Effect of Atrial and Ventricular Fibrillation and Ventricular Tachycardia on Carbohydrate Metabolism of the Heart

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The discovery that an increase in rate of stimulation of skeletal muscle leads to a rise in the level of active phosphorylase has focused interest on the relationship between integrated enzyme activity and mechanical activity of muscle. This enzyme, phosphorylase, catalyzes the reaction: glycogen plus inorganic phosphate = glucose-1-phosphate.

It was found that phosphorylase can be obtained in two forms: (1) phosphorylase a, the active form, which possesses 60 to 70 percent of its activity without addition of adenylic acid, and (2) phosphorylase b, the inactive form, which can be activated by the addition of adenylic acid. Increase in the active form results from injection of epinephrine, while strong muscular contraction and tetanic stimulation causes a decrease.

The presence of phosphorylase in heart muscle was first demonstrated by Kail and Sutherland, and it was discovered that the response of phosphorylase to epinephrine was similar to that in skeletal muscle. Catecholamines appear to play an important role in the correlation between force of contraction and phosphorylase a activity. Thus, Kukovetz and co-workers, working on heart muscle, found that a direct relationship exists between the per cent increase in heart muscle tension as produced by sympathomimetic amines and phosphorylase a activity. Mayer and Moran obtained similar results, but decided that it was not possible to state whether the primary effect of catecholamine action was on phosphorylase a activity or on the contractile mechanism. They discovered that the activation of myocardial phosphorylase was not related to sinus tachycardia induced by theophylline, but that stimulation of the cardiac sympathetic nerves caused a highly significant increase in enzyme activity. Belford and Feinleib found that in the isolated guinea pig auricle a three-hour period of one-second stimulation lowered the percentage of phosphorylase a. C. Cori, in summarizing the effect of stimulation on phosphorylase a content of skeletal muscle, stated that increasing the speed as well as the total number of contractions caused a progressively greater increase in the amount of active phosphorylase.

Increased rate of stimulation of skeletal muscle also produces major changes in carbohydrate intermediates of the Embden-Meyerhof cycle. In 1932, G. and C. Cori demonstrated that in the anaerobic muscle, glycogen disappeared, while lactate and hexose phosphate accumulated. Stimulation of skeletal muscle led to accumulation of hexosemonophosphate and the reaction of glycogen to hexosemonophosphate occurred more rapidly than the reaction of hexosemonophosphate to lactic acid; this pointed to phosphofructokinase as the rate-limiting step for lactic acid formation during contraction. Furthermore, the sum of lactic acid and hexosemonophosphate corresponded closely to the loss of glycogen. Similar results were published from this laboratory on the anoxic heart muscle.

The present discussion investigates the effect of changes in heart rate on the phosphorylase a activity and on glycogenolysis in heart muscle in vivo. Since cardiac rate, particularly ventricular fibrillation, profoundly alters coronary blood flow and myocardial oxygen consumption, the effect of changes in
heart rate on biochemical reactions was also investigated in the presence of supported coronary perfusion.

Methods

Experiments in Which Coronary Perfusion Was Not Maintained

A total of 57 experiments were performed. Phosphorylase activity was studied in six dogs with ventricular fibrillation, and six experiments were conducted to determine the effects of ventricular tachycardia and atrial fibrillation. Glycogen, G-6-P, and lactate were determined in six dogs with ventricular fibrillation and in eight animals with ventricular tachycardia. In four animals with ventricular fibrillation, and in three with ventricular tachycardia, pyruvate, lactate, alphaglycerol phosphate (alpha-GP), dihydroxyacetone phosphate (DHAP), and fructose-1,6-diphosphate (FDP) were determined.

Adult dogs, weighing from 15 to 25 Kg., were anesthetized by intravenous infusion of sodium pentobarbital (30 mg. per Kg. weight). Artificial respiration was carried out with a positive pressure pump through a tracheal cannula. Arterial pressures were determined by means of a Statham strain gauge connected to a catheter inserted into the femoral artery. Electrocardiograms were continuously recorded. After exposure of the heart and excision of the pericardium, ventricular fibrillation was induced by direct electrical stimulation (four volts with a frequency of 10 per second).

Auricular fibrillation was induced by application of a small amount of aconitine to one of the atria. Ventricular tachycardia of approximately 300 beats per minute resulted from direct electrical stimulation of the ventricle. Current strength was two to four volts with a rate of five per second.

For the studies on carbohydrate intermediates during ventricular tachycardia and fibrillation, three specimens, weighing approximately 400 mg., were removed from the apical area of the left ventricle, the first sample serving as control. The samples were removed at one-minute intervals. In six experiments, which served as control, three specimens of left ventricular muscle were removed at one-minute intervals without altering the heart rate.

During atrial fibrillation, the determinations were carried out on the right auricular appendage. Usually, the left atrial appendage served as a control. Table 2 illustrates that the percentage of phosphorylase a was identical in both atrial appendages. This is in line with the results of Belford and Feinleib.

Phosphorylase activity in the ventricle was studied in samples, weighing from 50 to 100 mg., taken from the apical area of the left ventricle.

Experiments in Which Coronary Perfusion Was Maintained

Twenty experiments were carried out. Figure 1 illustrates the perfusion system used in maintaining the coronary blood flow of the heart of the animal (dog 1) in which arrhythmias were produced. A total of three animals were used in this experiment, two of these (dogs 2 and 3) acting as blood donors for the coronary perfusion of dog 1. After anesthesia, all animals were heparinized (2 mg./Kg.). The coronary arteries of dog 1 were perfused with blood from the carotid arteries of the donor dog 2 by means of a Sigma motor pump which supplied a constant perfusion volume. Blood was returned from dog 1 to dog 2. In addition, dog 2 received blood from a third animal, dog 3, in order to maintain the necessary volume of perfusion. Venous blood from the right atrium of dog 1 drained into a reservoir to prevent dilatation of the right atrium. Both dogs 1 and 2 were maintained on artificial respiration with oxygen. During perfusion, the coronary inflow and pressure were maintained at about 160 ml./min. and 100 mm.Hg, respectively. Flow and pressure were measured with an electric flowmeter and a strain gauge, respectively. Coronary perfusion lasted three minutes before ventricular or atrial fibrillation was induced.

Analytical Methods

In the series of experiments in which coronary perfusion was not maintained, samples were imme-
diately dropped into liquid nitrogen. After performing these experiments, Mayer and Moran published a report stating that liquid nitrogen does not result in rapid enough freezing of the tissue. Consequently, cooled difluorodichloromethane (Freon-12) was used in the experiments in which the coronary circulation was maintained. This resulted in lower phosphorylase values in ventricular muscle. Therefore, no comparison can be made between the two experimental groups, but comparisons are possible within each of the two groups and also within each single experiment.

Phosphorylase was determined according to the method of Illingworth and Cori. All determinations were carried out in triplicate. Inorganic phosphate was measured according to the method of Fiske and Subbarow. Glycogen was assayed according to the method previously described. G-6-P was assayed with glucose-6-phosphate dehydrogenase according to the procedure of Kornberg. Lactate was estimated by a modified method of Hohorst. Instead of a semicarbazide buffer, a hydrazin buffer was used. Pyruvic acid, DHAP, and FDP were determined in a combined enzymic test. Alpha-GP was enzymically tested by a procedure outlined by Bücher and co-workers. Identical analyses were carried out on duplicate samples.

**Results**

**Experiments on Animals in Which Coronary Perfusion Was Not Supported**

*Effect of Ventricular Tachycardia and Fibrillation on Per Cent Phosphorylase Activity*

Table 1 illustrates that in six experiments in which specimens from normally beating left ventricle were taken at one-minute intervals, no significant alterations in the per cent phosphorylase activity could be detected. In contrast, ventricular tachycardia of 30-seconds duration resulted in a significant increase in phosphorylase activity (mean difference from control: +15.33, P < 0.01). In the specimen obtained one minute following onset of tachycardia, phosphorylase activity had again declined, although it still exceeded the control value (mean difference from control: +5.12, P < 0.025) (table 1). During prolonged stimulation, the percentage of active phosphorylase diminished. The results obtained during ventricular fibrillation showed the same trend (table 1). At the end of the first minute of fibrillation, the per-
Table 2

| Carbohydrate Intermediates During Ventricular Tachycardia and Fibrillation* |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | Glycogen        | Differences from control (0°) value | G-6-P           | Lactate         | Differences from control (0°) value |
| Time in minutes  | 0              | 0-1              | 0-2              | 0              | 0-1              | 0-2              |
| Control         | 2645            | -37.0            | -117             | 3.19           | -0.143           | +0.0233          | 121.37           | +0.12           | +4.8            |
| Significance of difference | 0.05 > $P$ > 0.025 | 0.2 > $P$ > 0.1 | 0.5 > $P$ > 0.4 | 0.2 > $P$ > 0.1 |
| Ventricular tachycardia† | 1944.5          | -80.0            | -99.4            | 2.31           | +1.80            | +3.1             | 86.4             | +128.0          | +190            |
| Significance of difference | 0.025 > $P$ > 0.01 | $P$ < 0.01      | 0.1 > $P$ > 0.05 | 0.05 > $P$ > 0.025 |
| Ventricular fibrillation‡ | 3357            | -458             | -657             | 2.99           | +26.7            | +56.8            | 89.8             | +524            | +870            |
| Significance of difference | $P$ < 0.01      | $P$ < 0.01       | $P$ < 0.01       | $P$ < 0.01     | $P$ < 0.01       | $P$ < 0.01       |

*Coronary circulation was not supported.
†Means of six experiments.
‡Means of eight experiments.

Table 2 illustrates that in six control experiments no significant difference in the concentrations of glycogen, G-6-P, and lactate in left ventricular muscle was observed. However, with ventricular tachycardia the concentration of lactate rose during the first and second minutes (mean increase of 128 and 190 μM per 100 Gm., $P$ < 0.01). In addition, the glycogen concentration fell significantly after two minutes of ventricular tachycardia (difference of -99.4 μM of units of glucose per 100 Gm., $P$ < 0.01). Glucose-6-phosphate rose slightly (rise of 1.8 μM per 100 Gm. after one minute, 3.1 after the second minute). These changes were, however, not statistically significant.

Results obtained during ventricular fibrillation were similar (table 2). There occurred a significant rise in lactate and glucose-6-phosphate after the first minute (mean rise of 26.7 μM per 100 Gm., $P$ < 0.01; and 56.8 μM of units of glucose per 100 Gm., $P$ < 0.01). Lactate and G-6-P concentrations continued to rise (table 2). In general, the changes encountered during ventricular fibrillation were more marked than those during ventricular tachycardia, but less significant. Overall, there was a significant increase in per cent phosphorylase a activity (table 1) following the onset of ventricular fibrillation (mean increase of 20.7, $P$ < 0.01). Experiments on Animals in Which Coronary Perfusion Was Supported

In four dogs the effect of artificial coronary perfusion on the myocardial glycogen, glucose-6-phosphate, and percentage of phosphorylase a activity was illustrated in table 1. A significant increase in per cent phosphorylase a activity occurred 30 seconds following the onset of artificial perfusion (mean increase of 29.2, $P$ < 0.01). Experiments on Animals in Which Coronary Perfusion Was Supported
lactate concentrations of the left ventricle were studied (table 3). After one and two minutes of induced ventricular fibrillation, the mean myocardial glycogen concentration was 1922 and 1985 μM glucose units, the mean G-6-P concentration was 4.00 and 3.98 μM, and the mean lactate concentration was 95.3 and 99.8 μM per 100 Gm. heart muscle. This shows that induced ventricular fibrillation in the presence of adequate coronary circulation has no effect on myocardial glycogen, G-6-P, and lactate concentration (table 3).

As shown in table 3, 30 seconds of induced ventricular or atrial fibrillation also failed to alter the mean per cent phosphorylase a activity in atrial or ventricular muscle, respectively.

**Discussion**

The experiments on hearts in which coronary perfusion was not maintained demonstrate that ventricular tachycardia and fibrillation result in a transitory increase in the ratio phosphorylase a/total phosphorylase. A similar increase in this ratio is observed during atrial fibrillation. These results agree with those of Cori on skeletal muscle that when the gastrocnemius muscle of the rat was stimulated at a rate of 20 per second for 12 seconds, there regularly occurred an increase in the level of active phosphorylase. The observation that phosphorylase a content first rises and then declines during ventricu-

lar fibrillation and tachycardia can be explained on the basis of activation of the two enzymes which catalyze the conversion of the phosphorylases. Apparently at the onset of ventricular tachycardia and fibrillation, heart muscle becomes alkaline, thus favoring formation of phosphorylase a. Accumulation of lactic acid then lowers the pH, depressing phosphorylase a content.

The changes in carbohydrate intermediates of the glycolytic shunt observed in these hearts during ventricular tachycardia and fibrillation are also similar to those described by Cori during rapid stimulation of anoxic skeletal muscle. There is an increase in lactate and a fall in glycogen concentration in heart muscle. Glucose-6-phosphate rises slightly; no appreciable changes occur in the concentrations of FDP, DHAP and pyruvate. Cori has found that during anoxia, stimulation of the frog gastrocnemius resulted in accumulation of G-6-P, indicating that the reaction glycogen to G-6-P occurs more rapidly than the reaction G-6-P to lactic acid; this points to phosphofructokinase as the rate-limiting step for lactic acid formation during contraction. Similar results were found in the arrested perfused heart muscle. It is likely that these changes in carbohydrate intermediates, observed here, are the result of anoxia. This is supported by the oxidation reduction potential as calculated from the ratio lactate/pyruvate.
as well as the ratio alpha-GP/DHAP, which becomes more negative during ventricular tachycardia and fibrillation (fig. 2). It is not possible to state whether these results are partially due to the release of catecholamines liberated as a result of anoxia.

Further support that the results described above are the effect of anoxia is furnished by the experiments in which the coronary circulation of the heart was maintained during ventricular and atrial fibrillation. No changes in phosphorylase a activity, nor in the myocardial concentrations of glycogen, glucose-6-phosphate, or lactate are noticed.

Summary
Changes in the ratio phosphorylase a/total phosphorylase in heart muscle, as well as the myocardial concentrations of glycogen, glucose-6-phosphate (G-6-P), lactate, pyruvate, fructose-1,6-diphosphate (FDP), dihydroxyacetone phosphate (DHAP), were followed during artificially induced ventricular tachycardia, and ventricular fibrillation, and atrial fibrillation. These experiments were carried out with and without support of the coronary circulation. When coronary circulation was not maintained, active phosphorylase increased, then diminished in heart muscle during ventricular and atrial fibrillation and during ventricular tachycardia. The myocardial concentration of lactate and glucose-6-phosphate rose, while that of glycogen diminished. No appreciable changes occurred in the concentration of fructose-1,6-diphosphate, dihydroxyacetone phosphate, and pyruvate. These changes were similar to those reported on skeletal muscle. When coronary circulation was maintained, no changes in carbohydrate intermediates or in phosphorylase a activity were recorded. These results demonstrate that the metabolic changes encountered in heart muscle during these arrhythmias are the result of anoxia.

References

Circulation Research, Volume IX, July 1961
ARRHYTHMIAS AND MYOCARDIAL METABOLISM


BOOK REVIEWS


This is a collection of reports on experiments performed on monkeys at the Institute for Experimental Pathology and Therapeutics of the Academy of Medical Science of the USSR. The following topics are covered: physiology and pathology of higher nervous activity, pathological morphology and physiology, and infectious pathology. The chapter on induction of hypertension and coronary insufficiency would be interesting to investigators who have been studying other forms of experimental hypertension.


The application of right heart catheterization and selective pulmonary angiography to the study of pulmonary disease is described.


This is a thesis on the relationship of varicose veins, abnormal function of the colon, and diet.
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