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G. KINETIC STUDIES OF EPINEPHRINE EFFECTS IN THE PERFUSED RAT HEART[1]

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It is well established that a variety of drugs and hormones can affect the phosphorylase *b* to *a* interconversion in muscle. The question that I will consider here is whether or not energy produced from glycogenolysis, as a result of this interconversion, is involved in the initiation of the catecholamine-induced inotropic response in cardiac muscle.

This problem has been approached from a kinetic point of view, and data have been obtained which show that changes in the force of contraction and tissue content of cyclic 3′,5′-AMP precede the increase of phosphorylase *a* and subsequent mobilization of glycogen. These results support the concept, amply illustrated by Dr. Sutherland, that the multiple effects of epinephrine (E), even in the same tissue, represent separate actions of the hormone which are not necessarily associated with enhanced glycogenolysis.

A rat heart preparation, cannulated at the aorta, and perfused by gravity feed (8, 9, 11), has been used for these studies. A convenient kinetic monitor of the metabolic effects of E is provided by the continuous recording of the state of oxidation-reduction of the pyridine nucleotides (1, 4). This method makes use of the fact that reduced pyridine nucleotides, especially when bound, are highly fluorescent, whereas pyridine nucleotides in the oxidized form are much less fluorescent.

Figure 1 illustrates the type of response obtained when a single large dose of epinephrine is injected into the perfusion fluid passing to the coronary circulation. The tissue fluorescence is shown in the upper trace, while the lower trace shows the contractile force as measured with a strain gauge. It should be noted that in this figure, as in figure 2, an increase of fluorescence is recorded as a down-

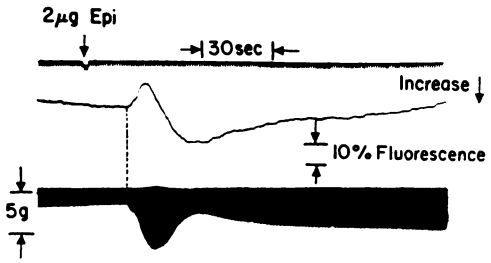

FIG. 1. Effect of epinephrine (Epi) on the tissue fluorescence (upper trace) and force of contraction (lower trace) of the perfused rat heart. The perfusion fluid contained no glucose, and was not recirculated.

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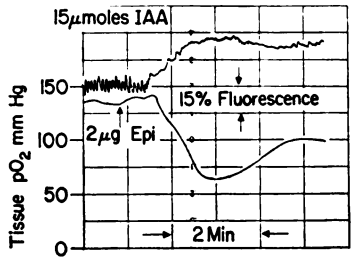

FIG. 2. Effect of epinephrine after prior treatment of the heart with 1 mM iodoacetate (IAA) for 20 min. The upper trace records the tissue fluorescence and the lower trace tissue oxygen tension. The heart was perfused with 15 ml of recirculating fluid containing 10 mM glucose.

ward deflection of the trace. Shortly after the addition of 2 μg E, there is an oxidation of pyridine nucleotide, which coincides with the onset of the inotropic response. After about 10 sec, there is a marked increase of reduced pyridine nucleotide, followed by a slower recovery to the initial steady state. The biphasic nature of the fluorescence trace illustrates that there are two competing reactions affecting the redox state: an initial oxidation of mitochondrial pyridine nucleotide as the respiratory activity increases, followed by an increase of cytoplasmic DPNH as glyceraldehyde-3-P—formed by glycogenolysis—reacts with its dehydrogenase. Reoxidation of the cytoplasmic DPNH occurs as it equilibrates with lactic dehydrogenase.

Confirmation that the pyridine nucleotide reduction is confined to the cytoplasmic space is shown in figure 2. The upper trace shows the fluorescence change while the lower trace records the tissue oxygen tension. In this experiment the heart was pretreated for 20 min with 1 mM iodoacetate prior to the recording shown. Iodoacetate slows the heart rate considerably, and individual heart beats may be seen as small cycles of pyridine nucleotide oxidation and reduction: the onset of oxidation occurring simultaneously with the onset of each contraction. Upon the addition of 2 μg E, the heart rate increases, and there is a marked and persistent oxidation of the pyridine nucleotides, while the tissue pO_2 falls as respiration is stimulated. Thus iodoacetate, by inhibiting glyceraldehyde-P dehydrogenase, has abolished the cytoplasmic response, and allowed the isolation of the mitochondrial response.

The oxidation of pyridine nucleotide in the mitochondria, which, as we have seen, coincides with the increased force of contraction and respiratory rate, is considered to be due to the release of ADP formed by the increased work of the heart, *i.e.*, a state 4–3 transition (3). This viewpoint is supported in a general way by measurements of the total content of the adenine nucleotides in hearts subjected for different times to stimulation by E. These results are shown in figure 3. ATP decreases immediately after the onset of the inotropic response, and there are corresponding increases in the levels of ADP and AMP. Inorganic phosphate also increases as a result of an increased rate of utilization of creatine-P. As noted from the figure, these changes are much greater than those of the adenine nucleotides since creatine-P serves as a buffer to prevent large changes of the ATP/ADP ratio (2). Included in figure 3 are values for the tissue pO_2, and it is seen that a

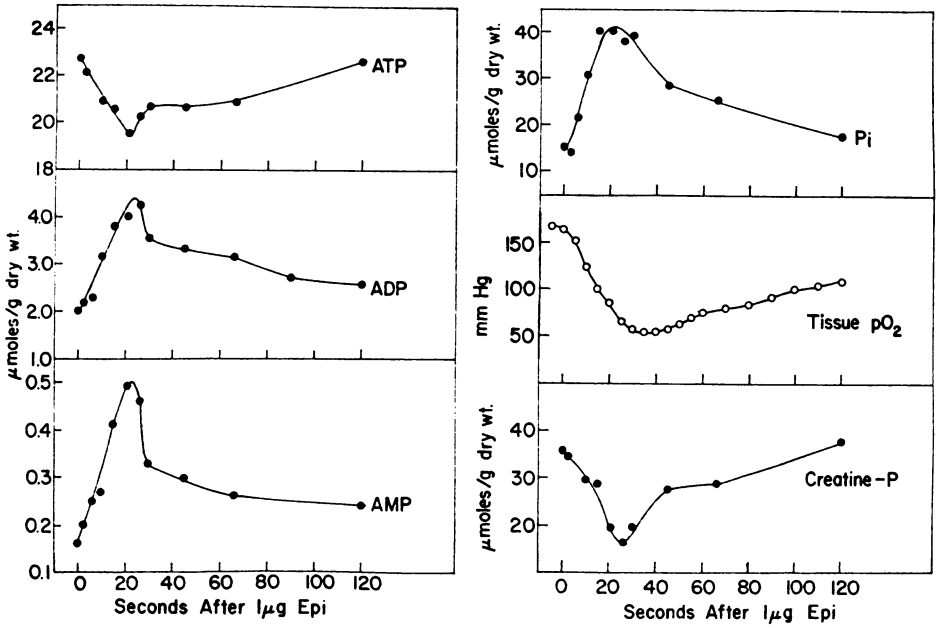

FIG. 3. Effect of epinephrine on phosphate compounds, and the tissue oxygen tension. The perfusion fluid contained 10 mM glucose and was not recirculated.

minimum is reached at about the same time as minima are observed in the ATP and Cr-P curves, and maxima in the ADP, AMP and P_i curves. These maxima and minima represent overshoots in the transient state before an increased rate of energy production can compensate for the increased energy demand due to the inotropic stimulus.

Analytical confirmation that the increased force of contraction precedes an increase of phosphorylase *a* and glucose-1-P, is shown in figure 4. The contractile force increases rapidly to a peak within 12 sec and declines, with an overshoot, to a plateau value 60% greater than the initial. Cyclic 3′,5′-AMP increases rapidly and in parallel with the contractile force from 1.2 to 7.1 mμmoles per g

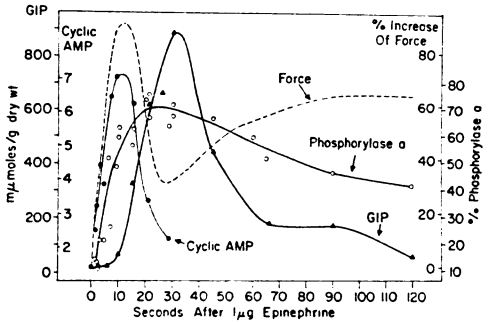

FIG. 4. Correlation of the force of contraction with the tissue content of cyclic AMP, glucose-1-P and phosphorylase *a*. Perfusion conditions as in figure 3.

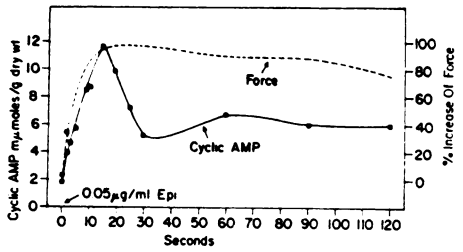

FIG. 5. Correlation of the force of contraction with the tissue content of cyclic AMP. Hearts were perfused without recirculation with fluid containing 10 mM glucose, 5×10^{-5} M EDTA and 0.05 μg/ml epinephrine.

dry weight, and subsequently declines to a value twice the initial after 30 sec. Phosphorylase *a* does not increase appreciably until 4 sec after the onset of the inotropic response, and reaches a maximum with 70% of the total phosphorylase in the *a* form after 20 to 25 sec. There is a slightly longer lag before glucose-1-P increases, and peak values are obtained after 30 to 35 sec. Thus, peak values of cyclic 3′,5′-AMP appear to correspond with the peak of the contractile force, while the phosphorylase *a* peak comes about 10 sec later, and the glucose-1-P peak occurs after a further 10 sec.

The relationship between the changes of contractile force and the levels of cyclic 3′,5′-AMP is of particular interest, and was studied further, as shown in figure 5. In this experiment, E at a concentration of 0.05 μg per ml was continuously present in the perfusate flowing to the heart. Under these conditions, the contractile force increases smoothly to a plateau value about 80% above the initial. The cyclic 3′,5′-AMP levels, however, increase rapidly to a peak, in much the same manner as in figure 4, and decline within 30 sec to a new steady state level about 3 times greater than the initial.

The close correlation between the rise of cyclic 3′,5′-AMP and the increase of contractile force on the one hand suggests that it is in some way involved in the inotropic action, but on the other hand the nonlinear relationship between contractile force and cyclic 3′,5′-AMP levels suggests that it is not the direct mediator. I would like to propose as a tentative hypothesis that Ca^{++} is the true mediator, and that cyclic 3′,5′-AMP exerts its effect by increasing the amount of ionized calcium in the heart. Further work in this area would clearly be of great value.

Finally, I would like to present the results of some preliminary experiments on the glycogen synthesizing system which were made in collaboration with Dr. J. Larner. Figure 6 shows the effects of E on the levels of glycogen and uridine diphosphoglucose (UDPG), and on the percentage of the total UDPG-α-glucan transferase in the glucose-6-phosphate independent ("I") form. Glycogen is rapidly mobilized as shown in the lower part of the figure, with a rather smaller decrease observed in the presence of glucose. The most interesting feature of these experiments is that while the percent of transferase "I" activity falls in the absence of glucose, it shows a pronounced, though transient, rise in the presence

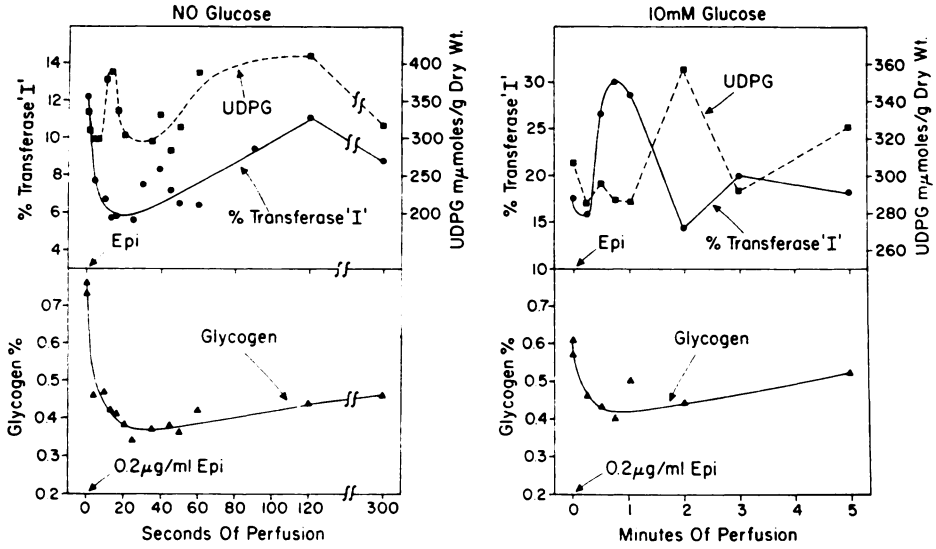

FIG. 6. Effect of epinephrine on the tissue content of glycogen, UDPG and UDPG-α-glucan transferase "I" activity.

of glucose. Since the rate of glycogen synthesis is critically dependent on the level of transferase "I", these results imply that in the presence of glucose there is not only an increased rate of glycogen breakdown, but also a more rapid rate of synthesis. These studies are in accordance with the findings of Williams and Mayer (7) that the percent of transferase "I" is increased by E in the intact rat heart, and previous studies of my own showing that E increases the rate of incorporation of C^{14}-glucose into glycogen (8).

Glucose-6-P has been shown not only to increase the activity of the transferase dependent ("D") form of the enzyme, but also to promote the "D" to "I" inter conversion (6). However, glucose-6-P levels increase to a similar extent in the presence and absence of glucose, and reach peak values after 30 to 35 sec (10, 11). There thus appears to be a specific effect of glucose on the transferase "D" to "I" interconversion. As seen from figure 6, the UDPG level shows rapid, but small fluctuations, as may perhaps be expected from the complex nature of the enzyme reactions determining its rate of production and utilization (5, 6).

SUMMARY

The application of methods with a fine time resolution to the study of metabolic events following administration of E to the perfused rat heart has shown that the increased force of contraction occurs prior to an elevation in the level of phosphorylase *a*. It is concluded, therefore, that neither energy produced from glycogenolysis nor an intermediate of glycogen breakdown is involved in the initiation of the inotropic response. Cyclic 3′,5′-AMP does, however, appear to be involved, but in a manner which remains to be elucidated. In cardiac muscle, when glucose is present, E enhances both the rate of glycogen breakdown and the rate of glycogen synthesis.

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