Imaging Left Ventricular Muscarinic Receptor Heterogeneity

A Tool to Evaluate Individuals at Risk for Sudden Death?

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As pointed out by Mazzadi et al in this issue of Circulation: Cardiovascular Imaging, parasympathetic tone plays a critical role as modulator of the cardiac sympathetic nervous system in both health and disease. Decreased tone, as reflected by decreased heart rate variability (HRV), has an important role in prognosis after myocardial infarction (MI). HRV is also associated with worse outcome in some patients with heart failure. Sudden increases in parasympathetic tone, identified by increase in HRV, may precipitate ventricular fibrillation in selected individuals. Other investigators have not found any of a multitude of HRV components to be predictive of outcome. The predictive value of HRV is insufficient to be used alone and must be part of a multivariate assessment.

Given the wealth of data demonstrating that HRV and its derivatives provide useful but not highly predictive clinical information and at a relatively low cost, what added value is gained by the use of a complicated and expensive imaging method? There are limitations to measurement of HRV that generally require the patient to be in sinus rhythm without sinoatrial dysfunction, to have ≤20% ectopic beats, have good quality tracings and, ideally, at least 24 hours of recording. The requirement for sinus rhythm limits its application in the largest population in which it is most likely beneficial, those with heart failure. Also, imaging of presynaptic sympathetic function has been shown to better predict adverse events. The results of the Mazzadi et al study suggest that imaging of parasympathetic function also may be predictive.

Probably the most important rationale for the use of imaging to measure muscarinic cholinergic function relates directly to the regional heterogeneity of myocardial perfusion and sympathetic and parasympathetic function. As noted by Mazzadi et al, heterogeneity appears to play a major role in the arrhythmias associated with myocardial infarction and ischemia and probably some of the nonischemic cardiomyopathies. Our own observation, using positron emission tomography (PET), that the magnitude of heterogeneity of presynaptic and postsynaptic sympathetic function is associated with adverse outcome in ischemic cardiomyopathy, supports the importance of functional heterogeneity. HRV and its components reflect both systemic and global cardiac parasympathetic and sympathetic function interaction but do not distinguish between the two. Moreover, HRV cannot measure regional dysfunction. In this report, Mazzadi et al make the case that there are regional differences in muscarinic receptor density in the post-MI patient, even in the absence of significant residual scar, and by inference regional differences in parasympathetic function. Their observations were made in only 11 patients and 9 normal subjects. If their observation of increased receptor density remote from MI location is substantiated in larger populations and can be related to arrhythmic events or other adverse outcome, muscarinic imaging should have value as a tool for identifying the highest-risk individuals in populations identified at increased risk by current, less complex prognostic tools. It is also likely that knowledge of presynaptic sympathetic function and perhaps β-receptor density and blood flow in the same myocardial regions will be necessary to best identify those at various levels of risk. PET is currently unique in its ability to provide this information.

Imaging the parasympathetic system is not new. More than 25 years ago, Maziere et al reported imaging cardiac muscarinic receptors using 11C-methyl-quinuclidinyl benzilate (MQNB) and PET. This was shortly after the initial biodistribution and characterization studies by Gibson et al. However, since then, muscarinic imaging has focused on the brain, and there have been relatively few (<50) reports of cardiac muscarinic imaging as compared with the more than 800 relative to imaging the presynaptic sympathetic component. There are a number of reasons for this disparity.

A method for imaging presynaptic function, methyl-iodobenzylguanidine (MIBG), a marker of norepinephrine uptake and clearance, was developed at about the time that the clinical importance of the sympathetic nervous system in the morbidity/mortality associated with congestive heart failure and sudden death was recognized. In contrast, the role of the parasympathetic system and muscarinic receptor subtypes were not as well understood. Furthermore, the γ single-photon emission computed tomography tracers for muscarinic imaging, primarily 123I-QNB derivatives, were lipophilic, and cardiac uptake was not readily quantified. Thus, there were no readily available and interpretable γ-labeled radiotracers for muscarinics, whereas MIBG can be labeled.
with $^{123}$I or $^{131}$I and is easily interpreted. A radioligand that can be imaged using the widely available $\gamma$-camera makes it more likely that it will be used and the results reported. Additionally, the need to use PET to image the muscarinics and the requirement for a relatively short half-life ($T_{1/2}$ 20 minutes) positron-labeled compound that could only be made by a select group of investigators markedly restricted potential application.

Equally important is the fact that imaging receptors is inherently more complex than imaging tracer uptake/retention, the common case with myocardial perfusion imaging or presynaptic sympathetic imaging. This is because imaging receptor ligands at a single time, as done for perfusion imaging, is a sum of all the possible states of the radioligand at that time within the imaging voxel. That is, some activity will be in the blood, some will be in the interstitial space, some specifically bound to receptors, and some nonspecifically bound. Although a static image for presynaptic radiotracer uptake normalized to the delivery by blood flow will show presynaptic concentration of the tracer into the neuronal space, receptor imaging is limited to 1 molecule bound to 1 receptor, but the concentration of ligand in the blood or interstitial space may constitute a greater fraction of the imaged activity. The ligands can also bind to and be released from a receptor and then bind again to the same or nearby receptors all within a small myocardial region before leaving the heart. A static image of a receptor ligand might be the result of ligand binding to a large number of low-affinity receptors or a small number of high-affinity receptors. To understand receptor function, one needs to quantify the receptor density ($B_{\text{max}}$) in absolute terms as well as $K_D$, and that can only be done by injection of a radioligand at a minimum of 2 different specific activities at 2 time points with dynamic imaging and the application of a kinetic nonequilibrium mathematical model to the measured time-activity curves.

**How Do the Mazzadi et al Results Fit With Earlier Studies?**

The approach used for quantification of muscarinic receptor density is based on the 2-radioligand injection method proposed by Delforge et al. This was a simplification of a 3-injection approach and a kinetic nonequilibrium model. The model has been validated, so far as we have been able to determine, only by comparing the model parameter values that provided the best fit to the PET time-activity curves in a few dogs and men to literature values for $M_2$-receptor density from tissue samples by other investigators. It is disappointing that the validation was not done against tissue samples from the same heart, but it is not a fatal flaw as related to the current article. Comparing $B_{\text{max}}$ for muscarinic receptor density between regions of the same heart is appropriate, even if the PET $B_{\text{max}}$ value is not identical to tissue measured $B_{\text{max}}$. However, if the model behaves differently in the 2 regions because of factors important in modeling of PET data, then there would be concern. Are there suggestions that this might be the case? The most compelling evidence that the model is behaving correctly is the comparison of results in the normal volunteers to that of the remote region of the patients. Mean $B_{\text{max}}$ in the remote region is approximately twice that of the normal subjects, even when one excludes the patient with a remote value almost one-third higher than the next closest. Is there anything besides the suggested upregulation of the muscarinic receptors that could account for this? Age difference is not a factor. All patients were taking $\beta$-blockers, whereas the normal subjects were not. Although the authors and these reviewers are unaware of reports of $\beta$-blocker effect on vagal action, animal studies suggest that any effect of $\beta$-antagonists would decrease, not increase, muscarinic receptor concentration. Given the tight relationship between norepinephrine release at the myoneural interface and the $M_2$ receptors, one could postulate a situation in which $\beta$-blockade in the normal region was causing the sympathetic nerve terminal to release more norepinephrine in an attempt to overcome the $\beta$-blockade. This would increase norepinephrine concentration in the myoneural junction, which, through feedback mechanisms, might result in increased parasympathetic function. If the observed increase in $B_{\text{max}}$ in the remote region is anything other than a true physiological response, the most likely methodological reasons relate to the method of partial volume correction and uncertainty in the model parameter estimation.

The method (geometric transfer matrix) of correcting for partial volume/spillover was designed for the brain. As noted by the authors, there is no inherent reason why it should not work in the heart. However, there are no reports on the geometric transfer matrix behavior on a dynamic image set in which the target has intrinsic as well as respiratory motion. Could this method have led to overcorrection in both the remote and infarct regions that would have the effect of overcorrecting the time-activity curves? The geometric transfer matrix is a different method than one used in the original articles by Delforge et al and might account for the observation of a higher $B_{\text{max}}$ in the normal subjects in the current manuscript compared with the earlier values. The authors correctly note that the different methods may account in part for the higher normal values but do not address any potential for regional differences.

As noted earlier, the 2-injection protocol used for this study has been shown to have comparable estimates of $B_{\text{max}}$ relative to direct tissue sample, as does the more complex 3-injection protocol. However, for the 2-injection protocol, the other model parameter values have much poorer estimation (wider confidence intervals) compared with the 3-injection protocol. This raises the question of covariance between parameter estimates that are not addressed in the original model descriptions. What is the uniqueness of the individual parameter estimates? Is it possible the reported results are secondary to nonuniqueness?

Can $B_{\text{max}}$ in infarcted regions be normal? MRI delayed contrast enhancement (DCE) is clearly the gold standard for noninvasive determination of MI size/severity. However, the MRI analysis used by Mazzadi et al was validated against perfusion images using $^{99m}$Tc-sestamibi and single-photon emission computed tomography. One can hypothesize that it might underestimate true infarct size/severity, accounting for the patient with no scar by MRI. Normal muscarinic receptor density is unlikely to be the case for those with very large areas of scar, but there was limited scar size in this study.
population, so it cannot be determined. It seems unlikely that the timing of the PET and MRI studies relative to the MI event is responsible. The mean of 43 days between event and imaging should be sufficient to allow for the known 3-fold decrease in DCE that occurs over 8 weeks after an experimental MI. Thus, one would anticipate that the amount of DCE truly reflected the final amount of infarction. As suggested by the authors, heterogeneous areas of DCE mixed with neuronal damage within the damaged regions could account for the measured “normal” $B_{\text{max}}$ as could the possibility that the “normal” receptors are in the peri-infarct region. Which scenario is true will require further studies.

It is well known that sympathetic neurons are more susceptible to ischemia than are the myocytes, and imaging extent of deinnervation is greater than that of malperfusion or ultimate scar. This peri-infarct zone of sympathetic deinnervation undoubtedly leads to a response of the parasympathetic system. When subsequent studies to better understand the parasympathetic are done, it is imperative that there are companion studies of the sympathetic system.

Finally, infarcted myocardium is not simply scar, particularly in the reperfused myocardium. There are endothelial cells and fibroblasts in addition to islands of myocytes. Endothelial cells can have M₁ and M₃ receptors. It would seem likely that the same is true for fibroblasts, which are known to have accompanying sympathetic nerve terminals. Is the MQNB activity in the region of delayed contrast enhancement a function of non-M₂ receptors on other cell types, since MQNB is not 100% specific for the M₂ receptors?

In summary, this report emphasizes the importance at looking at the heart on a regional basis when trying to understand the effects of ischemia and infarction. Although PET is not alone among imaging methods in its ability to evaluate regional abnormalities, it is unique in its ability to evaluate receptor pathophysiology. If the described increase of muscarinic receptor density in regions of the heart remote from the apparent damage are confirmed in subsequent investigations and in larger patients cohorts, we will be compelled to elucidate the mechanism(s) and the potential relationship to sudden death. It seems unlikely that quantification of muscarinic receptor density alone will be sufficient for these tasks. Combined with imaging the presynaptic norepinephrine kinetics and/or the postsynaptic $\beta$-receptors, we may potentially use these tools in the management of patients at risk for sudden death.

**Disclosures**

None.

**References**


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