions or sepsis in the heart and in the vascular wall, resulting in a marked decrease in blood pressure associated with a negative inotropic effect on cardiac myocytes [13].

iNOS itself does not seem to be deleterious, since a substantial, cardiospecific overexpression of iNOS in transgenic mice is not associated with harmful effects on cardiac hemodynamics and energetics and does not result in heart failure [14]. The heart in vivo can functionally deal with pathological NO production, which includes rapid intracellular metabolism. Nitrosative stress induced by overexpression of iNOS is attenuated by myoglobin. This was demonstrated in the double transgenic mouse model of Gödecke et al. [15], where cardiac-specific iNOS overexpression in myoglobin-deficient mice leads to cardiac hypertrophy, ventricular dilatation, and interstitial fibrosis.

Studies examining the role of NO on myocardial ischemia/reperfusion (I/R) injury have demonstrated both pro- and anti-apoptotic effects, depending on the source of NO or the experimental conditions. Therefore, iNOS expression during myocardial I/R has protective but also detrimental effects. Langendorff preparations from iNOS−/− mice subjected to cardiac ischemia showed no acute differences in cardiac contractility, heart rate, coronary dynamics, or leakage of intracellular creatine kinase or lactate dehydrogenase, suggesting no significant role for iNOS in the early phase of cardiac ischemia [16]. Use of an adenoviral vector encoding human iNOS demonstrated that myocardial overexpression of iNOS provided profound protection against I/R injury, and this salubrious effect was dependent on the upregulation of COX-2 [17] and remained detectable for two months, with no evidence of inflammation or cardiac contractile depression [18]. In addition, the response during myocardial I/R injury of iNOS transgenic mice revealed that overexpression of iNOS confers protection, as witnessed by a decrease in infarct size after 30 min global hypoxia followed by 40 min reperfusion in isolated mouse hearts [19]. However, inhibition of iNOS by aminoguanidine during prolonged reperfusion for 48 h decreased infarct size and improved myocardial function [20].

Most in vivo studies have shown that iNOS plays a prominent role in the protective effect of late-phase ischemic preconditioning. Ischemic preconditioning involves exposure of the heart to brief periods of ischemic insult that in turn generates a cardioprotective effect against a later, more prolonged ischemic episode. This protective effect can be distinguished into an early phase, which is protective for 2–3 h after the initial insult, and a late phase, which is effective 1–3 days after the initial ischemic insult [21]. Recent research has identified several of the key signalling molecules involved in late preconditioning, including NO, the protein kinase C α isoform, two members of the Src family of protein tyrosine kinases Src and Lck, nuclear factor κB, and iNOS [21]. In this issue, Obansanjo-Blackshire et al. [3] have described a Src/iNOS protecting mechanism. Interestingly, here calcineurin is positioned at the beginning of this cascade and signals via NFAT. Regulation of iNOS via calcineurin/NFAT signalling has already been shown in other cell types [22,23]. Based on the data of Obansanjo-Blackshire and these other studies, it can be assumed that calcineurin initiates a signalling cascade similar to that operating during preconditioning, thereby protecting cardiomyocytes against simulated I/R injury. Besides this favourable effect of calcineurin, the authors noticed an increase in cell size beginning three days after infection with adenovirus expressing calcineurin. Interestingly, the protective effect of iNOS expression is an early response of cardiomyocytes to NFAT activation (within 24 h), whereas hypertrophic effects appear at later time points.

3. Conclusions

For iNOS and calcineurin/NFAT signalling, involvement in beneficial as well as detrimental pathways in the heart is being discussed in the literature. As shown in this issue [3], a link between iNOS and calcineurin/NFAT signalling has been established. In this context, induction of iNOS by calcineurin primarily has protective effects against ischemic injury. This finding gives rise to the question whether anti-apoptotic effects of calcineurin/NFAT signalling may also be mediated by iNOS. Furthermore, the described protective effect is reminiscent of the preconditioning phenomenon in which iNOS is involved, and calcineurin activation might be the primary, not yet identified mediator in this scenario.

What becomes evident from the sum of these studies is that NFAT activation primarily results in beneficial effects in the heart, protecting it against cell damage and contractile dysfunction. However, if sustained and strong NFAT expression persists, the protective side of calcineurin/NFAT is overruled and NFAT may contribute to decompensated hypertrophy and heart failure progression. In this context,
activation of additional factors, such as MEF-2, AP-1, GATA, or other transcription factors known to interact with NFAT, may be important, and the expression of individual NFAT isoforms may be relevant.

References