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EDITORIAL

PET Imaging of Carbon-11-S-Adenosylhomocysteine: A Measure of Myocardial Energy Balance?

In the past decade, a variety of positron-emitting tracers have been developed, making it possible to study myocardial flow and metabolism noninvasively. The application of these tracers in man has expanded our understanding of the pathophysiological processes associated with ischemic heart disease. Myocardial blood flow can be assessed in relative and absolute terms (*1–3*), and substrate utilization can be studied (*4*). During ischemia, acetate utilization diminishes in parallel with oxidative metabolism (*5*), the oxidation of palmitate diminishes (*6*), and the uptake of glucose is enhanced relative to blood flow (*7*). Other techniques permit the assessment of cardiac receptors (*8*) and tissue oxygenation (*9,10*). In the current issue of the *Journal*, Deussen and colleagues demonstrate the feasibility of an important and novel strategy for probing cardiac metabolism, namely the detection of increased cytosolic adenosine (*11*).

An abundant literature has shown that an imbalance between myocardial oxygen supply and utilization causes a net degradation of adenine nucleotides leading to increased cytosolic adenosine. Increased cytosolic adenosine cannot be measured directly because of intracellular compartmentalization (*12*). The administration of excess homocysteine causes the formation of s-adenosylhomocysteine (SAH) from adenosine, via the enzyme SAH hydrolase, at a rate that reflects the intracellular adenosine concentration. Therefore, measurement of SAH during the administra-

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tion of excess homocysteine should provide a sensitive marker of impaired myocardial energy balance.

Figure 1 shows the relationship between SAH and high energy phosphate metabolism. The SAH reaction, mediated by SAH hydrolase, an enzyme present in cardiomyocytes (*13*), is the last step in cellular transmethylation reactions. Although the equilibrium constant for the reaction (1 μM) greatly favors SAH synthesis, the net SAH reaction runs in the hydrolytic direction (to the left) under normal in vivo conditions. However, the net SAH reaction is readily reversed by excess homocysteine, and SAH is formed at a rate dependent on the cytosolic concentration of adenosine (*14,15*). Homocysteine is usually administered as homocysteine thiolactone (HCTL) which is readily available intracellularly due to its high membrane permeability (*16*) and is rapidly hydrolyzed to homocysteine by esterase enzymes (*17*). Because SAH is not metabolized by other enzymes and has a low membrane permeability (*18*), SAH accumulates locally in the presence of excess homocysteine.

The SAH technique takes advantage of the sensitivity of myocardial adenosine production to reductions in tissue oxygenation (*19–21*). If mitochondrial ATP synthesis is limited by oxygen delivery, the resulting net ATP hydrolysis increases cytosolic concentrations of ADP and AMP, causing increased adenosine production via the hydrolysis of AMP by 5′-nucleotidase (*22*). Because free cytosolic concentrations of ATP are normally 100,000-fold higher than those of adenosine, the net hydrolysis of only small amounts of ATP causes large increases in adenosine. SAH, by integrating the adenosine signal, increases the sensitivity for ischemia.

In the current study (*11*), Deussen and colleagues administered racemic ^{11}C-homocysteine thiolactone (HCTL) supplemented with "cold" HCTL to open chest dogs following the onset of regional ischemia. Ischemia was produced by tightening an occluder around the coronary artery until diastolic coronary perfusion pressure was reduced to 20 mmHg. In one additional animal, a complete coronary occlusion was employed. In three of the five animals with partial coronary stenoses, PET images obtained between 5 and 60 min following the administration of ^{11}C-HCTL showed enhanced tracer activity in areas of the myocardium supplied by the stenosed artery. In two of the five, a regional accumulation of tracer did not occur, presumably because a lower dose of unlabeled HCTL (10 mg/kg versus 30 mg/kg) was given and the resultant total plasma homocysteine levels were insufficient to reverse the SAH hydrolase reaction. In a parallel study using ^{35}S-HCTL and HPLC analysis of tissue biopsies, it was confirmed that the increased radiotracer activity in the ischemic zone was due to the accumulation of labeled SAH. Thus, during ischemia, the administration of excess homocysteine in the form of ^{11}C-HCTL causes the formation of ^{11}C-SAH from adenosine, and the enhanced accumulation of ^{11}C activity in the ischemic region can be detected using PET.

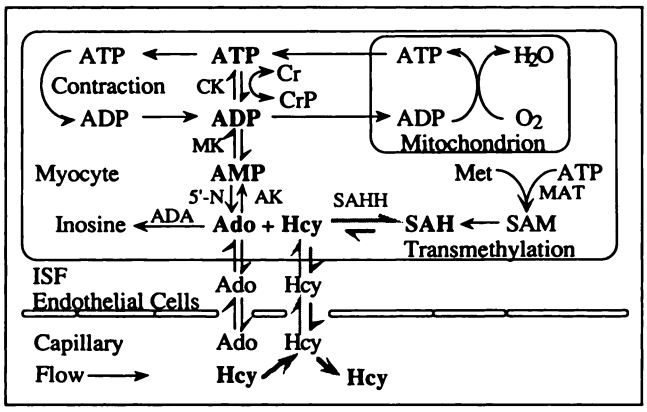

FIGURE 1. The SAH method for assessing myocardial energy balance is schematically shown. ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine monophosphate; Cr = creatine; CrP = creatine phosphate; CK = creatine kinase; MK = myokinase; 5′-N = 5′-nucleotidase; AK = adenosine kinase; ADA = adenosine deaminase; Ado = adenosine; Hcy = homocysteine; SAHH = SAH hydrolase; Met = methionine; SAM = s-adenosyl methionine.

While quite encouraging, these initial studies leave a number of important questions unanswered regarding the possible application of this technique to study regional myocardial energy balance in man. One question relates to the sensitivity of this technique. Coronary perfusion pressures distal to the stenosis were only moderately reduced (60 systolic, 20 diastolic) suggesting that the technique may be quite sensitive. Unfortunately, no information about the effects of the stenosis on myocardial blood flow, mechanical function or other metabolic parameters is given. The authors do state that global cardiac function was preserved, but did not measure regional function or state how global function was evaluated. Compensatory hyperkinesis of nonischemic areas might have prevented hemodynamic deterioration even if the regional ischemia was quite severe. It would also be important to know whether increased SAH formation was restricted to small areas of severely ischemic subendocardial areas and whether such areas showed histological evidence of irreversible injury at the end of 60 min. In the two studies which failed to show increases in SAH, additional metabolic or flow measurements showing ischemia would make it possible to conclude with more certainty that the dose of cold HCTL was insufficient.

The maximum ratio of total tracer activity in ischemic myocardium to

that in blood between 5 and 60 min (about 1.4), while sufficient to produce an image "hot spot" in these open chest studies, is not high. A concern is that adequate target-to-background ratios may be more difficult to obtain when imaging is performed under less ideal circumstances or if the severity of ischemia is less than in the current study. Fortunately, it is likely that the method can be substantially optimized in several ways to provide better signal to noise. First, the PET-SAH method is based on the accumulation of ^{11}C activity into an expanded cellular SAH pool in ischemic myocardium. Expansion of the SAH poor size requires administration of at least 0.01 mmol/kg HCTL. In the current study, the plasma homocysteine concentration was enhanced by the addition of unlabeled HCTL to the injectate to yield a final dose of 30 mg/kg (four studies) or 10 mg/kg (two studies). It is noteworthy that dilution of the ^{11}C label represents a departure from usual practice in nuclear medicine, where tracers of the highest possible specific activity are desired. However, if the enzyme Km for homocysteine (Km = 150 μM) is greatly exceeded, unlabeled HCTL will competitively inhibit the incorporation of ^{11}C-HCTL into the SAH pool because of enzyme saturation. It is possible that better labeling of the SAH pool can be achieved by using a dose of unlabeled HCTL which is different from those used in the current study. Secondly, as mentioned by the authors, the use of nonracemic ^{11}C-HCTL would substantially decrease the proportion of unreacted tracer molecules. Finally, the use of a continuous infusion of ^{11}C-HCTL rather than the bolus injection used by Deussen et al. would keep the plasma HCTL concentration more constant and possibly provide for better incorporation of the label into the SAH pool. Much further work is needed to optimize the dosing strategies for this tracer.

In the Deussen study (*11*), visual analysis of the images and semiquantitative analysis of the time-activity data were used to identify areas of ischemia and increased SAH formation. However, the application of an appropriate tracer kinetic model should allow the PET-SAH method to be used to a fuller capability to assess regional energy balance, or tissue oxygenation. The model will need to account for the effects of flow, the enzymes adenosine deaminase, adenosine kinase and SAH hydrolase, transport of adenosine and homocysteine between tissue regions, and tissue production of adenosine. In theory, the use of an appropriate mathematical model (*23*) would make it possible to estimate the cytosolic concentration of adenosine in underperfused myocardium by fitting the tissue time activity curve, using the blood-pool, time-activity curve as the model input function and by knowing the tracer specific activity and coronary flow. In the current study, images were obtained for 60 min, but there are clear differences in the shapes of the time-activity curves for ischemic and nonischemic myocardium during the first 20 min. The application of an appropriate kinetic model might dramatically increase the sensitivity of the technique while allowing for shorter imaging protocols which make full use of the early portions of the time-activity curve.

Deussen and colleagues are to be congratulated for pioneering what appears to be a most promising new PET technique. In addition to a possible role in regulating coronary blood flow, there is an increasing recognition that adenosine has other important physiological functions, including antiadrenergic effects (*24,25*), inhibition of electrical excitability (*26*), cardioprotective effects during ischemia and reperfusion (*27,28*) and as a mediator of preconditioning (*29*). Thus, there is considerable interest in adenosine kinetics during ischemia and the SAH technique may provide an index of free cytosolic adenosine that can be detected noninvasively using PET.

In addition to the noninvasive study of adenosine, the PET-SAH method also has the potential to be applied clinically as a unique diagnostic tool. The kinetics of the PET-SAH technique suggest its potential diagnostic application with acute or provoked ischemia. In this regard it is interesting to speculate about the use of this technique with dipyridamole, the primary mechanism of which appears to be the blockade of cell membrane adenosine transport. Because the PET application of ^{11}C-HCTL is designed to signal cytosolic adenosine concentrations, there are two reasons to expect dipyridamole to enhance the PET imaging of regional ischemia. First, redistribution of flow away from the ischemic region due to the vasodilating effects of dipyridamole should transiently worsen energy balance, leading to further increases in cellular adenosine production. The finding that dipyridamole provokes overt signs of ischemia in 20% of patients supports this reasoning. Second, inhibition of membrane transport of adenosine by dipyridamole should block its cellular efflux, causing additional increases in cytosolic adenosine concentration in the ischemic region. In nonischemic areas, intracellular adenosine levels should remain normal despite elevated plasma adenosine concentrations because dipyridamole blocks the cellular uptake of adenosine. A potential advantage of this technique over methods which primarily measure blood flow is that the SAH method should be sensitive to the balance between flow and metabolism. Possible clinical uses include the detection of ischemic but viable myocardium, the determination of the physiological significance of a coronary stenosis, and the study of regional myocardial energetics. In conclusion, the preliminary studies reported by Deussen et al. (*11*) warrant cautious optimism. Much further work is needed to assess the potential of this technique in its application to the study of regional myocardial energy balance in man.

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