Effects of Isoproterenol on Carbohydrate Metabolism of Isolated Canine Heart
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Circ Res. 1963;12:76-84
doi: 10.1161/01.RES.12.1.76

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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Early studies of the effects of epinephrine on the isolated and metabolically supported canine heart demonstrated increases in heart rate, contractile force, and myocardial oxygen consumption.\(^1\) The increases in myocardial oxygen consumption following epinephrine administration were roughly proportional to the increase in heart rate.\(^2\) In heart-lung preparations epinephrine administration resulted in increased myocardial glucose utilization.\(^3\) This increase was somewhat dependent on arterial glucose concentration and was associated with a myocardial glycogen depletion unless glucose was administered with the epinephrine. In the isolated heart, stimulated by either epinephrine or pressure work loads, both lactate and glucose utilization were increased,\(^4\) but epinephrine stimulation did not effect pyruvate utilization.\(^5\) Increased work loads applied to heart-lung preparations resulted in no change in myocardial glycogen content when arterial glucose concentrations were maintained.\(^6\) Under these conditions only 25% to 60% of the oxidative metabolism can be accounted for by carbohydrate utilization. Stimulation of the stellate ganglion increased heart rate, blood pressure, and coronary blood flow, but did not effect myocardial utilization of lactate.\(^7\)

Isoproterenol, or isopropyl norepinephrine, described in 1941,\(^8\) has marked excitatory and enhanced inhibitory sympathomimetic activity as compared to epinephrine. It was found to be several times more potent than either epinephrine or norepinephrine with respect to chronotropic and inotropic effects on isolated perfused hearts of various species.\(^9\), \(^10\) The inhibitory effects of isoproterenol on various types of smooth muscle\(^11\), \(^12\) as well as the stimulatory effects of isoproterenol on myocardial contractile force and rate were classified by Ahlquist\(^13\) as beta receptor responses in contrast to alpha receptor responses resulting from sympathetic excitation in all organs but the heart.

Isoproterenol is as effective in cardiac patients, even with congestive failure, as in normals, in increasing cardiac output while lowering diastolic filling pressure.\(^14\) These hemodynamic responses simulate those of exercise. It has been used in clinical investigations to compare the marked similarities in hemodynamic responses with those of exercise in the same cardiac patients.\(^15\) In certain hypokinetic states following cardiopulmonary bypass for open heart surgery, it has also been useful in restoring cardiac output, without concomitantly imposing an increased pressure load on the ventricles. The effective dose for these responses is critical and excesses initiate ventricular arrhythmias. Toxic doses in mammalian experiments produce acute myocardial necrosis and infarction.\(^16\)

It is apparent that isoproterenol is an extremely potent sympathomimetic amine. It is useful for clinical cardiac investigation and for therapy in certain hypokinetic states. The effective therapeutic dosage range is narrow and only moderate increases in the quantity of drug administered may rapidly precipitate toxic effects. The purposes of the investigation reported herein were to study cardiac metabolism before and during isoproterenol...
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infusion, to elucidate some of the metabolic substrates used by the myocardium under extended maximal non-toxic isoproterenol stimulation, and to determine whether this stimulation resulted in significant depletion of myocardial substrate stores. Doses of isoproterenol infusion were selected that would produce an extended maximal inotropic effect similar to that produced in the clinical situation.

Methods

Two dogs were used for each experiment. They were anesthetized with 12 mg of pentobarbital per kilogram of body weight, intubated, and ventilated with a Palmer respirator. Pentobarbital was chosen as the anesthetic agent since it produces no significant increase in circulating adrenergic amines. The heart and great vessels of the smaller, experimental dog, from which the heart was to be excised, were exposed by a bilateral transsternal thoracotomy. The animal was heparinized with 3 mg heparin/kilogram of body weight. A large #22-28 perfusing catheter was inserted through the brachiocephalic artery into the ascending aorta immediately above the aortic valve. A small pressure recording catheter was inserted into the ascending aorta via the left subclavian artery. A Walton strain gauge arch was sutured obliquely across the left ventricle.

The larger or perfusing dog was heparinized with 3 mg heparin/kilogram of body weight and a femoral artery and vein were catheterized with #18-22 catheters. The femoral artery catheter of the large perfusing dog and the brachiocephalic artery catheter of the small experimental animal were connected by Tygon tubing from which the air was displaced by blood. This tubing was passed through an occlusive Sigmamotor pump for control of the perfusion. Three-way stop-cocks inserted into the line permitted arterial sampling, and constant infusion of isoproterenol during the experimental period. When these preparations were completed, the cavae of the small experimental dog were occluded; the aorta was ligated distal to the left subclavian artery, and perfusion of the isolated heart was started immediately. The heart was quickly excised and suspended in a large plastic constant temperature chamber (fig. 1). Venous blood flowed from the coronary sinus and Thesbian veins through a wide opening in the right atrium and by gravity into the bottom of the constant temperature chamber. It then returned to the large perfusing dog by gravity via a defoaming chamber.

The perfusion was maintained at approximately 1 ml/g heart weight per min.* This perfusion resulted in a mean aortic bulb pressure of 80-100 mm Hg.

The physiological responses of heart rate, aortic bulb pressure, base-apex electrocardiogram, and myocardial contractile force were all continually monitored. Frequent measurements of the blood flow through the perfused heart were made by collecting the venous blood for one minute in a graduated cylinder. This rate of blood flow remained unchanged throughout each experiment since the

*Perfusions greater than this quickly caused myocardial edema, diminished utilization of metabolites, deterioration of myocardial contractile force, arrhythmia, and early failure of the preparation. Perfusion rates less than 1.0 ml/g of heart weight per min were characterized by gradual bradycardia and electrocardiogram change suggesting the onset of myocardial hypoxia. The perfusion rate is in the range of normal canine coronary flow as observed by many authors and compiled by Gregg.

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

A schematic diagram of the experimental preparation used in this study. The excised heart with its wide-open right atrium is suspended in a constant temperature chamber. The coronary arteries are perfused by the arterial catheter fixed in the ascending aorta. The perfusion is controlled by an occlusive Sigmamotor pump. Isoproterenol was infused at a constant rate during the experimental period into the arterial line between the Sigmamotor pump and the isolated heart. Arterial samples were taken from this line and simultaneous venous samples from the coronary sinus. Venous blood returned to the large perfusing dog by gravity via a defoaming chamber.

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ISOLATED CANINE HEART

Effect of Intrarterial Isoproterenol 0.8 μg/100 gm/min.

<table>
<thead>
<tr>
<th>Control</th>
<th>24 min</th>
<th>Control</th>
<th>1 min</th>
<th>3 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECG</td>
<td>Contractile Force</td>
<td>Coronary Arterial Pressure</td>
<td>ECG</td>
<td>Coronary Arterial Pressure</td>
</tr>
<tr>
<td>1.3 μg/100 gm/min.</td>
<td>1 mln</td>
<td>3 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 2

This shows examples from single experiments of myocardial response to isoproterenol infusion at 0.8 μg/100 g heart weight/min, 1.3 μg/100 g heart weight/min, and 1.9 μg/100 g heart weight/min.

Sigmamotor pump speed and blood volume of the perfusing dog were kept constant. At regular intervals small fragments of left atrial myocardium were excised for determination of intracellular glycogen concentration (Montgomery). The excised heart was perfused for one hour, during which time several arterial and coronary sinus blood samples were simultaneously taken.

The arterial sample was taken via a three-way stop-cock placed in the arterial line proximal to the aortic bulb. The venous sample, taken simultaneously, was obtained by passing a heparin-filled non-occluding polyethylene catheter into the coronary sinus and aspirating the blood. Diligent caution was exercised during these samplings to avoid contamination of the sample by air.

to establish control measurements. These samples were analyzed for the following components: 1. pH (Beckman model G pH meter); 2. oxygen and carbon dioxide contents; 3. glucose; 4. pyruvic acid; 5. lactic acid. Blood samples for lactic and pyruvic acids were precipitated in cold trichloracetic acid within 15 seconds after withdrawal. Huckabee's method of determining excess lactate (XL) concentration was used to evaluate anaerobic metabolism. The blood samples were analyzed by technicians in the biochemical laboratories of the Department of Surgery and Division of Cardiology, Department of Medicine of the University of Washington School of Medicine. Since relatively large volumes of blood were withdrawn for analytical procedures, the perfusing animal was repeatedly transfused with equal vol-
TABLE 1
This tabulates the myocardial utilization of various metabolites during the control and isoproterenol infusion periods. Negative values indicate that the myocardium released this compound. The asterisk indicates the metabolites that changed significantly \( (P < 0.01) \) when isoproterenol was administered.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control ( \times 10^{-7} \text{ M/100 g/min} )</th>
<th>Isoproterenol ( \times 10^{-7} \text{ M/100 g/min} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>18.3</td>
<td>26.3*</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>-17.2</td>
<td>-24.3*</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.6</td>
<td>-7.2*</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Glycogen conc.</td>
<td>469 ( \times 10^{-7} \text{ M/100 g} ) (as 6 C units)</td>
<td>524 ( \times 10^{-7} \text{ M/100 g} ) (as 6 C units)</td>
</tr>
</tbody>
</table>

\* \( P < 0.01 \)

TABLE 2
The oxygen equivalents in \( M \times 10^{-6} /100 \text{ g of heart weight/min} \) for the three carbohydrate substrates measured are shown. The sum of these oxygen equivalents equals the predicted \( O_2 \) utilization if all the aerobic metabolism were derived from these substrates. The difference between the predicted \( O_2 \) utilization and the observed \( O_2 \) utilization during isoproterenol infusion suggests the metabolism of some other substrate.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Control</th>
<th>Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>15.3 (83%)</td>
<td>15.3 (58%)</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.7 (8%)</td>
<td>. .</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>1.9 (9%)</td>
<td>2.3 (9%)</td>
</tr>
<tr>
<td>Predicted ( O_2 ) utilization</td>
<td>18.9</td>
<td>17.6 (67%)</td>
</tr>
<tr>
<td>Observed ( O_2 ) utilization</td>
<td>18.3</td>
<td>26.3</td>
</tr>
<tr>
<td>Non CHO sources</td>
<td>. .</td>
<td>(8.7) (33%)</td>
</tr>
</tbody>
</table>

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It was imposed on either ventricle.† Together, these three substrates apparently accounted for all the oxygen utilization (Table 2).

At the end of one hour, a constant infusion of isoproterenol was begun and continued for 30 minutes. The same physiological parameters were monitored, and serial paired A-V blood samples were withdrawn for analysis at periodic intervals. At the end of each perfusion, the great vessels were excised and the heart weighed.

Results

The expected stimulatory effects on the beta receptors of the isolated myocardium varied with time and dosage of isoproterenol. When only 0.8 \( \mu \text{g} \) or less/100 g heart weight per min was administered, usually over 20 minutes elapsed before there was a definite increase in heart rate and contractile force (fig. 2). Increasing the dose of isoproterenol to 1.3 \( \mu \text{g} /100 \text{ g heart weight per min} \) was usually accompanied by a prompt cardiac ac-

Under these conditions of coronary artery perfusion no blood was perfused into the left ventricle because of the competence of the aortic valve in the presence of a steady high level aortic bulb pressure. In the few experiments where intermittent aortic incompetence occurred and left ventricular ejection of blood was present, the aortic bulb pressure tracing approximated an arterial pulse contour instead of the normal sine wave. Such experiments were not included in the compiled data since the left ventricle did have a pressure load, its work would obviously be increased, and its metabolite utilization altered,
TIME-DOSE RELATIONSHIP TO PHYSIOLOGICAL RESPONSES

This is a graph of the dose of isoproterenol infused, plotted against the time of onset of inotropic myocardial response. The hyperbolic regression line as calculated from the rough data is shown. At high concentrations, i.e., more than 1.9 \( \mu \text{g} \) isoproterenol/100 g heart weight/min, the inotropic response was instantaneous and if the infusion was continued, arrhythmias rapidly occurred. At doses below 0.8 \( \mu \text{g} \) isoproterenol/100 g heart weight/min no response was apparent even after as much as one hour of infusion.

The results of 25 experiments in which doses of isoproterenol ranging from 0.8 to 1.9 \( \mu \text{g} \)/100 g heart weight per min were infused, the onset of cardiac responses is delayed over 20 minutes.

At doses below 0.8 \( \mu \text{g} \)/100 g heart weight per min, usually no cardiac response could be initiated even after 60 minutes of infusion. These relationships are graphically expressed in figure 3.

The response of heart rate and contractile force during the isoproterenol infusion period as compared to the control period.

When the dose of isoproterenol infused is plotted against the time of onset of inotropic stimulation, a hyperbolic relationship is suggested. At high doses of the drug, e.g., 1.9 \( \mu \text{g} \)/100 g heart weight per min, the onset of cardiac responses is immediate and if such a dose is continued, arrhythmias and conduction defects soon supervene. At low doses of the drug, e.g., 0.8 \( \mu \text{g} \)/100 g heart weight per min, the onset of cardiac responses is delayed over 20 minutes.

When the dose of isoproterenol was maintained near this level, the prompt inotropic response was maintained throughout the 30-minute period of infusion without deleterious effects. If a dose of 1.9 \( \mu \text{g} \) of isoproterenol/100 g heart weight per min was infused, the characteristic cardiac responses were initiated in 20 seconds and reached maximal levels in the next few seconds (fig. 2). Continued infusion of this concentration of drug soon produced severe arrhythmias and conduction defects.

When the dose of isoproterenol infused is plotted against the time of onset of inotropic stimulation, a hyperbolic relationship is suggested. At high doses of the drug, e.g., 1.9 \( \mu \text{g} \)/100 g heart weight per min, the onset of cardiac responses is immediate and if such a dose is continued, arrhythmias and conduction defects soon supervene. At low doses of the drug, e.g., 0.8 \( \mu \text{g} \)/100 g heart weight per min, the onset of cardiac responses is delayed over 20 minutes.

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The results of 25 experiments in which doses of isoproterenol ranging from 0.8 to 1.9 \( \mu \text{g} \)/100 g heart weight per min were infused, are included in the data. In each of these experiments the inotropic effects of the infused drug occurred promptly and were sustained for the 30-minute infusion without evidence of arrhythmias.

The various physiological (heart rate, contractile force, and aortic bulb pressure) and metabolic parameters (oxygen, carbon dioxide, glucose, lactic acid and pyruvic acid utilizations, and tissue glycogen) were plotted against time during the control and isoproterenol infusion periods. The observations for the control period can be best expressed as linear regressions. In each instance these linear regressions had slopes that did not differ significantly from the slope of a horizontal line. The linear regressions for the
Various physiological and metabolic parameters of the control period are expressed in figures 4-8 as broken lines labeled "Control."§

The plotted observations for the isoproterenol infusion period showed marked initial changes in the various parameters followed by sustained response at a new level. Such responses suggest an exponential relationship to time. The exponential regressions for the physiological and metabolic parameters of the isoproterenol infusion period were derived by standard statistical methods and expressed in figures 4-8 as solid lines labeled "Isoproterenol." Cardiac rate and contractile force responded to isoproterenol infusion with initial rapid acceleration to plateaus of maximal response, which were reached after five minutes (fig. 4). This differed very significantly from the control observations. A similar, but less significant, reduction in coronary arterial pressure was observed (fig. 5). Since the coronary artery flow was held constant, this represented the reduction in coronary arterial resistance.

§Since the various parameters did not change significantly during the control period, the Y-intercepts for both the control period and the isoproterenol infusion period are shown as originating at the same point in figures 4-8. The P values given on figures 4-8 express the significance between the cardiac response in the isoproterenol infusion period and the control period.

Myocardial utilization of oxygen, and excretion of carbon dioxide increased exponentially with time (fig. 6). These changes in oxidative metabolism were significantly different from the controls. There was no significant change in mean RQ of 0.92 over a period of 30 minutes. Although glucose utilization tended to fall initially, there was no difference in average values (fig. 7). The increase in intracellular glycogen was small and only bordered on significance.

Isoproterenol decreased lactate and increased pyruvate utilization (fig. 8). The change in lactate from utilization to excretion was significantly different from control observations.

Myocardial oxygen consumption during the plateau of maximal response to isoproterenol increased on the average by more than 40% (table 1). Yet there was no change in glucose utilization, and lactate was excreted rather than utilized. Pyruvate utilization increased 22%, however. In terms of oxygen equivalents (table 2), the predicted oxygen utilization, based upon glucose and pyruvate, was 67% of the observed oxygen utilization. The remaining 33% of observed oxygen utilization was, therefore, derived from other substrates than those measured.

Discussion
The observations for the control period of
the 25 experimental preparations used in this study were compared with those of 35 other similar preparations employed for other purposes. The control observations in the present study exhibited less variability and more stability over the period of time studied. Thus, perfusion of the isolated heart as described in this preparation at physiological pressures and rates of flow provided reproducible results.

The effects of isoproterenol under the conditions of these experiments resembled the physiological responses to epinephrine on the isolated and perfused heart first described by Barcroft and Dixon,\textsuperscript{1} that is to say, heart rate and contractile force increased, as did myocardial oxygen consumption. The changes in glucose and lactate utilization differed in that there was no increase with isoproterenol, as has been described for epinephrine by Bogue et al.\textsuperscript{4} Yet the lack of significant increase in lactate utilization corresponded with the findings of McGinty and Miller\textsuperscript{7} with respect to the effects of stimulation of the stellate ganglion on myocardium.

Isoproterenol, therefore, increased oxidative metabolism and heart rate by approximately 40%, yet did not increase the utilization of carbohydrate metabolites studied, with the possible exception of pyruvate. In the absence of significant changes in tissue glycogen concentration it is unlikely that significant anaerobic glycolysis was occurring. These responses suggested that other metabolites than those measured were involved in the increased oxidative metabolism. Quite possibly, lipids, especially fatty acids, were being utilized in increased amount under these circumstances.

During the control period, lactate utilization accounted for a small part of the oxidative carbohydrate metabolism. This utilization probably represents an oxidation to pyruvate and its metabolism as a three-carbon compound, since lactate participates only in the DPN:DPNH\textsubscript{2}, lactic dehydrogenase system in the cell, Huckabee.\textsuperscript{20,28,29,30} The inotropic response of the myocardium to isoproterenol was, however, associated with a very significant excretion of lactate. Huckabee asserts that lactate production depends on two separate factors, pyruvate concentration, and the adequacy of oxygen supply on a cellular level. Thus the observed changes in lactate metabolism reflect the changes in pyruvate and DPN:DPNH\textsubscript{2}, the lactic dehydrogenase system. A calculation of the "excess lactate" concentration in this preparation before and after isoproterenol stimu-

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FIGURE 7
The response of glucose utilization and changes in tissue glycogen concentration during isoproterenol infusion as compared to the control period.

FIGURE 8
The response of lactate and pyruvate metabolism during isoproterenol infusion period as compared to the control period.

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lation reveals that the lactate metabolism remains essentially unchanged. This necessitates an assumption that the equilibrium of the DPN:DPNH₂, lactic dehydrogenase system is unchanged. Katz et al. have reported that with the increased oxidative metabolism associated with epinephrine myocardial stimulation there is an increased coronary flow and venous oxygen concentration rises, instead of falling. Certainly the oxygen available in the intravascular compartment is adequate. These authors suggest then that the necrosis produced by myocardial hypoxia as a consequence of large doses of epinephrine may result from an inadequate oxygen supply at the cellular level. The increased oxidative metabolism of isoproterenol stimulation may, in the high dosage range, produce a similar relative cellular hypoxia. If such were the case, the equilibrium of the DPN:DPNH₂, lactic dehydrogenase system would be shifted and part of the lactate excretion could result from this relative cellular hypoxia.

This compound is more potent than either epinephrine or norepinephrine in respect to both pharmacological and pathological effects. Its use clinically should, therefore, be carefully supervised.

Summary

Chronotropic and inotropic effects of intraarterial infusion of isoproterenol were monitored in the isolated, metabolically supported preparations of the canine heart.

The time-dosage relationship was found to be hyperbolic over a narrow range of concentration of isoproterenol. Increasing concentration beyond the optimal range by a factor of about two produced arrhythmias and A-V conduction defects.

Oxidative metabolism increased significantly and proportionally to heart rate.

Since utilization of glucose remained the same, pyruvate utilization increased slightly and lactate was excreted; the augmented oxidative metabolism could not be accounted for by the observed changes in carbohydrate substrates.

Myocardial glycogen concentrations were insignificantly changed during this inotropic stimulation.

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