Fueling the Heart

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A
n invitation from Eduardo Marbán, on the occasion of the 50th anniversary of Circulation Research, provides an opportunity to reflect on my understanding of the most important advance in regulation of cardiac metabolism. By the early 1950s, the major findings were the observation by Locke and Rosenheim in 1907 that glucose is used as a fuel by the isolated perfused heart and the observation by Bing and coworkers in 1954 that normally beating human hearts prefer fatty acid to any other substrate for oxidation. Bing’s in situ experiments involved measurements of the myocardial extraction of various substrates and oxygen and of coronary flow. Although there is no substitute for studying metabolism of the heart working in a normal environment, these studies do not allow for the exploration of individual enzymatic reactions.

The most important advance in understanding the regulation of cardiac metabolism was the discovery, in the post–World War II era, of the pathways of glycolysis and fatty acid metabolism including the role of membrane transport in glucose uptake. Although heart muscle slices and homogenates were used for studies of glycolysis, some or all of the cardiac muscle cells were disrupted and prevented rigorous studies of membrane permeability.

Regulation of Glucose Transport
I began my research career in 1953 to test the hypothesis that the effect of insulin on glycolysis in muscle is due to acceleration of glucose transport into the cell. After completing a medical school at Johns Hopkins and a residency in Obstetrics and Gynecology at Vanderbilt, I joined the laboratory of Charles R. Park, Professor of Physiology at Vanderbilt. My long-term goal was to define the mechanisms of placental transport of nutrients. Dr Park had demonstrated that insulin accelerated the uptake of glucose and nonmetabolized sugars in the isolated rat diaphragm, a preparation that was not ideal for transport studies because all of the muscle cells were cut. As a result, I began modifications of the method to perfuse the isolated rat heart as a Langendorff preparation. All of the cells are intact and the perfusate is delivered via the normal vascular bed. The goals were to maintain a stable heart rate and coronary flow over 3 hours of perfusion and to reduce the volume of bicarbonate buffer recirculated through the heart so that uptake of glucose over time could be measured. The studies confirmed the initial hypothesis that insulin accelerates transport of glucose and nonmetabolized sugars into the cell and that transport acceleration is a major factor in the hormone’s effect on glycolysis.

After 2 years of required service in the Army, as Assistant Chief of Obstetrics and Gynecology at Fort Campbell, Ky, in 1955 to 1957, my studies of heart metabolism were extended by exploring the hypothesis that anoxia, another potent stimulator of glycolysis in heart, accelerates transport of glucose and nonmetabolized sugars. Perfusion with oxygen-deficient buffer markedly increased the rate of glycolysis and entry of a nonmetabolized sugar into the heart cells. However, the effects of insulin and anoxia on intracellular glucose phosphorylation were quite different. Addition of insulin resulted in accumulation of intracellular free glucose, while anoxia resulted in a fast rate of anaerobic glycolysis but much lower levels of intracellular free glucose. These findings confirmed the hypothesis that anoxia accelerates both glucose transport and phosphorylation. In 1959, I decided to remain as a full-time physiologist/biochemist and to cease clinical activities.

Regulation of Glycolysis
Acceleration of glucose phosphorylation in anoxic hearts indicates that the activity of a glycolytic enzyme is increased. Identification of important steps in a metabolic pathway is possible if the equilibrium position is known. Near-equilibrium reactions have been defined by Newsholme and Crabtree as those reactions in which the “rate of the forward catalytic process is similar to the rate of the reverse catalytic process.” “Both rates must be considerably greater than the flux.” Nonequilibrium reactions are those “reactions in which the rate of the reverse component is very small compared with the forward component.” Substrate concentrations are well above the $K_{m}$ and product concentrations have little effect on net flux through a nonequilibrium reaction. Nonequilibrium reactions are potentially regulated by means other than substrate or product concentrations. Measurements of the substrate and product concentrations of the glycolytic enzymes allow for calculation of mass action ratios. The potential compartmentation of the substrates and products must be considered in these calculations. Comparison of the mass action ratio with the equilibrium constant of the purified enzyme indicates that the reaction is nonequilibrium if the two values are not similar and near equilibrium if the values are approximately equal.

Regulatory/flux-generating reactions can also be identified by measurements of flux through the pathway together with steady-state contents of intermediates. The steady-state rate of glycolysis is increased 10- to 20-fold to a new steady-state rate by the induction of anoxia. The steady-state contents of intracellular free glucose, glucose-6-P and fructose-6-P de-
crease in anoxic hearts, while the product of the phosphofructokinase reaction, fructose-1,6-P increases. These findings indicate that the activity of phosphofructokinase is increased in anoxic hearts. Chance and Williams first proposed this principle and applied it to identification of regulatory reactions in the oxidative phosphorylation pathway. This principle is known as the “crossover” theorem. Phosphofructokinase also is a nonequilibrium reaction, as judged by the comparison of the mass action ratio and the equilibrium constant of phosphofructokinase.

Enzyme activity reflects the amount of enzyme present and the control of enzyme activity by covalent modification and allosteric interactions. This type of regulation as opposed to enzyme synthesis provides for rapid changes in catalytic activity because regulators interact directly with existing enzyme. Sites on the enzyme that can be regulated include the substrate site, active or catalytic site, and the regulatory or allosteric site. Allosteric regulators include cofactors and substrates from other reactions in the pathway. As discussed above, in isolated hearts perfused with glucose-containing buffer under anoxic conditions, glycolytic flux is faster. Several mechanisms contribute to this change, including an increase in the activity of phosphofructokinase that results from higher concentrations of allosteric activators such as ATP, AMP, and inorganic phosphate and decreased concentrations of the allosteric inhibitor ATP.

**Regulation of Glycogenolysis**

Induction of anoxia in the perfused heart not only accelerates glucose transport and glucose phosphorylation but also leads to a rapid rate of glycogen breakdown. Glycogenolysis is catalyzed by glycogen phosphorylase, which occurs in two forms, an active a-form and an AMP-dependent b-form. Phosphorylase kinase, when phosphorylated by cAMP-dependent protein kinase, phosphorylates the b-form and converts it to the fully active a-form.

When phosphorylase activity was measured in extracts of hearts perfused under anoxic conditions, no increase in phosphorylase-a could be detected. This finding led to the hypothesis that phosphorylase-b activity is increased in anoxic hearts by changes in the intracellular concentrations of allosteric activators and inhibitors and in a substrate, inorganic phosphate. Purified phosphorylase-b was assayed in the presence of the allosteric activator AMP, and potential allosteric inhibitors were sought. Glucose-6-P and ATP were found to inhibit the AMP-dependent activity of phosphorylase-b. In anoxic hearts, concentrations of the allosteric inhibitors, glucose-6-P and ATP, fall, whereas concentrations of the allosteric activator, AMP, and the substrate, inorganic phosphate, increase suggesting that activated phosphorylase-b catalyzes the rapid breakdown of glycogen.

The studies discussed thus far using the isolated rat heart perfused by the Langendorff technique demonstrate the activation of glucose transport by insulin and a shift in the control of glycolysis to glucose phosphorylation. Anoxia also increases glucose transport into the cell, but in addition phosphorylation of the sugar is accelerated. Activation of phosphofructokinase and phosphorylase-b by higher tissue contents of allosteric activators and substrates AMP, ADP, and inorganic phosphate and reduced contents of allosteric inhibitors ATP and glucose-6-P result in rapid glycolysis and glycogen breakdown.

**Heart Work, Ischemia, and Glycolytic Regulation**

In order to study the effects of cardiac work on substrate utilization, Dr James Neely and I developed a working heart preparation in which the left atrium and aorta are cannulated and heart work is elevated by increasing left atrial filling pressure or outflow resistance. Increased heart work and intraventricular pressure development increase the consumption of oxygen and glucose. The ability to correlate changes in ventricular function with metabolic events during exposure to drugs, hormones, or anoxia is a major advantage of the working heart preparation. Since coronary flow is linearly related to ventricular pressure development, as in the Langendorff preparation, these preparations are not well-suited for studies of the effect of ischemia on ventricular function and metabolism. By modifying the working heart model to maintain left atrial inflow while preventing coronary flow during diastole by insertion of a ball valve in the outflow tract immediately above the remnant of aorta, ejection of perfusate and development of intraventricular pressure during systole were maintained initially. Aortic diastolic pressure was reduced from 60 to 20 mm Hg and coronary flow fell by 60%. Ventricular failure began after 4 to 6 minutes and progressively worsened and became irreversible after about 30 minutes. If coronary perfusion pressure was maintained at 25 mm Hg, reduced, reasonably stable ventricular function was maintained for at least 1 hour.

An increase in ventricular pressure development accelerates the uptake and oxidation of glucose from the perfusate. More rapid rates of glucose transport and phosphorylation account for the faster glucose uptake. Transport of glucose and nonmetabolized sugars is accelerated by higher levels of intracellular free glucose do not accumulate, indicating that glucose phosphorylation accelerates along with glucose transport. A 4-fold increase in glycolytic flux is accompanied by an increase in fructose-1,6-P but not in glucose-6-P, indicating that phosphofructokinase is accelerated. Since depletion of ATP and accumulation of ADP, AMP, and inorganic phosphate do not occur in working hearts, the factors accounting for activation are undefined. In other experiments in which both palmitate and glucose were provided to hearts developing higher levels of ventricular pressure, fatty acid was used in preference to glucose. Preferential utilization of fatty acid involves inhibition of phosphofructokinase and glucose transport.

In contrast to a sustained increase in glycolysis in anoxic hearts, induction of ischemia results in a transient increase in glycolysis followed by inhibition. Inhibition of glycolysis occurs even though the intracellular levels of ADP, AMP, and inorganic phosphate are increased. In ischemic hearts, lactate accumulation is much more marked than in anoxic hearts because lactate washout is slow, NADH accumulates, and glycolysis appears to be controlled by removal of the reduced product of the pathway.
Current Challenges

Studies of cardiac metabolism in the perfused heart involve invasive techniques and the attempt to simulate in vitro the conditions to which the heart is exposed in vivo. Noninvasive methods for studying intermediary metabolism in humans and intact animals will allow for assessment of the mechanisms proposed as the result of invasive studies. NMR can be used to measure metabolite concentrations and unidirectional flux rates. NMR is insensitive and metabolite concentrations must be 0.5 to 1.0 mmol/L in order to be detected. The metabolite must contain one of the more sensitive nuclides ($^1$H, $^{31}$P, $^{23}$Na, $^{19}$F, $^{13}$C). PET scans record, in three dimensions, the distribution in organs of radioactive isotopes that emit positrons. By labeling physiological substrates with isotopes of naturally occurring substances such as $^{15}$O, $^{13}$N, $^{18}$F or $^{13}$C, important information concerning regional cardiac metabolism can be gained.

Reference


Key Words: cardiac metabolism  myocardial work  myocardial ischemia  insulin  anoxia