Adrenergic Excess, hNET1 Down-Regulation, and Compromised mIBG Uptake in Heart Failure

Poverty in the Presence of Plenty*

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An increased adrenergic drive contributes to augmented myocyte contractility and altered peripheral vasoreactivity in heart failure (HF) and appears intuitively compensatory (1). The increased adrenergic contents in the synaptic cleft eventually result in desensitization of the post-synaptic myocardial adrenergic receptors (2), cardiac remodeling, worsening HF, poor exercise tolerance, increased susceptibility to arrhythmias, and sudden cardiac death (3). Systemic norepinephrine (NE) levels correlate with mortality (4,5), and the beneficial effects of beta-receptor blockade in HF support the role of the adrenergic system in the progression and pathophysiology of HF (6–9).

In the sympathetic neuron, tyrosine is actively delivered into the axoplasm, where it is converted to dopamine by cytoplasmic enzymes. Dopamine is then transported to intraneuronal vesicles where NE is synthesized and stored (Fig. 1). The action potential at the nerve terminal triggers the release of NE in the synaptic cleft, where it activates the post-synaptic beta1-adrenergic receptors on the sarcolemmal membrane of the cardiac myocyte; cleft NE also activates the pre-synaptic alpha2a- and alpha2c-adrenergic receptors to inhibit further NE release.

Almost all NE is actively removed from the synaptic cleft by a transporter, human norepinephrine transporter 1 (hNET1).

In patients with HF, there are increased quantities of NE in the synaptic cleft. A small proportion of this NE spills into the bloodstream (increasing plasma NE levels), and the remainder is transported by hNET1 back into the axoplasm. However, there is gradual down-regulation of the transporter and increased levels of cleft NE, an excess that results in further impairment of NE reuptake (10). This concept has been best portrayed by radionuclide metaiodobenzylguanidine (mIBG) imaging; mIBG is an NE analog that demonstrates storage, transport, and reuptake characteristics similar to NE in sympathetic neurons.

Reduced NE reuptake results in low mIBG concentration in the neurons, and the accelerated release kinetics lead to an enhanced mIBG washout rate. In clinical imaging, myocardial mIBG labeled with radioactive iodine uptake is commonly quantified in terms of the heart to mediastinal (H/M) uptake ratio. The lowest H/M (or the highest washout rate) in patients with HF is associated with the worst prognosis; mIBG uptake has been proposed to be a superior predictor of outcomes than left ventricular (LV) ejection fraction or B-type natriuretic peptide. Beta-blockers have been shown to improve myocardial retention of mIBG (11,12), and the improved mIBG uptake correlates with the morphologic characteristics of reverse remodeling.

In this issue of JACC, Drakos et al. (13) employed mIBG imaging to substantiate the role of the adrenergic system in both the pathogenesis and recovery of patients with HF. A markedly reduced mIBG uptake in end-stage HF was substantially...
reversed by unloading of the ventricle after left ventricular assist device (LVAD) implantation; the H/M mIBG uptake ratio on delayed images and the washout rate were both significantly improved. These results suggest that LVAD restores NE re-uptake mechanisms. These data are consistent with our recent observations of the significant reduction in hNET1 expression in myocardial samples obtained from patients with advanced HF and marked improvement after ventricular unloading with LVAD.

To determine the status of hNET1 expression in end-stage HF, we analyzed 8 hearts explanted from heart transplant recipients, 4 with idiopathic dilated and 4 with ischemic cardiomyopathy; hNET1 expression in cardiomyopathic hearts was compared with 8 unused donor (normal) hearts. We determined hNET1 protein levels by immunoblot analysis; hNET1 protein expression was significantly reduced in HF hearts compared with control hearts (p < 0.001) (Figs. 2A and 2B). We also analyzed paired specimens from 5 additional patients before and after LVAD placement. The hNET1 protein was virtually absent in 4 of 5 pre-LVAD cardiac tissues, and the post-LVAD samples showed a dramatic increase in the hNET1 proteins levels (p < 0.05) (Figs. 3A and 3B). The molecular data indicated the feasibility of restoration of hNET1 expression in assisted hearts.

Norepinephrine-induced endoplasmic reticulum stress and an impaired post-translational glycosylation of NET can accelerate its degradation and may result in lower hNET density in HF. To elucidate this possibility, we analyzed the hNET glycosylation profile in normal and HF tissues. There was no difference in NET1 glycosylation in normal and HF hearts (Fig. 2C), suggesting that there was no defect in surface expression of hNET on the sarcolemma. Almost all of the hNET protein was glycosylated in these heart samples; a barely detectable unglycosylated fraction of hNET was present in the supernatant.

It is expected that the superfluous NE in the synaptic cleft will normally be efficiently reabsorbed by the hNET1. In fact, patients with α2c polymorphism who exhibit a retarded ability for the feedback control of NE release from the neurons and release excessive amounts of NE in the synaptic cleft demonstrate a higher delayed mIBG H/M ratio. It is surmised that an increased activity of hNET1 serves as a protective molecular adaptation to partially offset the deleterious consequences of an incessant bombardment of the myocyte with toxic amounts of NE. However, such compensatory mechanisms are gradually overwhelmed, and persistent NE excess leads to beta-adrenoceptor desensitization and worsening of HF. Data from transgenic models of exaggerated NE release support the importance of NE reuptake mechanisms. Three months after aortic banding, dramatically reduced survival was observed in alpha2A-knockout (52%) and alpha2C-knockout (47%) mice owing to HF with enhanced LV hypertrophy, myocardial fibrosis, and elevated circulating catecholamines, compared with wild-type and alpha2B-deficient animals (86%).

Multiple experimental studies have demonstrated that genetic alterations in the NE receptors or their downstream signaling pathways may be associated with cardiomyopathy, with the myocytes possibly the innocent victims. A cardiomyocyte-specific transgenic overexpression of Gαq results in mal-adaptive myocardial hypertrophy and is also associated with reproducible peripartum cardiomyopathy and HF; inhibition of Gαq-induced signaling at-
tenuates mitogen-activated protein kinase activation and cardiac hypertrophy. Genetic polymorphism of the rate-limiting enzyme tyrosine hydroxylase also induces structural changes in the myocardium. The exact regulation of the enzymatic cascade through pre-synaptic adrenergic and muscarinic receptors, hNET1 efficiency, and deficits of intraneuronal monoamine oxidase have not been elucidated. It is noteworthy that the reduction of myocardial NE uptake and loss of neuronal NE in HF is not due to the loss of structural integrity of the cardiac sympathetic nerves, as exemplified by the unaffected expression of the pan-neuronal protein gene product 9.5.

Evolutionarily, the sympathetic neurons, as compared with the parasympathetic neurons, are primitive. Unlike parasympathetic neurons, which have evolved with cholinesterase protection, sympathetic neurons rely completely on the neuronal reuptake mechanism to manage NE excess and hence are easily overwhelmed. In absence of a metabolic inhibitor of NE in the synaptic cleft, the excess NE results in adrenoceptor modulation, myocyte apoptosis (14), and myocellular loss, further worsening myocardial function. It seems that we pay the price for the lack of evolution in the sympathetic system.

Nerve growth factor overexpression in mice facilitates NE reuptake and prevents onset of HF. Injection of nerve growth factor into the stellate ganglia of rats with HF resulted in improved NE uptake, replenishment of cardiac NE stores, and
improvement in LV function (15); this NE consolidation occurred without any change in the number of sympathetic nerves. Similarly, overexpression of NET1 resulted in marked improvement of HF in a rabbit model (16). The NET1 gene transfer normalizes not only protein expression of the NET1 but also beta1-adrenergic receptors and sarcoplasmic reticulum Ca\(^{2+}\)/H\(^{-}\)ATPase, thus replicating some of the molecular effects that typically accompany pharmacologically managed HF. Another important finding was the complete recovery of NE uptake by LV myocardial tissue. These experiments suggest that modulating hNET1 expression may serve as an attractive alternative therapeutic target in the management of HF and may lead to development of pharmacologic agents that facilitate NE re-uptake mechanisms in the myocardium.

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