Environmental Health Perspectives
Vol. 26, pp. 169–174, 1978

Myocardial Metabolism for the Toxicologist

by Robert G. Merin*

Drug effects on myocardial contractile function are obviously of considerable practical importance for the toxicologist. The basic mechanism of such actions must reside at some point in the metabolism of cardiac muscle. Interference in the liberation of energy from the fuels that the heart uses may be implicated. It is possible that drugs may interfere with the storage (conservation) of that energy as the high energy phosphates (ATP and CP). Finally, the utilization of that stored energy by the contractile proteins themselves may be altered. The latter process is highly dependent on intracellular calcium ion kinetics. Anesthetic drugs, which produce reversible depression of myocardial contractile function is a dose-dependent fashion, have been shown to interfere to some extent with all three processes. However, the most important mechanism probably involves utilization of energy and intracellular calcium ion movement. A basic knowledge of the biochemistry of cardiac muscle is necessary for the understanding of drug action and toxicity at the subcellular level.

My basic research interest in the past 10 years has centered on the effects of anesthetic drugs on cardiac metabolism (*1–5*). I have been trying to correlate the depressant effects of anesthetics on ventricular function with their effect on cardiac metabolism, coronary blood flow, and oxygenation. Although I did not consider myself a toxicologist heretofore, I suppose in a sense I have been studying a "toxic" effect of anesthetics on the heart. However, these negative inotropic effects are almost always reversible, and if not carried to extreme, apparently leave no permanent sequellae. At least this is true of the functional cardiac depression. In patients with ischemic heart disease, however, it is possible that a temporary decrease in myocardial perfusion may result in sufficient decrease in oxygen delivery to produce permanent damage in the form of myocardial infarction. Certainly these patients are at great risk for myocardial infarction after anesthesia and surgery (*6, 7*). In addition to anesthetic effects on ventricular function and myocardial perfusion and oxygenation, disturbances in heart rate and rhythm can also result in "cardiac toxicity." Inasmuch as the latter has not been one of my major interests, I will not discuss this further, except to indicate that a number of drug

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Table 1. Cardiac toxicity: rate and rhythm.

Drugs	Mechanism	Duration
Anesthetics	Overdose	Usually transient
Cardioactive	Overdose	Usually transient
Cations (K^+, Li^+, Ca^{2+})	Overdose	Usually transient
ANS drugs	Overdose	Usually transient
Others (tricyclics, phenothiazines)	Overdose + ANS effect	Usually transient

Table 2. Cardiac toxicity: ventricular function.

Drugs	Mechanism	Duration
Anesthetics	Overdose	Usually transient
Antiarrhythmics	Overdose	Usually transient
ANS drugs	Overdose	Usually transient
Anticancer (Adriamycin)	Cardiomyopathy	Permanent
Methysergide	Fibrosis + VHD	Often permanent

Table 3. Cardiac toxicity: perfusion and oxygenation.

Drugs	Mechanism	Duration
Anesthetics	O_2 supply/demand	Often permanent
ANS drugs	O_2 supply/demand	Often permanent
Antihypertensives	O_2 supply/demand	Often permanent
Oral contraceptives	? clotting abnormality	Permanent

types can produce such toxicity (Table 1) (*8, 9*). Likewise, several drug classes have been associated with "toxic" effects on ventricular function (Table 2) and perfusion and oxygenation (Table 3) (*8, 9*).

Olson has divided the phases of cardiac energy metabolism into three stages: (1) the liberation of energy from the fuels the heart uses; (2) conservation of that energy as the high energy phosphates, adenosine triphosphate (ATP), and creatine phosphate (CP); and (3) utilization of the energy by the contractile proteins of cardiac muscle (Fig. 1) (*10, 11*). If a drug or chemical poisons cardiac contractile function, the subcellular mechanism must lie somewhere within this inclusive schema.

Liberation of Energy

The heart is an aerobic organ. This is a consequence of the efficiency of the oxidative pathways of energy liberation and conservation as compared with the only major anaerobic mechanism in heart muscle, glycolysis. For example, 1 mole of glucose liberates 2 moles of ATP through anaerobic glycolysis, while the same mole of glucose can produce 36 moles of ATP through oxidative pathways (*12*). Obviously then, the continual high energy demands of the heart are best satisfied by the latter and hence, oxygen is essential for adequate cardiac function.

Heart muscle can liberate energy from numerous fuel sources (Fig. 1). Free fatty acids, ketone bodies, triglycerides, lactate, pyruvate, and glucose can all be extracted from the blood by the heart if the arterial concentrations are high enough. The heart also stores and utilizes both lipids in the form of triglycerides and carbohydrate as glycogen, although not nearly as extensively as skeletal muscle (*13*).

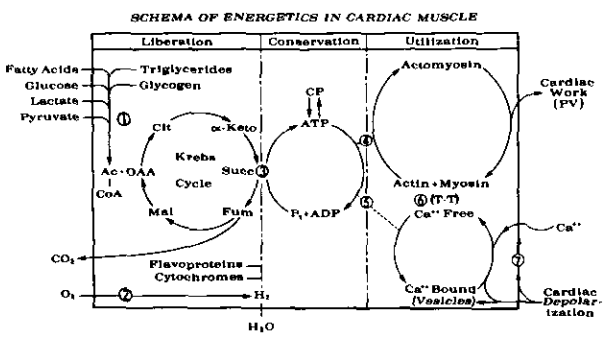

FIGURE 1. Schema of cardiac energetics. Cit = citrate; a-Keto = α-ketoglutarate; Succ = succinate; Fum = Fumarate; Mal = malate; AcCoA = acetyl Co A; OAA = oxalacetate; CP = creatine phosphate; ATP = adenine triphosphate; ADP = adenosine diphosphate. From Olsen et al. (*11*).

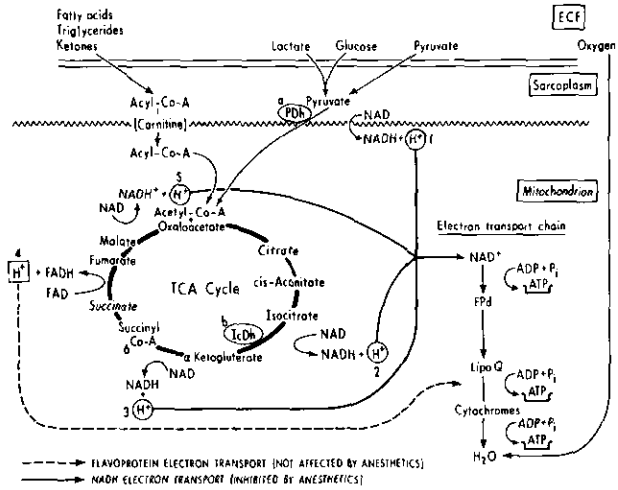

FIGURE 2. Schema of oxidative metabolism in cardiac muscle: (1-5) sites of hydrogen ion release for electron transport and oxidative phosphorylation; (6) Site of "direct" production of ATP; (a+ b) rate-limiting steps in TCA cycle. NAD = nicotine adenine dinucleotide; FAD = flavin adenine dinucleotide; TCA = tricarboxylic acid; ATP = adenosine triphosphate; ADP = adenosine diphosphate; P_i = inorganic phosphate; PDh = pyruvate dehydrogenase; IcDh = isocitric dehydrogenase; FPd = flavoprotein; ECF = extracellular fluid; H+ = hydrogen ion.

Oxidative Metabolism

As mentioned before, all of these fuel sources liberate the majority of their energy through oxidative metabolism. Glucose and lactate are first converted to pyruvate; the former through anaerobic glycolysis, and the latter by oxidation, with nicotine adenine dinucleotide (NAD) accepting the hydrogen ion (Fig. 3). Thus this step for lactate is dependent on oxygen for a continuing supply of NAD. The next step is the formation of the two-carbon fragment, acetyl Co-A (Fig. 2). For both lipids and carbohydrates, the conversion takes place in the mitochondria. Pyruvate is converted to acetyl Co-A by the action of the enzyme complex known as pyruvate dehydrogenase in the outer mitochondrial membrane (*14*). The fatty acids are activated with Co-enzyme A to acyl Co-A and transported across the mitochondrial membrane by the 7-carbon organic acid, carnitine (Fig. 2). Acyl-Co-A is then beta-oxidized, catalyzed by an enzyme complex in a manner analogous to pyruvate dehydrogenase, to the same acetyl Co-A, which then combines with oxaloacetate to begin the tricarboxcylic acid (TCA) cycle. The actual liberation of energy and conservation as ATP occurs at six points in the TCA cycle; direct phosphorylation of succinyl-Co-A is responsible for 1 mole of ATP; 3 moles NADH and 1 mole $FADH_2$ are produced in the TCA cycle, and 1 mole

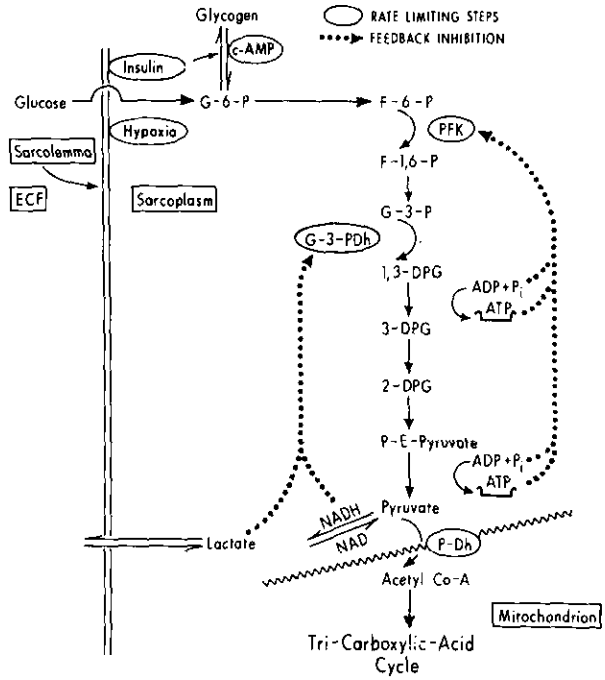

FIGURE 3. Schema of glycolysis: c-AMP = cyclic 3-5-adenosine monophosphate; G-6-P = glucose 6-phosphate; F-6-P = fructose 6-phosphate; PFK = phosphofructokinase; F-1,6-P = fructose 1,6-diphosphate; G-3-P = glycerol 3-phosphate; G3-PDh = glycerol 3-phosphodehydrogenase; 1-3-DPG = 1,3-diphosphoglycerate; ADP = adenosine diphosphate; P_i = inorganic phosphate; 3-DPG = diphosphoglycerate; 2-DPG = 2-diphosphoglycerate; P-E-pyruvate = phosphoenol pyruvate; P-Dh = pyruvate dehydrogenase NAD = nicotine adenine dinucleotide; ECF = extra-cellular fluid.

NADH is generated by the oxidation of pyruvate to acetyl Co-A (Fig. 2). These hydrogens then combine with oxygen along the electron transport chain, catalyzed by the various cytochrome and flavoprotein enzymes to form ATP and water. Several moles of ATP are produced by each of the oxidations through oxidative phosphorylation. The control of oxidative metabolism is governed primarily by the availability of substrate and oxygen (both for the final electron transport and for the oxidation of NADH to provide NAD for the continuing activity of the TCA cycle). In addition, the products of energy utilization, adenosine diphosphate (ADP), adenosine monophosphate (AMP), and inorganic phosphate (P_i) stimulate the rate-limiting enzymes in the TCA cycle, isocitric dehydrogenase (Fig. 2b) and pyruvate dehydrogenase (Fig. 2a). Finally, the electron transport chains themselves may be inhibited or uncoupled by various drugs and chemicals.

Glycolysis

The only mechanism by which heart muscle can liberate energy anaerobically is through the Embden-Meyerhof glycolytic pathway (Fig. 3). Either glycogen or glucose may be broken down to pyruvate, producing 4 moles of ATP per mole of glucose (or glucose-1-phosphate from glycogen) at a cost of 2 moles of ATP without molecular oxygen. The first rate-limiting step in this process is the membrane passage of glucose which is normally insulin-dependent (Fig. 3). However, hypoxia in the absence of insulin will also stimulate the membrane transport of glucose. The next rate-limiting step is the conversion of fructose-6-phosphate to fructose-1-6-diphosphate, catalyzed by the enzyme phosphofructokinase (PFK). This enzyme is responsive to the energy balance of the heart. Excess ATP and CP inhibits its activity, decreasing glycolysis, and rising levels of ADP, AMP and P_i stimulate the enzyme, increasing glycolytic production of energy. Cyclic AMP stimulates, and hydrogen ion inhibits PFK, providing influences outside the actual energy supply-demand relationship. As mentioned, the pyruvate-lactate balance is exquisitely sensitive to the oxygen supply, with hypoxia driving the reaction towards lactate production. Hypoxia actually stimulates the whole glycolytic pathway (membrane transport and PFK), producing increasing amounts of pyruvate. In addition, metabolic oxygen is necessary for the oxidized NAD concerned in the conversion of pyruvate to acetyl Co-A. Hypoxia markedly inhibits this conversion (in spite of the stimulation of pyruvate dehydrogenase by ADP, AMP, and P_i) and without metabolic oxygen, the electron transport chain and the TCA cycle grind to a halt, increasing acetyl Co-A levels and further inhibiting pyruvate oxidation. Consequently, the concentration of pyruvate builds, driving the pyruvate-lactate reaction towards lactate. The end result is lactate production by hypoxic heart muscle, whereas the well oxygenated heart efficiently uses lactate. Finally in the ischemic heart, however, inhibition of glycolysis occurs. This is a result of the build-up of hydrogen ion and NADH resulting in the inhibition of the enzyme glyceraldehyde-3-phosphate dehydrogenase (G-3-PDh) (*13*). Under normal circumstances, this step is not rate-limiting, but the mechanism appears to be important in the ischemic heart.

Glycogen and Triglycerides

As mentioned above, the heart stores fuel from glucose as glycogen and from fatty acids as triglycerides. The control of these processes is still not well delineated, but it appears that insulin directly stimulates the synthesis of glycogen and promotes esterification of fatty acids to triglycerides by sup-

pressing lipolysis. The best documented stimulus to glycogenolysis and lipolysis is cyclic 3-5 AMP produced primarily by beta adrenergic stimulation but perhaps by other mechanisms as well (glucagon and growth hormone). In general increased energy demand by the heart will stimulate oxidation of both glycogen and triglycerides. Unlike skeletal muscle, however, cardiac muscle stores relatively little fuel, uses it only in times of great stress, and rapidly depletes the stores, relying predominantly on exogenous fuel sources (*13*).

Conservation (Storage) of Energy

Heart (and skeletal) muscle obtain their energy for contraction through the hydrolysis of ATP, which is generated in the mitochondria as we have discussed, and appears to be utilized in the interaction of actin and myosin. The heart stores this energy both as ATP and CP, with the latter serving as a buffer store. CP is rapidly and easily converted to ATP by reaction with the ADP generated from ATP hydrolysis, stimulated by creatine-phosphokinase (CPK). A further mechanism of maintaining stable ATP level is through the formation of ATP directly from ADP catalyzed by the enzyme myokinase (*15*). Theoretically, interference with energy liberation and conservation should result in decreased tissue high energy phosphate levels, and block in energy utilization should lead to no change or an increase in CP and ATP concentrations. However, as indicated in the previous section, the control of myocardial energy supply is tightly linked to the levels of ATP, ADP, AMP, and P_i levels, as they exert the major controls on both glycolysis and oxidative metabolism. Hence, heart muscle attempts to maintain these levels constant even in face of influences to the contrary.

Utilization of Energy

The whole purpose of energy liberation and conservation is the interaction of the contractile proteins which are the basis for the contractile function of the heart. Although much is known about the anatomy and physiology of these subcellular components of the muscle cell, some of what I will discuss below is still supposition and hypothesis as concerns cardiac muscle.

The two basic contractile proteins are the "thin" actin filament and the "thick" myosin filament (Fig. 4). Myosin is composed of a thick stalk and globular heads which contain the ATPase and the actin-binding sites. Myosin ATPase is relatively inactive and, although sensitive to calcium, cannot develop the requisite activity to hydrolyze sufficient ATP for the energy needs of contracting muscle. Only when the cross-bridging (binding) to actin has occurred does the enzyme become sufficiently active for this purpose (when stimulated by calcium ion). The thin actin filament is composed of a double helix of actin globules, with a long thin tropomyosin protein located in the helical grove. At intervals along the tropomyosin corresponding roughly to the location of the projecting myosin heads, another protein, troponin, is bound to tropomyosin. This association is crucial to the control of contractile function. During rest (diastole in the heart), the troponin-tropomyosin complex inhibits actin-myosin crossbridging (Fig. 4). When the muscle cell membrane (sarcolemma) is activated by an action potential, the membranes barrier to calcium ion is temporarily removed, and the high extracellular calcium ion concentration provides a gradient for intracellular influx, probably triggering further release of an intracellular calcium ion store. Sarcoplasmic calcium ion level rises rapidly from less than $10^{-7}M$ (the level during relaxation) to somewhere between 10^{-6} and $10^{-5}M$. Calcium ion binds to the troponin–tropomyosin complex, releasing the inhibition of the myosin binding site, and crossbridging occurs, resulting in tension development and shortening of the muscle (Fig. 4). The rising calcium ion concentration also stimulates the actomyosin ATPase activity so that ATP hydrolysis may release the requisite energy for contraction. The source of this intracellular calcium ion is still controversial. It may be from the sarcoplasmic reticular (SR) membrane system as in skeletal muscle. However, SR is much less abundant and widely distributed in cardiac muscle, so it may be that calcium ion is released from sarcolemmal binding sites (*16, 17*). In order for the cardiac cycle to be completed, the crossbridging must be broken, and the muscle must relax. It seems likely that active sequestration of calcium ion is necessary to lower sarcoplasmic calcium ion concentration back to less than $10^{-7}M$ in the short time available. Both SR and mitochondrial membranes possess the requisite enzyme and transport systems for this function.

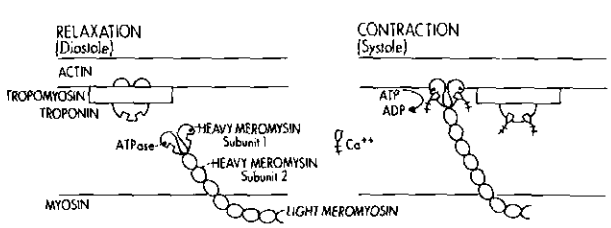

FIGURE 4. Schematic of contractile proteins. Ca^{2+} = calcium ion; ATP = adenosine tri-phosphate; ADP = adenosine diphosphate.

Again, however, the anatomy of cardiac muscle with the sparse SR and abundant sarcolemma in the form of the T-tubular system suggest that the calcium ion release and sequestration process may be predominantly a function of the sarcolemma.

Site of Action of Drugs

Calcium ion is central to this whole process (Fig. 5), releasing the inhibition of the troponin–tropomyosin mechanism so that actin–myosin crossbridging can take place, stimulating the actomyosin ATPase so that energy may be utilized for contraction, and possibly stimulating the reuptake of sarcoplasmic calcium ion which is necessary for relaxation and the recurrent cardiac cycle. Consequently, it is not surprising that some interference with calcium ion kinetics has been suggested as a locus of action of anesthetic drugs (*18*). Likewise, the calcium-sensitive ATPase as a crucial part of energy utilization may also be involved (*19*). Interference in energy liberation and supply is also possible, although the evidence seems less convincing at present (*18–22*). The most likely sites for drug or chemical action would be at the rate-limiting steps (Figs. 2 and 3), although anesthetics (*20*) and several known poisons (rotenone, cyanide) interfere with electron transport systems assistance or uncouple oxidative phosphorylation.

Myocardial Perfusion and Metabolism

As emphasized previously, the heart needs continuous oxygen supply in order to function efficiently. The components of this supply include adequate arterial oxygen content and sufficient arterial blood pressure to provide enough coronary

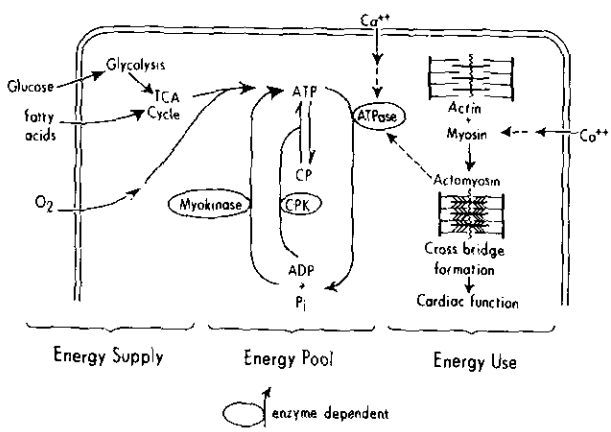

FIGURE 5. Cardiac metabolism schema.

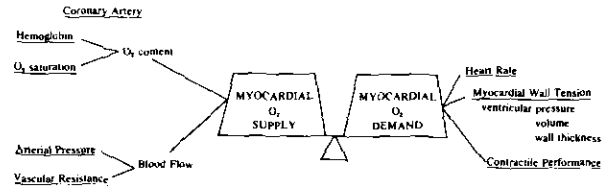

FIGURE 6. Myocardial oxygen supply and demand determinants.

blood flow against the coronary vascular resistance (Fig. 6). The crucial factor is the balance between myocardial oxygen supply and demand. If both are increased or decreased in concert, then there is no imbalance. This is the function of the autoregulation of coronary blood flow which is controlled predominantly by alterations in coronary vascular resistance. Increasing oxygen demand results in coronary vasodilation, probably through the activity of the breakdown products of ATP (adenosine or possibly AMP) (*23*). Decreasing demand means more ATP and coronary vasoconstriction (or at least reversal of vasodilation). In addition, one of the major causes of increased demand, sympathetic stimulation, may also produce beta adrenergically mediated coronary vasodilation, although this is a controversial subject (*24*).

Although decreased arterial oxygen content can cause myocardial tissue hypoxia, as long as blood viscosity is not too high (for instance, as a result of polycythemia from chronic hypoxia), amazingly low arterial oxygen contents can cause little functional impairment because of marked coronary vasodilation and increased coronary blood flow. The most common cause of myocardial tissue hypoxia is myocardial ischemia as found in ischemic heart disease as a result of coronary atherosclerosis. In this pathogenic situation, normal coronary autoregulation is ineffective because of the fixed coronary arterial obstruction. Hence decrease in arterial driving pressure, or increase in the determinants of oxygen demand (heart rate, myocardial wall tension, and contractile performance of the heart) (Fig. 6) can result in cardiac toxicity manifested as myocardial infarction. For investigational purposes, it is important to be able to detect such an imbalance before actual tissue death. As mentioned during the discussion of glycolysis, hypoxic heart muscle will produce lactate in contrast to well oxygenated tissue which extracts it. Other markers in coronary venous blood of tissue hypoxia include increasing concentrations of potassium, P_i, and the purine metabolites, inosine and hypoxanthine (*25*). Other methods of detecting cardiac tissue hypoxic injury include electrocardiographic ST segment analysis, nuclear scanning

techniques, and elevation of the mb-CPK isoenzyme (*26*). Thus far, the effect of anesthetics on myocardial oxygenation in both normal (*2, 5, 20, 27*) and ischemic (*28*) hearts appears to be related primarily to effects on oxygen demand. However, it is entirely possible that drugs and chemicals may specifically interfere with oxygen supply. Ergot derivatives (*29*) and vasopressin both can produce coronary vasoconstriction and uncouple the myocardial oxygen supply demand ratio. It is possible that substances may affect oxyhemoglobin association or dissociation thereby interfering with oxygen delivery. In order to uncover these effects, some method of quantitating myocardial tissue oxygenation must be used. Merely documentating the determinants of oxygen supply and demand is not sufficient.

Conclusion

Only by knowledge and review of the biochemistry and physiology of cardiac energetics and contractile function can the likely sites for the effect of toxic substances on this function be identified. This review, although necessarily superficial, is meant to stimulate interest. For actual projects, obviously considerably more resource material will be necessary. Hopefully, this is only the beginning.

This study was supported in part by Grant *HL13257* and Research Career Development Award 31752, National Heart, Lung and Blood Institute.

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