

## **Have Imagers Aptly or Inadvertently Overlooked the Neuronal Myocardial Compartment?**

Vasken Dilsizian & Jagat Narula

University of Maryland School of Medicine, Baltimore, Maryland and  
Icahn School of Medicine at Mount Sinai, New York, New York

Imaging of the myocardium has been immensely challenging but equally rewarding. The heart is a constantly moving organ, normally beating 60-100 times a minute and every beat studded with multiphasic physiology. The continuous imaging is pleasing but the quantitative measurements require meticulous gating. Contrast-based imaging requires precise timing to capture contrast transition and also methodology to preclude contamination from myocardial tracer uptake, and differentiation of the target uptake from the residual blood pool presence. All challenges of cardiac imaging have constantly honed the growth of imaging in cardiovascular system. The contractility of the myocardium is the most prominent trait related to the outcomes and various morpho-functional imaging strategies have focused on muscle mechanics for the assessment of global and regional ejection fraction, as well as the strain and strain rates.

Echocardiographic, magnetic resonance and radionuclide imaging have performed comparably well. Molecular imaging has targeted muscle ischemia, apoptosis and necrosis with impressive results. Inducible myocardial ischemia has allowed indirect assessment of significant coronary artery stenosis, which has been ably supplemented by the computed tomography angiography. The interstitial compartment has predominantly been conducive to magnetic resonance imaging, and has uniquely addressed the presence of interstitial edema and myocardial fibrosis. The neuronal component of the myocardium, which may directly or indirectly influence other aforementioned components or disintegrate due to other components, has been sparingly evaluated. This might be an inadvertent oversight, or an apt omission due to its interaction with the abnormalities produced by other myocardial compartments, that have been more easily measured.

The importance of neuronal dysfunction in the progression of heart failure pertaining to adverse outcomes including life-threatening arrhythmias is well established (1,2). The long-term pathophysiologic consequences of sympathetic excess include ventricular remodeling, and during this process the increased norepinephrine (NE) concentration in synaptic clefts results in adrenoceptor desensitization accentuating remodeling process. Improved clinical outcomes associated with adrenergic blocking drugs confirm the centrality of sympathetic dysregulation in HF. In the sympathetic nerve terminals including that in heart, tyrosine is actively transported, converted to intermediary dopamine followed by NE synthesis; NE is stored in intraneuronal vesicles and released by exocytosis into the synaptic cleft to activate adrenoceptors in the postsynaptic sarcolemmal membrane. In addition, cleft NE also activates presynaptic  $\alpha$ -receptors,

predominantly  $\alpha_{2c}$ , to inhibit NE release. Most of the NE in the cleft is actively taken back up by the neuron through human NE transporter (hNET1) and minimal NE amount diffuses into the plasma. The sarcolemma has  $\alpha_{(1a, b, d)}$  and  $\beta_{(1-3)}$ -adrenergic receptors;  $\beta$ -adrenoceptors through  $G_{\alpha_s}$  and adenyl cyclase catalyze ATP to cAMP, and the  $\alpha$  receptors through  $G_{\alpha_q}$  are coupled to phospholipase C for effector function. Whereas  $\beta$ -receptors are associated with positive inotropic, chronotropic, and hypertrophic effects, exposure of  $\beta_1$ -receptors to NE excess results in their desensitization by uncoupling from transducing G protein that is mediated by  $\beta$ ARK1 and  $\beta$  arrestins. The polymorphisms of  $\beta_1$ -receptors (Arg389) and  $\alpha_{2c}$ -receptors (del 322-325) have been described in HF populations that result in increased beta receptor sensitivity and decreased regulatory inhibition of neuronal NE release. With the supraphysiological NE contents of the cleft, in addition to  $\beta$ -receptor desensitization there is gradual down-regulation of hNET-1 that adds to the NE toxicity and further downregulation of hNET-1. Upon comparison of explanted hearts from transplant recipients with unused donor (normal) hearts, hNET1 expression by immunoblot analysis was significantly reduced in failing hearts (**Fig. 1**). In addition, paired specimens from patients before and after LVAD placement, the post-LVAD samples showed a dramatic increase in the hNET1 proteins levels (**Fig. 2**). It is therefore expected that an in vivo imaging strategy for the assessment of hNET1 integrity would mirror the remodeling process and recovery in HF.

This concept of hNET1 down-regulation has been best exemplified by radiolabeled metaiodobenzylguanidine (*mIBG*) imaging (3-7). *mIBG* is an NE analog that demonstrates storage, transport, and reuptake characteristics similar to NE in sympathetic neurons. Downregulation of hNET-1 and consequently reduced NE reuptake is reflected by low *mIBG* concentration in the neurons. On the other hand, increased NE demand in HF and accelerated NE release lead to exaggerated *mIBG* washout rate. For the purposes of clinical imaging, radioiodinated *mIBG* uptake in the failing myocardium has been quantified as the heart-to-mediastinal (H/M; mediastinal uptake presumed to be the background uptake) ratio. The low H/M uptake ratio (and a high washout rate) in HF patients is associated with the worse prognosis, and beta-blockers improve myocardial *mIBG* retention. The resolution of *mIBG* uptake correlates with reverse remodeling. A markedly reduced *mIBG* uptake in end-stage HF has also been demonstrated to be reversed by unloading of the left ventricular by the assist device (LVAD) implantation (7).

Evolutionarily, the sympathetic neurons, as compared with the parasympathetic neurons, are primitive (1). Unlike parasympathetic neurons, which have evolved with cholinesterase protection, sympathetic neurons rely completely on the neuronal hNET1 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, and myocellular loss, further worsening myocardial function. ADMIRE-HF and ADMIRE-HFX studies have confirmed the prognostic significance of imaging of neuronal dysfunction (2,3). Whereas ADMIRE-HF trial employed *mIBG* imaging, assessment of post-synaptic beta receptors and pre-

synaptic neuronal NE synthesis provide additional information about sympathetic integrity. This supplement brings together the first comprehensive review of the modern state of HF and neuronal imaging. It contains the distilled work of several decades of high quality laboratory and clinical work of scientists.

## References

1. Haider N, Baliga R, Chandrashekhar Y, Narula J. J Am Coll Cardiol Img. 2010;3:71-75.
2. Jacobson AF, Senior R, Cerqueira MD et al. Myocardial iodine-123 meta-iodobenzylguanidine imaging and cardiac events in heart failure. Results of the prospective ADMIRE-HF (AdreView Myocardial Imaging for Risk Evaluation in Heart Failure) study. J Am Coll Cardiol 2010; 55:2212-21.
3. Narula J, Gerson M, Thomas, GS, Cerqueira, MD, Jacobson AF. I-123 mIBG Imaging for Prediction of Mortality and Potentially Fatal Events in Heart Failure: The ADMIRE-HFX Study. J Nucl Med 2015 (submitted).
4. Klein T, Huang R, Smith MF, Abdulghani M, Asoglu R, Remo BF, Turgeman A, Mesubi O, Sidhu S, Saliaris A, See V, Shorofsky S, Chen W, Dilsizian V, Dickfeld T. Three-dimensional <sup>123</sup>I-Meta-iodobenzylguanidine Cardiac Innervation Maps to Assess Substrate and Successful Ablation Sites for Ventricular Tachycardia - A Feasibility Study for a Novel Paradigm of Innervation Imaging Circ Arrhythm Electrophysiol 2015 Feb 23.
5. Abdulghani M, Duell J, Smith M, Chen W, Asoglu R, Klein T, Bob-Manuel T, Slairis A, See V, Shorofsky S, Dilsizian V, Dickfeld T. Global and Regional Myocardial Innervation Before and After Ablation of Drug-Refractory Ventricular Tachycardia Assessed with I-123 Metaiodobenzylguanidine. J Nucl Med 2015; June (in press).
6. Dilsizian V, Chandrashekhar Y, Narula J. Introduction of New Tests: Low Are the Mountains, High the Expectations". J Am Coll Cardiol Img 2010; 3:117-9.
7. Drakos et al. Myocardial sympathetic innervation and long-term left ventricular mechanical unloading. J Am Coll Cardiol Img. 2010;3:64-70.

## Figure 1

### **hNET1 in Cardiomyopathic and Normal Human Hearts**

(A) Immunoblot analysis for the assessment of human norepinephrine transporter 1 (hNET1) expression demonstrates decreased levels in cardiomyopathic hearts as compared with control human hearts (1 control and 2 cardiomyopathy sample is shown). Equal protein in each well was confirmed by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein level. (B) Densitometric analyses of hNET1 immunoblot (Fluor-S Multilmager; Bio-Rad, Hercules, California) are expressed as a bar graph and represent the intensity of the hNET1 bands' mean  $\pm$  SEM (\* $p < 0.05$ ) of 4 IDCM, 4 ISCM, and 8 control hearts. Values were corrected by the respective values of GAPDH for each sample. (C) The hNET1 glycosylation analysis demonstrated that most of the hNET1 protein in cardiomyopathic and control human hearts was glycosylated, which bound and precipitated with con-A Sepharose beads (pellet); barely detectable unglycosylated hNET1 not bound to con-A Sepharose beads remained in the supernatant. GAPDH immunoblot confirmed equal protein in each well. AU = arbitrary unit; IDCM = idiopathic dilated cardiomyopathy; ISCM = ischemic cardiomyopathy. Reprinted with permission (1).

## Figure 2

### **hNET1 Before and After LVAD Placement in Human Hearts**

(A) Immunoblot showed very low levels of hNET1 protein in pre-left ventricular assist device (LVAD) sample, which were markedly increased in post-LVAD (1 representative sample is shown). Equal protein in each well was confirmed by GAPDH protein level. (B) Densitometric analyses of hNET1 immunoblot from 5 paired pre- and post-LVAD samples showed restoration of hNET1 levels in post-LVAD myocardial samples. Values were corrected by the respective values of GAPDH for each sample. Abbreviations as in Figure 1. Reprinted with permission (1).



