Lipid and Carbohydrate Metabolism of Myocardium During the Biphasic Inotropic Response to Epinephrine

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ABSTRACT

The metabolic response of the left ventricle to a 90 min regional infusion of 1-epinephrine (5 μg/min) was assessed in the intact anesthetized dog for 3 hr. The first hour was characterized by a rise in stroke output at constant filling pressure. Subsequently, contractility fell and there was release of K and PO₄ ions as well as SGOT from the myocardium that was attributed to tissue injury. The myocardial RQ rose for the first hour, presumably due to glycogenolysis, since carbohydrate extraction from blood was unaltered. Production of lactate by the myocardium during the RQ rise occurred without ischemia, since blood flow (Kr³¹ method) and O₂ uptake rose 10%. The proportionately greater increase in contractility was not consistent with "O₂ wasting" as a basis for the injury. After 1 hr the RQ fell to levels consistent with predominant lipid utilization. However, FFA extraction declined and the oxidation, extraction and incorporation of palmitate-L-C¹⁴ into tissue lipid were diminished during hormone exhibition. Plasma triglyceride uptake, insignificant in controls, was enhanced during the 3 hr without a rise in arterial concentration. Oxidation of this lipid was insufficient to prevent a threefold triglyceride increment in the myocardium. The authors discuss a possible relationship of these metabolic alterations to the pathophysiologic response induced by epinephrine.

ADDITIONAL KEY WORDS cardiac injury lipid transport triglyceride accumulation oxygen consumption myocardial ion transport free fatty acid anesthetized dogs

While the acute effects of L-epinephrine on myocardial function have been amply demonstrated,¹-² the influence of sustained infusions on left ventricular function have not been well characterized. It is known that evidence of tissue injury may appear after a suitably sized dose of epinephrine or other catecholamine is administered for a sustained period.³-⁴ However, the hemodynamic correlates of the morphologic alteration and their relation to the myocardial injury remain to be defined. Still more obscure are the metabolic processes which contribute to the enhanced contractile properties of the myocardium immediately after epinephrine. The relevance of the altered glycolytic reactions and substrate concentrations to the inotropic response are subject to considerable doubt.⁵,⁶ Recently, support for the postulated dependence of enhanced oxygen consumption on increased free fatty acid delivery to the myocardium has been advanced.⁷
This report is concerned with the temporal sequence of changes in left ventricular function in the intact animal during sustained intracoronary infusion of l-epinephrine. This mode of administration permits an analysis of the myocardial effects of this catecholamine without the large rise in oxygen uptake that occurs when cardiac rate and systemic arterial pressure are increased. Moreover, the relative effects on free fatty acid and triglyceride uptake assessed in this study are determined more readily when arterial concentrations of these substrates remain relatively constant.

**Methods**

Male mongrel dogs, 19 to 22 kg, were anesthetized 18 hours postprandial with morphine sulfate 3 mg/kg and pentobarbital (Nembutal 12 mg/kg), and studied without opening the chest. After insertion of an endotracheal tube, respiration was regulated with a Harvard respiration pump, facilitating the maintenance of arterial oxygen saturation and pH in the normal range. Catheters were placed in the coronary sinus, pulmonary artery, aorta and left ventricle for blood sampling and for pressure determinations, and in the left coronary artery for myocardial blood flow measurements. Although the catheters were filled initially with dilute heparin, slow saline infusions or intermittent flushes were used during the experiment to maintain patency of the catheters. Since arterial free fatty acids did not rise during the experiments, the earlier limited use of heparin did not appear to affect substrate concentrations.

Hemodynamic and metabolic studies were done before, during and after the intracoronary infusion of l-epinephrine bitartrate, 5 μg/min of base, for 90 min in water given at the rate of 0.1 ml/min. This provided about twice the minimal effective isotropic dose of epinephrine. When changes in heart rate or rhythm occurred, the 5 μg dosage was reduced for a few minutes and then resumed, with maintenance of the control rate. A control group was studied similarly, using saline for infusion. Minimal recirculation of the hormone occurred as judged by the lack of increments in heart rate, arterial pressure and arterial free fatty acid levels, presumably due to the large myocardial capacity for storage of catecholamines.

During the evaluation of cardiac performance in the intact animal in experiments lasting many hours, a direct measurement of contractility using the force-velocity relationship is not feasible but less direct methods may be employed to characterize myocardial function. Thus, contractility change has been deduced from the relation of stroke output to left ventricular end diastolic pressure (LVEDP).9-10

Ventricular and femoral arterial pressures were measured through 50 cm Codadale-Lubin 8F catheters connected directly to Statham strain gauge transducers P23Db. Photographic recordings were made from a multichannel oscilloscope recorder (Electronics for Medicine) at five-minute intervals. The frequency response of this recording system was linear from 0 to 30 cycles per second. The first derivative of the left ventricular pressure pulse (dp/dt) was computed continuously by an R-C differentiating circuit and converted into mm Hg/sec.11 The amplitude of dp/dt was a linear function of frequency to 70 cycles per second. Ventricular diastolic pressure was recorded at sufficient sensitivity so that 1 mm Hg equaled 5 mm on the tracing. All measurements were made at the end-expiration phase of the respiratory cycle. Cardiac output was measured by dye dilution, and the control values were not obtained until 2.5 to 3 hours after induction of anesthesia when further decline of cardiac output after pentobarbital is no longer observed.12

Simultaneous blood samples from the artery and coronary sinus were taken for determination of arteriovenous differences of substrates. In the interpretation of arteriovenous differences the requirements for a relatively constant coronary blood flow and arterial substrate concentration10, 13 were met after 15 minutes of epinephrine infusion. The blood was placed in chilled tubes containing EDTA and, after separation in a refrigerated centrifuge, the plasma was stored at -15°C prior to analysis. Samples were drawn at 25, 15 and 5 minutes before epinephrine infusion was begun, and at 15-minute intervals for three hours.

Substrate determinations were done in duplicate and included glucose,14 lactate,15 pyruvate,16 free fatty acid,17 triglyceride,18 and phospholipid.19 The triglyceride method was modified using a florisil column in place of silicic acid to exclude phospholipid more effectively from the chloroform eluate,20 which was verified by thin-layer chromatography. To obtain left ventricular tissue for lipid analysis, the heart was arrested with iced saline and a transmural sample (about 1 g) from the apex was carefully trimmed to remove pericoronary adipose tissue before placing the sample in liquid nitrogen. The tissue was homogenized in phosphate buffer and the lipid extracted as for plasma. Plasma samples without significant hemolysis were also analyzed for glutamic oxaloacetic transaminase21 and phosphate ion.22 Plasma potassium was analyzed on a Beck-
man B spectrophotometer with a flame attachment and the hematocrit was measured by the glass capillary method. Duplicate determinations of blood oxygen and carbon dioxide were performed by the method of Van Slyke and arterial pH was determined on a Beckman meter at 37°C. Donor animals were used for 35 ml blood replacements after each arterial-coronary sinus sampling. This procedure had no apparent effect on substrate extraction in the control observations when saline was infused, 0.1 ml/min, into the left coronary artery catheter.

To assess the changes in left ventricular blood flow, sequential flow measurements were made using krypton injected into the left coronary artery via a polyethylene tube within a Sones catheter. With the side-holes of the catheter in the left main coronary artery and the end-hole in the circumflex proximal to the atrial artery, angiographic dye readily filled both main branches. By infusing C14-labeled epinephrine it was found that isotope concentrations were similar in the respective tissue sites perfused by the two major branches. Also, the accumulation of triglyceride after hormone infusion (see Results) was the same in the area perfused by the anterior descending and circumflex branches, so that there appeared to be a relatively uniform distribution of the epinephrine infused into the Sones catheter. In six separate animals, using electromagnetic flow probes on the circumflex artery just distal to the atrial artery, placement of the catheter in this position showed no change from a mean control coronary flow of 38 ± 4 to 39 ± 5 ml/min. Control animals infused with saline in the coronary artery over a period of three hours showed no evidence of ischemia (see Results).

To clarify the apparent changes in myocardial extraction of plasma free fatty acid, 24 μc of isotopic palmitate with a specific activity of 7 to 10 mc/mmole were infused for 40 min at a constant rate after priming with 6 μc in seven control and seven epinephrine-treated animals. The palmitate-1-C14 used in this study was purified by preparative thin-layer chromatography (TLC) to 99+ per cent. The purified fraction, after methylation, was established as palmitic acid by gas liquid chromatography. Albumin-bound palmitic acid-1-C14 was prepared by dissolving the acid in ethyl alcohol, adding twice the molar equivalent of K2CO3 in saline and heating gently to remove the alcohol. After complexing with human albumin, the molar ratio of palmitate to albumin was 5.

Separation of lipid groups in the plasma and tissue lipid extracts was accomplished by TLC. Ascending thin-layer chromatography was done on activated silica gel impregnated with dichlorofluorescein, using a petroleum ether, ethyl ether, acetic acid (90:20:1) solvent. The C14 O2 content was determined in paired arterial-coronary sinus blood samples by modification of a previous method. All C14 radioactivity measurements were performed in a Nuclear-Chicago liquid scintillation counter at room temperature, using an external standard. Fifteen ml of 0.5% PPO and 0.03% POPOP in toluene were used as the phosphor.

Results

Myocardial Function

The changes in left ventricular function during the infusion of 5 μg/min of epinephrine in the left coronary artery for 90 min are shown in figure 1 (solid lines). A substantial rise of the left ventricular pressure dp/dt max was evident by 15 minutes, ac-

![Figure 1](http://circres.ahajournals.org/)

*PPO: 2,5-diphenyloxazole.
†POPOP: 2-p-phenylenebis-4 methyl-5 phenyloxazolone.
accompanied by a small decline in left ventricular end diastolic pressure, which is consistent with a direct inotropic action of epinephrine on the myocardium. There was an associated stroke output increment and shortened duration of ventricular systole from 162 ± 7 to 140 ± 6 msec. Subsequently, both stroke output and dp/dt declined and end diastolic pressure rose progressively. This was evident before the end of the epinephrine infusion and thereafter became more pronounced, so that by three hours the left ventricular end-diastolic pressure was twice the control value and was accompanied by a significantly diminished stroke output and dp/dt. No significant changes in arterial pressure or heart rate occurred during the infusion. These findings are indicative of depressed left ventricular function.

In the control group of eight animals, infusion of saline into the left coronary artery caused no significant changes in these parameters of left ventricular function. Biphasic changes in myocardial ion transport occurred after epinephrine (fig. 2). After an initial period of uptake of potassium and phosphate ions, with significant positive arterial-coronary sinus differences persisting through 30 min, loss of these ions from left ventricular muscle was evident. Significant negative arterial-coronary sinus differences were present by one hour and fifteen minutes, and these persisted throughout the period of observation without significant alteration of arterial concentrations. Concentrations of the transaminase enzyme were also increased in coronary sinus blood. The late rise of arterial transaminase levels probably resulted in underestimation of enzyme loss from the left ventricle. Between 75 and 180 min, ventricular ectopic beats were seen in six of eight hormone-treated animals at a frequency of 3 to 17/min, persisting for 7 to 44 min. The previously described ST-T changes after epinephrine were seen in all animals. Control infusions of saline produced no significant changes in the extraction of ions or enzyme nor in the epicardial electrocardiogram.

MYOCARDIAL METABOLISM

The general metabolic response of the left ventricle to the epinephrine infusion was indicated by a significant rise in the respiratory quotient between 30 and 45 min, presumably a reflection of the glycogenolytic properties of this hormone (fig. 3). The changes of respiratory quotient (RQ) were not accounted for by a significantly altered cumulative uptake of the carbohydrate substrates as measured by the product of coronary blood flow and arteriovenous differences (table 1). During the phase of rising RQ, there was a significant production of excess lactate by the left ventricle, but this reverted subsequently to control values. The peak change in lactate extraction at this time was reduced from \( +0.64 \pm 0.14 \) to \( 0.03 \pm 0.07 \) mmoles/liter. In three of the animals these values had become negative, so that actual lactate production appears to be the basis for the excess lactate changes. Lactate production was not related directly to a decrease in coronary blood flow (fig. 3), since a significant increase in flow occurred during the period of epinephrine infusion. The myocardial arterial-coronary sinus...
differences of oxygen decreased from 14.1 ± 0.92 to 12.9 ± 1.0 volumes per cent, and did not increase as would have been expected if the blood flow response had been inadequate during the positive inotropic response to epinephrine.

Despite the fact that the oxygen consumption of the ventricle was enhanced by approximately 10%, free fatty acid, as the major substrate extracted by the myocardium in the fasting state, was extracted by the heart to a lesser degree than during the control period. Diminished fatty acid extraction persisted throughout the period of observation and was not accompanied by significant changes in the arterial concentration of this substrate (fig. 4). An increase in the arteriovenous differences of triglyceride, however, indicated that this substrate provided for a major part of the oxidative needs of the myocardium during the epinephrine response without an elevated arterial concentration. The enhanced extraction of triglyceride persisted after interruption of the epinephrine infusion and after blood flow and oxygen consumption were restored to approximately control levels. As is evident in figure 5, the altered uptake of lipid by the myocardium was significantly

**TABLE 1**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Saline (8)</th>
<th>Epinephrine (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>7.11 ± 0.1*</td>
<td>6.94 ± 0.73</td>
</tr>
<tr>
<td>Lactate</td>
<td>14.8 ± 0.92</td>
<td>14.1 ± 1.3</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>1.38 ± 0.09</td>
<td>1.45 ± 0.12</td>
</tr>
</tbody>
</table>

*Uptakes were calculated as product of coronary blood flow and the arterial to coronary sinus differences of substrate for three hours.
†Numerals in parentheses indicate numbers of animals.
‡Means ± SEM.

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Lipid uptake in left ventricle. Decline of free fatty acid uptake by the myocardium under the influence of epinephrine was associated with enhanced extraction of triglyceride persisting for the three hours of observation.

The persistence of the enhanced triglyceride extraction in epinephrine-treated animals was accompanied by accumulation of this lipid in the myocardium (fig. 5). By three hours, the concentration of triglyceride was three times that in the saline-treated animals. Phospholipid did not appear to participate in the tissue lipid accumulation which occurred after epinephrine, since concentrations in controls were $19.7 \pm 1.7 \, \text{mg/g}$ and $20 \pm 2 \, \text{mg/g}$ in the hormone-treated animals.

To evaluate the possibility that the diminished extraction of fatty acid was due to release of free fatty acid into coronary venous blood by virtue of the hydrolysis of plasma or cardiac muscle triglyceride, palmitic acid-1-C\(^14\) was infused systemically over a period of 40 min. The extraction of this fatty acid was substantially less than that observed in control animals (fig. 6). There was no A-CS difference in specific activity, suggesting that the altered palmitate extraction was representative of the group of plasma free fatty acids. Production of C\(^14\)O\(_2\) from the labeled fatty acid was reduced to an equivalent degree during epinephrine infusion. Incorporation of C\(^14\) palmitate into tissue lipids was

Cumulative lipid uptake by myocardium over 3-hour period. The bar graph on the left represents the calculated free fatty acid and triglyceride uptake by the myocardium over three hours observation, derived from the product of coronary plasma flow and substrate A-V difference. Tissue triglyceride concentrations are shown on the right.
Myocardial metabolism of palmitate-1-C<sup>14</sup>. During the infusion of 30 µg for 40 min, substantial extraction of labeled free fatty acid occurred. This was significantly reduced in animals receiving epinephrine. Oxidation, as judged by C<sup>14</sup>O<sub>2</sub> production, was proportionately diminished. Cumulative uptake and production were calculated as the product of the arterial to coronary sinus difference and the coronary plasma flow in the case of fatty acid, coronary blood flow in the case of C<sup>14</sup>O<sub>2</sub>.

Discussion

The rise in the stroke output and dp/dt max during the early phase of a sustained intracoronary infusion of epinephrine is typical of the response to brief catecholamine stimulation. Attempts have been made to relate the metabolic basis of this response to activation of glycogenolysis by the phosphorylase system and the tissue increment of hexose phosphates. However, the fact that this metabolic reaction is not readily detected at lower inotropic doses of epinephrine, or may be inhibited without apparent effect on the contractile increments, would tend to minimize this reaction sequence as a critical determinant of the mechanical response. Although egress of potassium ions from muscle has been postulated as a mechanism for enhancing contractility and is seen during the positive inotropic response to strophanthinidin, it is noteworthy that uptake of this ion by the myocardium occurs early during epinephrine infusion, so that a net loss of potassium from the muscle is not essential for increased contractility.

While impaired ventricular function would be anticipated after histologic evidence of muscle necrosis produced by catecholamines, it is apparent that functional decline occurs relatively early under the conditions of this study. Evidence for this is found in the progressive rise of ventricular filling pressure to twice control levels, while stroke output and dp/dt max are declining. Since this functional decline is related temporally to the appearance of biochemical evidence of injury, it may well be viewed as a consequence of such injury, as is seen in ischemic necrosis.

Selective release of ions and protein has been described early in the course of injury to noncardiac tissue. While negative arterial-coronary sinus differences of potassium may be observed after strophanthinidin in nontoxic doses, the ion egress is limited to a period of five to ten minutes. In the present state of our knowledge, the sustained release of ions for a period of at least two hours seen after epinephrine does not occur in a normally functioning ventricle.

Since coronary blood flow increased during the epinephrine infusion, an absolute decrease in coronary blood flow is not the basis for the myocardial injury. However, the question of a relative deficit of blood flow remains. There was an approximate 60% increase in all the measured parameters of left ventricular activity, while the oxygen consumption increment was about 10%. Unlike the situation when an absolute reduction in flow occurs, there was no increase in extraction of oxygen by the left ventricle and no indication of reactive hyperemia during or after epinephrine. Further, the production of excess lactate after exhibition of the hormone occurred during the maximal positive inotropic effect, whereas during ischemia it occurs as contractility is declining. Assuming a similar pathogenesis of necrosis produced by the different catecholamines, in contrast to ischemia no alterations of mitochondria have been detected during...
the early phases of isoproterenol-induced necrosis.\textsuperscript{33} Another metabolic contrast with ischemic necrosis lies in the finding that free fatty acid uptake and incorporation into tissue triglyceride is not affected substantially at moderate levels of ischemia,\textsuperscript{34} whereas, during epinephrine-induced necrosis, fatty acid uptake and incorporation into tissue triglyceride were reduced.

That a contractility increase may occur without an increment of oxygen consumption has been observed during the positive inotropic response to strophanthinidin without subsequent evidence of injury.\textsuperscript{29} Further evidence that the positive inotropic response is not necessarily related to the induction of necrosis by catecholamines is found in the observation that methoxamine can produce such a tissue response without enhancing contractility.\textsuperscript{35} For these reasons, it is unlikely that the injury to cardiac muscle produced by epinephrine is related directly to an oxygen supply deficit.

The converse effect, namely a myocardial injury related to "oxygen wasting" has been postulated,\textsuperscript{8} because during systemic administration of catecholamines the rise in oxygen consumption may be substantial\textsuperscript{14} and out of proportion to the increase of cardiac work.\textsuperscript{1} In view of the relatively small increase in oxygen consumption after epinephrine and the proportionately larger rise in left ventricular stroke output and work, a low efficiency and high oxygen consumption response to catecholamines would not appear to be a prerequisite for the cardiac injury produced by this hormone.

The peak increase of contractility after epinephrine was associated with the maximal rise in the myocardial respiratory quotient, which is attributable to the utilization of cellular carbohydrate during glycolysis. The production of excess lactate by the heart was increased significantly during this time, and was related presumably to the increased formation of DPNH\textsuperscript{*} during the oxidation of glyceraldehyde and its subsequent interaction with the lactate-pyruvate system.\textsuperscript{9} This effect of the hormone was not related to demonstrable hypoxia in the myocardium.

Uptake of free fatty acid (FFA) usually provides the myocardium with its major substrate in the fasted state. Unexpectedly, in view of the previous correlation of the calorigenic effect of catechols with FFA usage,\textsuperscript{7} the arteriovenous differences were reduced by epinephrine, which effect persisted throughout the three hours of observation. As is evident from the isotopic studies reported here, this is not a reflection of hydrolysis of glyceroles but appears to be a transport effect similar to that produced by ethanol.\textsuperscript{10} Increased tissue levels of carbohydrate intermediates during glycolysis may account for this effect, since various carbohydrate substrates have been shown to inhibit competitively the uptake and oxidation of FFA.\textsuperscript{8} Persistent inhibition of oxidation of FFA entering the cardiac cell\textsuperscript{17} could result in a tissue triglyceride increment, but this seems unlikely in view of a respiratory quotient which indicated predominant dependence upon lipid for oxidative needs after 90 min of epi-nephrine administration.

Extraction of triglyceride by the myocardium in the fasting state is controversial.\textsuperscript{88, 89} Our own data and those of an earlier study using direct measurement of triglyceride\textsuperscript{89} have failed to show significant uptake of triglyceride. The enhanced uptake of triglyceride after epinephrine occurred without increased arterial concentrations and would appear to account in large measure for the tissue increment of triglyceride. The exact mechanism of transport of the glyceride fatty acid is unknown, but may involve hydrolysis by an extracellular lipase, or cellular uptake of the intact triglyceride molecule. While this lipid may serve as the major source of oxidative energy during epinephrine infusion, the accelerated transport produced eventually an accumulation of triglyceride in left ventricular tissue by three hours (fig 5), which, on the basis of electron microscopic findings would appear to be distributed diffusely throughout the myocardium.\textsuperscript{90} This implies a

\textsuperscript{*}Reduced diphosphopyridine nucleotide.
limit to the capacity for triglyceride oxidation by the myocardium, initiated at an undetermined time after epinephrine exhibition.

Although lipid increments in heart muscle are known to occur in a variety of pathologic situations, it is generally assumed that this is a secondary or late phenomenon that does not contribute to the impaired function of cardiac muscle. The early alteration of lipid transport during sustained epinephrine infusion raises the possibility that triglyceride may participate in the pathophysiologic response, particularly since this lipid appears to have ready access to the contractile protein region of the cell.

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