Increased eNOS expression as a compensatory mechanism reducing β-adrenergic responsiveness?

Frank U. Müller

Institute of Pharmacology and Toxicology, University of Münster, Domagkstrasse 12, D-48149 Münster, Germany

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See article by Danson et al. [13] (pages 613–623) in this issue.

Nitric oxide (NO) is an important regulatory factor of the cardiovascular system mediating endothelium-dependent vasodilatation and modulating different facets of cardiac function including heart rate, contraction, relaxation, cell growth, and survival [1–3]. NO is synthesized from L-arginine by NO synthases (NOS), a family of isoenzymes with characteristic functional and regulatory properties [4]. All three NOS isoforms, i.e. the neural isoform (nNOS), the inducible isoform (iNOS, induced upon stimulation with appropriate inflammatory mediators), and the endothelial isoform (eNOS), can be expressed within various cell types in cardiac muscle. In the heart, eNOS was identified in endocardial and endothelial cells, in cardiomyocytes, and in specialized cells of the conducting system of the sinus and atrioventricular nodes [1–4]. Therefore, NO derived from different isoenzymes expressed in diverse cell types may modulate cardiac function in a paracrine/autocrine manner. As a consequence, differential local NO levels may contribute to the conflicting paradigms on cardiac function of eNOS which were deduced from studies on different experimental models, namely isolated cardiac myocytes, isolated cardiac muscle preparations or assessment of cardiac function in vivo, in eNOS-deficient or eNOS-overexpressing mice (see below) [1,3].

Under basal conditions (i.e. in the absence of agonist stimulation, e.g. by catecholamines), a concentration-dependent, biphasic inotropic effect of exogenous NO was reported by different studies, while endogenous NO may exert a positive inotropic effect (for a detailed review on eNOS function in the heart and for further references, see Ref. [3]). The majority of studies on eNOS-deficient mice consistently reported that eNOS gene deletion does not affect basal cardiac inotropy; however, the cardiomyocyte-directed overexpression of eNOS led to a reduction in basal contractile state of Langendorff preparations [5]. Basal heart rate was normal or decreased in most studies on eNOS-deficient mice [3], and was not altered in eNOS-overexpressing mice [5]. Direct nitrosylation of the ryanodine receptor (RyR2) or of the voltage-operated L-type calcium channel as well as cGMP-dependent and -independent increases in cAMP levels are potential mechanisms contributing to the positive inotropic effects of NO [3]; the negative inotropic effect seen in eNOS-overexpressing mice possibly involves a decreased cardiac myofilament Ca2+ sensitivity [5]. Several groups reported an increased inotropic response to β-adrenergic receptor stimulation in eNOS-knockout mice from different strains in vivo or in isolated hearts [6–9]. However, this effect was not observed in papillary muscle preparations [10] or in isolated cardiac myocytes [11]. In eNOS-overexpressing mice, the inotropic response to β-adrenergic receptor stimulation was unchanged or reduced in Langendorff preparations [5] and in ventricular cardiac myocytes [12]. Effects of β-adrenergic receptor stimulation on heart rate were not altered in eNOS-deficient [9,10] or-overexpressing [5] mice. It is not fully understood which mechanisms contribute to inhibitory effects of eNOS on β-adrenoceptor-mediated positive inotropy in cardiac myocytes. A PKG-dependent phosphorylation of troponin I and a subsequently decreased Ca2+ sensitivity of myofilaments, a direct inhibition of RyR2 by NO, or a cGMP-dependent inhibition of voltage-operated L-type Ca2+ channels were suggested as pathways that are potentially involved [3]. Several studies on eNOS-deficient mice postulated a role of eNOS in mediating the attenuating effect of muscarinic stimulation on the inotropic response to
β-adrenergic receptor stimulation in vivo and in isolated cardiomyocytes [9,11], whereas other studies refute this hypothesis based on experiments on Langendorff hearts [8], papillary muscle preparations [10], and cardiac myocytes [10]. Taken together, these results indicate that eNOS does not play a major role in the regulation of basal heart contraction and rate, but may reduce contractile response to β-adrenergic receptor stimulation under certain experimental conditions. It is not clear to what extent eNOS is involved in the muscarinic receptor-mediated attenuation of β-adrenergic receptor-mediated inotropic effects.

In the current issue of Cardiovascular Research, Danson and colleagues [13] report that eNOS expression was increased in atria of mice pretreated with pertussis toxin (PTx), which leads to disruption of the signaling mediated by inhibitory GTP-binding proteins (Gi). Both positive inotropic and chronotropic effects of β-adrenergic stimulation were attenuated in isolated atrial preparations from PTx-pretreated mice. However, basal atrial contraction and beating frequency were not altered by pretreatment with PTx. The abrogation of this attenuation of β-adrenergic effects in the presence of the NOS inhibitor L-nitroarginine or in atria from eNOS-deficient mice suggested that (increased) eNOS plays a role in the reduced β-adrenergic response in PTx-pretreated atria. In conclusion, the authors propose increased eNOS expression as a compensatory mechanism limiting β-adrenergic responsiveness when cardiac parasympathetic control is impaired.

A similar increase in eNOS expression was previously reported in isolated rat hearts pretreated with PTX [14]; however, it was accompanied by increased basal and β-adrenoceptor-stimulated values of maximal left ventricular velocities of contraction (dP/dt max). Thus, it remains to be elucidated whether this discrepancy is due to the different species observed, unequal experimental settings (Langendorff preparation vs. isolated atria), or different functions of increased eNOS in ventricle and atrium. In general, inhibition of β-adrenergic receptor-stimulated contraction in PTx-pretreated atria is well in line with previous results from eNOS-deficient or overexpressing mice [5–9,12], although β-adrenergic receptor-stimulated heart rate was not altered in those studies. The reason for the disparate effects of increased eNOS protein levels on β-adrenergic receptor-stimulated chronotropy is not clear, but it is possible that the αMHC promoter used for eNOS overexpression [5] is less efficient in the sinus node, as discussed by Danson and colleagues [13]. Finally, data from eNOS-deficient mice (group without PTX-pretreatment) support previous studies that eNOS is not implicated in the muscarinic attenuation of the inotropic response to stimulation of the β-adrenergic receptor [8,10].

The results presented by Danson and colleagues indeed suggest another level of interaction between Gi-coupled signaling and the β-adrenoceptor-mediated chronotropy and inotropy requiring the expression of eNOS. It is an intriguing idea that eNOS enters the stage to counterbalance increased sympathetic activity when other protective mechanisms mediated by muscarinic or adenosine receptors are impaired. It may be speculated that this mechanism is relevant in human heart failure associated with increased sympathetic activity [15], impaired parasympathetic activity [16], and increased expression of eNOS [17]. Moreover, eNOS in general might represent an important mechanism of cardioprotection, e.g. as recently suggested in a study on rosuvastatin up-regulating eNOS in rat heart [18]. However, in order to evaluate the relevance of this mechanism for the pathophysiology of heart failure, several questions require further experimentation: (I) Does PTx-pretreatment also increase eNOS in ventricular mouse myocardium and – if yes – is this effect also accompanied by inhibition of inotropic response to β-adrenergic stimulation? The study of Danson and colleagues is limited to atrial effects but dysfunction of ventricles certainly is the more important factor in heart failure. (II) To what extent can conclusions from experiments with PTX-mediated disruption of Gi signaling be transferred to the pathological situation where parasympathetic activity is decreased and Gi protein is increased? (III) A reduction in the entry of Ca2+ via voltage-operated L-type Ca2+ channels was ruled out as a mechanism suppressing β-adrenergic response in PTx-pretreated mice. However, the question about other mechanisms involved remains open. In addition, more needs to be learned about the regulation of eNOS gene expression.

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