Regulation of Glycogenolysis in Muscle

EFFECTS OF GLUCAGON AND ANOXIA ON LACTATE PRODUCTION, GLYCOGEN CONTENT, AND PHOSPHORYLASE ACTIVITY IN THE PERFUSED ISOLATED RAT HEART*

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The activity of phosphorylase in muscle appears to regulate glycogenolysis (1, 2). The enzyme has been found to exist in two forms, a and b (3-5). Phosphorylase b is active only in the presence of adenosine 5'-monophosphate, whereas phosphorylase a is active in the absence of the nucleotide. Epinephrine and other catecholamines are known to increase phosphorylase activity by accelerating conversion of phosphorylase b to a. This effect is mediated by increasing the production of adenosine 3',5'-phosphate, which then increases the activity of the enzyme responsible for the b to a conversion, phosphorylase b kinase (1, 6-10). Anerobic work also accelerates breakdown of muscle glycogen by increasing conversion of phosphorylase b to a (11, 12).

Glucagon increases breakdown of liver glycogen by promoting formation of adenosine 3',5'-phosphate, which activates dephosphorylase phosphorylase kinase (7, 13). The effect of glucose on peripheral glycogenolysis has been doubted and considered to be of little importance.

In the present study, glucagon and anoxia have been shown to accelerate breakdown of glycogen, to increase output of lactate, and to increase phosphorylase activity in the perfused rat heart. These effects have been demonstrated in hearts from reserpinized animals. The measurement of phosphorylase activity in rat heart is discussed.

EXPERIMENTAL PROCEDURE

Heart Perfusion—Hearts were obtained from albino Wistar or Sprague-Dawley rats of 200 to 300 g, which had been deprived of food for 18 hours. Rats were given intraperitoneal injections of heparin (2000 i.u.) 20 minutes or more before they were killed. In experiments involving glycogen analysis with use of Wistar rats, pentobarbital sodium was used to anesthetize the animals.

Isolated hearts were perfused via the coronary circulation with Krebs-Henseleit bicarbonate buffer (14) at 37° gassed with either O2 or N2 containing 5% CO2. The perfusate flowed by gravity from a reservoir 60 to 70 cm above the heart. At the end of perfusion, hearts were frozen by clamping between two 270-g blocks of aluminum cooled in liquid nitrogen (15) or were cut from the cannula and frozen immediately either in a mixture of acetone and solid carbon dioxide or in liquid nitrogen. When lactate production was measured, the effluent medium was collected in graduated cylinders at intervals shown in the figures and tables.

Materials—Crystalline glucagon (Lot 258-234-S-167-1), a gift of Eli Lilly and Company, was dissolved in dilute NaOH or Tris, pH 9.5, to yield a stock solution of 1 mg per ml. This was added to perfusion medium at the desired concentration immediately before use. Heparin, a gift of Evans Medical, Ltd., Liverpool, England, was dissolved in either distilled water or pentobarbital sodium (80 mg per ml) to 10,000 units per ml. AMP and glucose-1-P were obtained from Sigma Chemical Company; Dowex 1-X4 (CI), 200 to 400 mesh, from Bio-Rad Laboratories; and reserpine (Serpasil, 2.5 mg per ml) from Ciba Pharmaceutical Products, Inc.

Analytical Methods—Lactate was assayed by the method of Barker and Summerfield (16). Glycogen was measured on frozen hearts, crushed into small pieces. The polysaccharide was isolated and hydrolyzed (17) and assayed by the glucose oxidase method (18).

Phosphorylase Assays—For assay of phosphorylase, frozen hearts were powdered in a percussion mortar chilled in solid carbon dioxide. Weighed samples of powder, equivalent to about 0.1 g of dry heart, were extracted in either of the following ways: (a) The powder was sprinkled onto 6 ml of extraction fluid (35 mM glycero-phosphate, 30 mM cysteine, 1 mM EDTA, 20 mM fluoride, pH 6.1) in a porcelain mortar at room temperature and was stirred gently with a pestle until the powder was thawed and extracted. (b) The powder was mixed with 2 ml of 60% glycerol, pH 6.1, containing 20 mM NaF and 1 mM EDTA (12) in a mortar at -25° by grinding with a pestle. The glycerol extract was then diluted with 4 ml of the extraction fluid described above. After centrifugation, the extracts were diluted to approximately 175 ml per g, dry weight, with extraction fluid. Phosphorylase activity was estimated by measuring the release of inorganic phosphate at 30° in reaction mixtures containing 0.1 ml of 64 mM glucose-1-P with or without AMP (1 mM), 0.1 ml of 4% glycogen (shaken with Dowex 1-Cl before use), and 0.2 ml of extract (19). After either 5 or 10 minutes, or both, the...
reaction was stopped by the addition of 5 ml of acid molybdate (5 g of ammonium molybdate plus 25 ml of concentrated H2SO4 per liter), and the whole reaction mixture was used for phosphate analysis. In certain cases, when it was necessary to dilute the extract to 420 ml per g, dry weight, too little phosphate was released to be measured accurately with 0.2 ml of extract and the release of phosphate was linear for only 5 minutes. Under these conditions, therefore, the volume of reaction mixture was doubled and estimates were made at 2.5 and 5 minutes. To correct for the hydrolysis of glucose-1-P by acid molybdate and the production of inorganic phosphate from sources other than glucose-1-P during the incubation, each phosphorylase assay tube was accompanied by a control containing the same additions except that the glucose-1-P was not added until immediately after the acid molybdate. These controls were particularly important at higher dilutions of extract. Inorganic phosphate was assayed photometrically by the addition of 1 ml of Elon reducer (20) to the acid molybdate solution followed by dilution to 10 ml with water. The solutions were read at 810 mp after 45 minutes. Basal phosphorylase activity was expressed as micromoles of inorganic phosphate released per g of heart, dry weight, per minute of incubation measured in the absence of added AMP. Total phosphorylase activity was measured in the presence of 1 mM AMP.

Assay of AMP in Phosphorylase Assay Reactions—Duplicate assay tubes were deproteinized either (a) at the moment when the phosphorylase assay reaction was started with glucose-1-P, (b) at the midpoint of the assay period, or (c) at the end of the assay period, by the addition of trichloroacetic acid (final concentration, 5%). The extracts were centrifuged and the supernatant solution was extracted three times with volumes of ether. The aqueous phase was adjusted to pH 7.0 and applied to a column of Dowex 1-X4 (formate), 20 X 1 cm. The column was washed with 50 ml of water and the AMP was eluted by a continuous gradient of formic acid in water (final concentration, 1 M). The fractions containing AMP were pooled, and extinction at 260 mp was measured with the fractions immediately preceding and following the peak as blanks. After freeze-drying and redissolving in a small volume of water, the material in this peak was identified as AMP by ascending chromatography against AMP, ADP, ATP, NADP, and UMP in isobutyric acid-concentrated NH4OH-water (66:1:33 by volume), pH 3.7, and in 0.1 M sodium phosphate (pH 6.9)-66% ammonium sulfate-n-propanol (100:60:2) on Whatman No. 1 paper. Only one spot, having the RF of the AMP standard, was detected in each solvent system.

Determination of Dry Weight—A sample of frozen heart or frozen muscle powder was assayed for dry weight by weighing before and after drying in a vacuum. Rat heart muscle, after perfusion under aerobic conditions, has a water content of 3.76 ml per g, dry weight (14).

Calculation of Fraction of Phosphorylase in a Form—Basal phosphorylase activity (V1) was described by the equation

\[
V_1 = E_1 \left[ f_a V_a \left( 0.65 + \frac{0.35 A_1}{K_a + A_1} \right) + f_b V_b \left( 0.65 + \frac{0.35 A_1}{K_b + A_1} \right) \right]
\]

where \( E_1 \) equals the total quantity of enzyme; \( f_a \), the fraction in the a form; \( f_b \), the fraction in the b form; \( V_a \), the maximal velocity of phosphorylase a, taken as 2200 units per mg of protein at 30° (21); \( V_b \), the maximal velocity of phosphorylase b, taken as 1600 units per mg of protein at 30° (21); \( A_1 \), the concentration of AMP observed in the assay system; \( K_a \), the \( K_m \) for activation of phosphorylase a by AMP, taken as 5 x 10⁻⁴ M; and \( K_b \), the \( K_m \) for activation of phosphorylase b by AMP, taken as 5 x 10⁻⁴ M (22). Phosphorylase a was assumed to have 65% of maximal activity in the absence of AMP. Total enzyme activity (V2) was described by the equation

\[
V_2 = E_2 \left[ f_a V_a \left( 0.65 + \frac{0.35 A_1}{K_a + A_1} \right) + f_b V_b \left( 0.65 + \frac{0.35 A_1}{K_b + A_1} \right) \right]
\]

where \( A_1 \) equals the concentration of AMP in the assay system after the addition of 1 mM AMP. These equations were solved for the fraction of enzyme in the a form (f_a).

\[
f_a = \frac{R}{1 + R}
\]

where

\[
R = \frac{V_2 \left( \frac{V_a A_2}{K_a + A_2} - \frac{V_b A_1}{K_b + A_1} \right)}{V_1 \left( 0.65 + \frac{0.35 A_1}{K_a + A_1} \right) - V_1 \left( 0.65 + \frac{0.35 A_1}{K_b + A_1} \right)}
\]

RESULTS

Glycogenolysis and Lactate Production—At the beginning of perfusion, lactate production was high (Fig. 1). This initial high rate of lactate production was reduced by about 50% in hearts from reenergized animals, suggesting that epinephrine

1 The authors wish to thank Dr. E. G. Krebs for making this manuscript available before its publication.
reached had occurred during preparation of the heart. Under aerobic conditions lactate production fell rapidly and by 8 minutes had reached a low level which was maintained in control hearts to the end of perfusion (22 minutes). When perfusion was switched at 8 minutes to buffer containing glucagon, lactate production increased abruptly to reach a peak in 2 minutes. After 5 minutes it had fallen to about twice the control rate. Anoxia stimulated lactate production much more strongly than glucagon, but the pattern of response was similar.

Glycogen content fell slowly during the course of perfusion in control hearts (Fig. 1). Glycogenolysis was stimulated by glucagon and at the end of the experiment, the glycogen content was about 80% of that of control hearts. Anoxia resulted in a more prompt and complete disappearance of glycogen. A more detailed evaluation of rates of glycogenolysis was made difficult by the variation in glycogen content of individual hearts within a single experiment and from day to day.

The possibility that these effects of glucagon and anoxia might depend on tissue stores of catecholamines was investigated with hearts from animals previously treated with reserpine (Table I). The dose of reserpine used has been shown to deplete cardiac stores of catecholamines (23). Treatment with reserpine did not interfere with the effect of either glucagon or anoxia.

Mechanical Activity—In hearts perfused with glucagon, the rate and force of cardiac contraction were increased as described by Farah and Tuttle (24). These effects diminished after 10 minutes of exposure to the hormone. When the heart was perfused under anaerobic conditions, the rhythm became irregular and contractions slowed and finally stopped.

Phosphorylase Activity of Rat Heart Muscle—The measurement of phosphorylase activity presented technical difficulties which appear worthy of comment. In particular, it was found important (a) to freeze the tissue rapidly, (b) to powder the frozen heart finely in a percussion mortar, and (c) to mix extracting fluid and muscle powder in such a way that the enzyme was extracted at temperatures well below 0°C. Freeze-clamping as compared with immersion in liquid nitrogen did not influence the qualitative differences between groups of hearts, but did reduce the basal activity. Since the rapidity of freezing is much faster with the clamp (15), these values could be assumed to represent more nearly the basal activity in situ of the enzyme.

Reduction of the frozen tissue to a fine powder was essential for complete extraction. With the percussion mortar used in these studies, as much as 3 g of frozen tissue could be successfully powdered at a time. The glycerol extraction method, recently described by Danforth, Helmarich, and Cori (12), would appear to represent the best current method for extracting the frozen powder. As shown in Table II, glycerol extraction produced a consistently lower basal activity while decreasing the total phosphorylase activity only slightly.

Since basal phosphorylase activity could be increased either by conversion of b to a or by AMP activation of phosphorylase b, the AMP concentrations existing in the assay mixture were measured (Table II). Reaction mixtures prepared from aqueous extracts had a significantly higher AMP level than those prepared from glycerol extracts. This increase would appear to be the most important factor accounting for the higher basal activity found in the aqueous extracts. The AMP concentrations of the aqueous extracts were similar regardless of the perfusion conditions, but the levels in the glycerol extracts appeared to be lower in hearts exposed to glucagon. The levels of AMP observed in the reaction mixtures were 10 to 20 times higher than would have been expected from the AMP content of the heart. Under aerobic conditions, the perfused heart has approximately 0.4 μmole of AMP per g, dry weight, when assayed in trichloroacetic acid extracts (25), but the values in the phosphorylase assays would give an AMP content of 3 to 9 μmole per g of dry heart. These results indicate that AMP is formed rapidly during the preparation of the extracts and during the phosphorylase assay (20). When reaction mixtures were deproteinized at the beginning and end of the 10-minute assay, an increase in AMP concentration of approximately 5 μm was observed during this period.

The effect of various concentrations of AMP on phosphorylase activity from control, anoxic, and hormone-treated extracts is shown in Fig. 2. Phosphorylase activity was increased when the concentration of AMP in the assay reached approximately 5 × 10⁻⁶ m, the Kₘ for AMP activation of crystalline phosphorylase b (22). The fraction of phosphorylase in the a form could then be calculated from the observed basal and total activities and AMP concentrations.

Phosphorylase was almost entirely in the a form when hearts were removed from normal rats and assayed without perfusion (Table II). The fraction in the a form fell rapidly during perfusion and by 8 minutes reached values near zero, paralleling lactate production under comparable conditions. The enzyme remained almost entirely in the b form between 8 and 18 minutes of perfusion. The increased lactate production and glycogenolysis induced by glucagon were associated with conversion of approximately 46% of the phosphorylase to the a form. The increase in enzyme activity, apparently maximal after only 2 minutes of exposure to the hormone, decreased with longer periods of perfusion but remained near 28%. Anoxia increased the fraction in the a form to a lesser degree. This difference

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**Table I**

Effects of anoxia and glucagon on lactate production and glycogen content of perfused heart

<table>
<thead>
<tr>
<th>Conditions of perfusion, 9th to 22nd minute</th>
<th>Lactate production, 9th to 22nd minute</th>
<th>Glycogen content at 22 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal hearts</td>
<td>0.4 ± 0.1* (14)</td>
<td>13.5 ± 1.0* (16)</td>
</tr>
<tr>
<td>Aerobic</td>
<td>6.9± ± 0.7 (13)</td>
<td>3.5± ± 0.5 (12)</td>
</tr>
<tr>
<td>Glucagon, 1 μg per ml.</td>
<td>3.4± ± 0.4 (13)</td>
<td>8.0± ± 1.0 (13)</td>
</tr>
<tr>
<td>Hearts from reserpinized animals</td>
<td>0.6 ± 0.3 (16)</td>
<td>15.5 ± 1.0 (16)</td>
</tr>
<tr>
<td>Aerobic</td>
<td>7.8± ± 1.5 (8)</td>
<td>6.0± ± 0.5 (15)</td>
</tr>
<tr>
<td>Glucagon, 1 μg per ml.</td>
<td>1.9± ± 0.3 (16)</td>
<td>8.0± ± 1.0 (13)</td>
</tr>
</tbody>
</table>

* Standard error of mean.
* *p < 0.01 versus aerobic control.
* *v < 0.01 versus normal anaerobic.
TABLE II
Effects of perfusion, glucagon, and anoxia on phosphorylase activity of normal rat heart extracted with aqueous and glycerol-containing fluid

Hearts were removed and perfused as described in Fig. 1. The tissue was frozen with the Wollenberger clamp, pulverized, and divided into two weighed portions. The powder from each heart was extracted with aqueous and glycerol-containing fluid and assayed at a dilution of 175 ml per g of dry heart as described in "Experimental Procedure." Results are expressed per g of heart, dry weight.

AMP concentrations were estimated in parallel reaction mixtures containing 1 ml of muscle extract, 0.5 ml of glycogen, and 0.5 ml of glucose-1-P. At the midpoint of the assay period, 5 minutes, the tubes were removed from the bath and deproteinized. The reaction mixtures from the hearts in each group were pooled and analyzed for AMP. The average difference between the AMP concentrations of aqueous and glycerol extracts was 10.5 ± 1.3 μM.

The fraction of phosphorylase in the α form was calculated as described in "Experimental Procedure." The 12-minute values for AMP were used for calculation of fraction α in the other aerobic control groups. The number of hearts in each group is shown by the figure in parentheses.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Extraction method</th>
<th>Phosphorylase activity (μmoles P_i/min/g)</th>
<th>(Basal activity X 100)/total activity</th>
<th>AMP concentration (μM)</th>
<th>Fraction in α form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct from animal: no perfusion</td>
<td>Aqueous (4)</td>
<td>75.2 ± 4.9*</td>
<td>81.7 ± 3.9*</td>
<td>29.3</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Glycerol (4)</td>
<td>61.2 ± 2.3</td>
<td>70.7 ± 2.1</td>
<td>19.4</td>
<td>0.83</td>
</tr>
<tr>
<td>Aerobic perfusion for:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 min</td>
<td>Aqueous (3)</td>
<td>21.1 ± 2.2</td>
<td>73.6 ± 2.6</td>
<td>28 ± 2</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Glycerol (6)</td>
<td>11.2 ± 1.0</td>
<td>64.9 ± 1.7</td>
<td>17 ± 2</td>
<td>0.01</td>
</tr>
<tr>
<td>12 min</td>
<td>Aqueous (6)</td>
<td>16.9 ± 2.5</td>
<td>76.0 ± 2.7</td>
<td>22 ± 4</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>Glycerol (6)</td>
<td>10.5 ± 1.6</td>
<td>75.2 ± 3.0</td>
<td>14 ± 2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>18 min</td>
<td>Aqueous (6)</td>
<td>21.6 ± 4.3</td>
<td>82.5 ± 5.2</td>
<td>25 ± 4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Glycerol (6)</td>
<td>14.6 ± 2.2</td>
<td>77.3 ± 4.5</td>
<td>19 ± 3</td>
<td>0.03</td>
</tr>
<tr>
<td>Aerobic perfusion for 8 min, followed by anaerobic perfusion for:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min</td>
<td>Aqueous (6)</td>
<td>43.2 ± 2.9</td>
<td>82.8 ± 3.0</td>
<td>53 ± 3</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td>Glycerol (6)</td>
<td>26.8 ± 2.0</td>
<td>78.7 ± 2.6</td>
<td>34 ± 3</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>Aqueous (6)</td>
<td>41.0 ± 4.7</td>
<td>92.9 ± 6.7</td>
<td>46 ± 4</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>Glycerol (6)</td>
<td>26.3 ± 4.3</td>
<td>85.8 ± 5.0</td>
<td>32 ± 3</td>
<td>11.3</td>
</tr>
<tr>
<td>Aerobic perfusion for 8 min, followed by aerobic perfusion with glucagon (1 μg/ml) for:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 min</td>
<td>Aqueous (6)</td>
<td>68.8 ± 7.6</td>
<td>99.1 ± 6.8</td>
<td>68 ± 4</td>
<td>22.3</td>
</tr>
<tr>
<td></td>
<td>Glycerol (6)</td>
<td>48.1 ± 6.7</td>
<td>93.1 ± 8.9</td>
<td>51 ± 4</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>Aqueous (6)</td>
<td>36.7 ± 4.6</td>
<td>82.0 ± 4.5</td>
<td>45 ± 5</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>Glycerol (6)</td>
<td>26.5 ± 4.9</td>
<td>79.4 ± 4.7</td>
<td>32 ± 5</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* Standard error of mean.

p < 0.05 versus aerobic (8 minutes, glycerol).

p < 0.01 versus aerobic (8 minutes, glycerol).

Fig. 2. Effect of AMP concentration on phosphorylase activity of perfused hearts. Hearts were perfused aerobically for 8 minutes and then for a further period of 5 minutes with either oxygenated buffer (O), oxygenated buffer containing glucagon (1 μg ml⁻¹) (□), or anaerobic buffer (○). Hearts were frozen by immersion in liquid nitrogen. Phosphorylase activity was assayed in aqueous extracts prepared from a pool of four hearts at a dilution of 420 ml per g of dry heart (see "Experimental Procedure"). The AMP contents were 3.75, 6.15, and 3.40 μmoles per g of dry heart in extracts of control, glucagon, and anoxic tissue, respectively, when determined at the midpoint of the 5-minute assay period. The AMP concentration is the sum of that added and of that present in the muscle extract.

between glucagon and anoxia was apparent after only 2 minutes of exposure to the agents; when in the anoxic muscle approximately 13% of the enzyme was found to be in the α form. These changes in phosphorylase activity were inversely related to the rate of glycogenolysis. Total phosphorylase activity varied somewhat from group to group, but no consistent change in activity was observed with perfusion, glucagon, or anoxia.

As shown in Table III, the lowest concentration of glucagon that increased basal phosphorylase activity after 2 minutes of exposure to the hormone was 0.1 μg ml⁻¹. The effects of other time periods were not investigated.

The increased basal phosphorylase activity induced by anoxia and glucagon were also evident in hearts from animals treated with reserpine (Table IV). Total phosphorylase activity was
Effects of Glucagon and Anoxia on Glycogenolysis in Rat Heart

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TABLE III

Effect of various concentrations of glucagon on basal phosphorylase activity of isolated perfused rat heart

Hearts were perfused for 8 minutes as described in Fig. 1 with substrate-free bicarbonate buffer. Perfusion was then switched to buffer containing various concentrations of glucagon, and was continued for 2 minutes. The hearts were frozen by immersion in liquid nitrogen and extracted in aqueous fluid. The number of hearts in each group is shown by the figure in parentheses.

<table>
<thead>
<tr>
<th>Glucagon content of medium (μg/ml)</th>
<th>Basal phosphorylase activity (per cent of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>17 ± 2a (6)</td>
</tr>
<tr>
<td>0.001</td>
<td>18 ± 1 (6)</td>
</tr>
<tr>
<td>0.01</td>
<td>16 ± 3 (6)</td>
</tr>
<tr>
<td>0.1</td>
<td>27 ± 2 (6)</td>
</tr>
<tr>
<td>1.0</td>
<td>52 ± 5 (6)</td>
</tr>
<tr>
<td>10.0</td>
<td>55 ± 2 (6)</td>
</tr>
</tbody>
</table>

a Standard error of mean.

TABLE IV

Effect of glucagon and anoxia on phosphorylase activity of hearts of reserpínized rat

Hearts were perfused aerobically for 8 minutes and then for a further 5 minutes aerobic with or without glucagon (1 μg per ml) or anaerobically. The hearts were frozen by immersion in liquid nitrogen and extracted in aqueous fluid. Phosphorylase was assayed in extracts diluted to 175 ml per g of dry heart in the presence and absence of 1 mM AMP. Four animals in each reserpín group were treated for 1 day (5 mg per kg) and four for 5 days (1 mg per kg). The number of hearts is shown by the figure in parentheses. Results are expressed per g of heart, dry weight.

<table>
<thead>
<tr>
<th>Conditions of perfusion</th>
<th>Animal reser-</th>
<th>Phosphorylase activity</th>
<th>Basal activity (per cent of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>eserptínized</td>
<td>Basal</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(μmoles P&lt;sub&gt;1&lt;/sub&gt;/min/g)</td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>9.8 ± 1.6c (8)</td>
<td>51.8 ± 2.2c (8)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>13.1 ± 2.4d (8)</td>
<td>52.7 ± 4.9 (8)</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0</td>
<td>30.1 ± 2.7c (8)</td>
<td>63.0 ± 6.2 (8)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>48.1 ± 4.5d (8)</td>
<td>81.0 ± 6.3 (8)</td>
</tr>
<tr>
<td>Anaerobic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>26.9 ± 3.5c (8)</td>
<td>64.1 ± 4.4 (8)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>39.9 ± 3.7c (8)</td>
<td>81.0 ± 7.5 (8)</td>
</tr>
</tbody>
</table>

a Standard error of mean.

b p < 0.01 versus glucagon-free medium.

c 0.02 < p < 0.05 versus nonreserpínized control.

unchanged by reserpín treatment under any of the perfusion conditions.

Anatomical Distribution of Glycogen—The anatomical distribution of polysaccharide within the heart was investigated to determine whether the relatively slow rate of glycogenolysis induced by glucagon could be explained by an effect of the hormone on only a particular type of cardiac cell. Hearts were rapidly fixed after 15 minutes of exposure to the hormone by injection of 75% ethanol containing 1% trichloroacetic acid and 4% formaldehyde into the coronary vessels. The heart was then cut from the cannula and immersed in the same solution. After sectioning, the tissue was stained with p-aminosalicylic acid and examined microscopically. Although it was difficult to identify the Purkinje system with certainty in rat heart, there was no doubt that the hormone had reduced the quantity of p-aminosalicylic acid-staining material throughout the ventricular muscle to approximately the same extent as the reduction in glycogen content. It appeared likely, therefore, that the hormone had activated phosphorylase uniformly throughout the cardiac muscle.

DISCUSSION

Estimation of the fraction of rat heart phosphorylase in the α form is complicated by the relatively low total enzyme activity as compared to skeletal muscle (12) and the rapidity with which ATP breaks down during extraction and estimation of enzyme activity (26). Basal activity, assayed in the absence of added AMP, appears to measure essentially total activity of phosphorylase α together with a small activity of phosphorylase β. At the usual dilution of 175 ml per g of dry heart, the AMP concentration in the assay mixture was approximately 10 μM, a concentration sufficient to induce 90% of the maximal activity of phosphorylase α and 15% of the maximal activity of β. With the addition of mM AMP, both enzyme forms are maximally active, but the maximal activities of the two forms do not appear to be equal (21). As a result, the usual calculation of percentage of α, based on a ratio of activity in the absence of added AMP to activity in the presence of AMP, appears to give an imprecise estimation of the fraction of enzyme in the α form. With these considerations in mind, we have discarded the usual per cent α terminology and have referred to the basal activity of the extract and the fraction in the α form as calculated from the measurements of activity and AMP concentrations.

In extracts prepared from aerobic hearts, basal activity was approximately 20% of total, indicating that virtually all of the enzyme was in the β form. This observation is consistent with the slow rates of glycogenolysis and lactate production observed in these hearts. Only a small portion (8%) of the glycogen that disappeared was recovered as lactate; the majority was presumably oxidized to CO₂ and if so would account for only 20 to 30% of the oxygen consumed, indicating the presence of other readily oxidizable substrates (27).

Glucagon and anoxia appear to accelerate glycogenolysis in heart muscle by accelerating conversion of phosphorylase β to α. With glucagon, approximately 50% of the enzyme is converted to the α form, presumably because of a greater activity of phosphorylase β kinase. By analogy with liver, glucagon would increase phosphorylase kinase activity by increased levels of adenosine 3',5'-phosphate, but experimental confirmation is lacking (7). Approximately 10 to 20% of the phosphorylase of the anoxic heart is found in the α form; the mechanism of this activation is unknown. Treatment of animals with doses of reserpín reported to deplete tissue levels of catecholamines did not interfere with phosphorylase β to α conversion induced by either agent.

Despite a greater increase in phosphorylase α activity, glucagon caused a slower rate of glycogenolysis than anoxia, indicating that factors other than conversion of phosphorylase β to α are important for glycogenolysis. Preliminary observations have indicated that increased tissue levels of AMP and inorganic
phosphate are responsible for the accelerated glycogenolysis induced by anoxia (25).

Glucagon has recently been shown to activate phosphorylase in adipose tissue (28) and, in the present study, in cardiac muscle. Concentrations of the hormone well in excess of those measured in blood plasma (29) are necessary in both cases. The physiological significance of these extrahepatic effects is therefore uncertain.

SUMMARY

Glycogenolysis in rat hearts perfused with oxygenated substrate-free buffer occurred at a slow rate. Under these conditions, the phosphorylase was almost entirely in the b form. Glucagon was found to stimulate glycogenolysis and lactate production and to increase the fraction of enzyme in the a form to 50%. Anoxia produced a still faster rate of glycogenolysis and lactate production, but a smaller increase in phosphorylase a activity. These observations suggest that other factors limit glycogen breakdown after conversion of phosphorylase b to a. This limitation is removed by anoxia. Both glucagon and anoxia produce increased rates of glycogenolysis and lactate production and elevated levels of phosphorylase a in hearts from reserpined animals.

Measurement of phosphorylase activity in the rat heart is discussed.

REFERENCES
Regulation of Glycogenolysis in Muscle: EFFECTS OF GLUCAGON AND ANOXIA ON LACTATE PRODUCTION, GLYCOGEN CONTENT, AND PHOSPHORYLASE ACTIVITY IN THE PERFUSED ISOLATED RAT HEART

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