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THE EFFECT OF EPINEPHRINE ON ISOMETRIC TENSION AND PHOSPHORYLASE ACTIVITY OF THE ISOLATED RAT HEART*

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Abstract—The effect of small doses of epinephrine on isometric tension and phosphorylase activity of the isolated perfused rat heart was studied. The results of the experiments support our previous conclusion that the positive inotropic action of epinephrine on the heart is associated with a simultaneous increase in phosphorylase *a* activity.

IT IS well known that epinephrine and other sympathomimetic amines in addition to their cardiovascular effects have marked action on carbohydrate metabolism. Sutherland and Cori[1] found that epinephrine stimulated glycogenolysis in various tissues by activating the enzyme, phosphorylase. The investigations of Rall and Sutherland[2, 3] led to discovery of the coenzyme cyclic 3′,5′-adenylic acid and to the realization that this substance plays an important role in the activation of phosphorylase by epinephrine.

The possibility that the functional and biochemical actions of epinephrine are related is a subject of considerable theoretical and practical importance. Studies by Lundholm and Mohme–Lundholm[4] and Axelsson *et al.*[5] provide support for the view that the effects of epinephrine on smooth muscle are related to its action on glycogen metabolism. Similar conclusions were reached by Ellis[6] in his studies with heart and skeletal muscle. Cori[7] demonstrated that contraction of gastrocnemius muscle produced by nerve stimulation was associated with an increase in the activity of phosphorylase *a*. The positive inotropic action of epinephrine and other sympathomimetic amines on the heart was found by Hess and Haugaard[8] and Kukovetz *et al.*[9] to be concomitant with the activation of phosphorylase. These observations were supported by the studies of Mayer and Moran,[10] Belford and Feinleib,[11] and Lacroix and Leusen.[12] However, in a recent communication Mayer *et al.*[13] reported that in anesthetized, open-chest dogs the intravenous injection of epinephrine in doses less than 0·5 μg/kg produced significant increases in contractile force but no change in phosphorylase activity. Because of the importance of the subject and the apparent discrepancy of results we restudied the problem of the relation between the action of epinephrine on phosphorylase activity and contraction of the isolated perfused rat heart.

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METHODS

Hearts were perfused with a modified Locke solution by the Langendorf method and isometric tension was measured with a strain gauge and recorded on a Sanborn oscillograph. Varying doses of epinephrine dissolved in 0·2 ml saline were injected through a catheter placed close to the coronary openings. When the maximum drug effect was observed, the heart was frozen in dry ice–alcohol and an extract made for enzyme assay. In control experiments, 0·2 ml saline was injected. The experiments were carried out over a period of several weeks, and the drug concentrations were selected at random. Phosphorylase *a* and total phosphorylase activities were determined according to the method of Cori and Illingworth.[14] The reaction was carried out in a final volume of 1 ml containing 3·3 mg of tissue. A detailed description of experimental methods is found in a previous publication.[9]

EXPERIMENTS AND DISCUSSION

The work reported here is similar to our earlier study except that emphasis was placed on the determination of the mechanical and enzymatic effects of small doses of epinephrine. All the results of this series of experiments, including a statistical analysis, are presented in Table 1.

TABLE 1. EFFECT OF EPINEPHRINE ON ISOMETRIC TENSION AND PHOSPHORYLASE ACTIVITY OF THE PERFUSED RAT HEART

Dose (μg)	N	Phosphorylase *a* $\pm$ s.e.m. (%)	P*	N	Increase in isometric tension $\pm$ s.e.m. (%)	P*
Control	18	27·3 $\pm$ 0·94		18	−1·0 $\pm$ 1·96	
0·05	8	31·0 $\pm$ 1·51	<0·05	8	10·4 $\pm$ 2·90	<0·005
0·06	7	27·0 $\pm$ 2·31	not sig.	7	14·6 $\pm$ 5·36	<0·005
0·10	8	31·8 $\pm$ 1·57	<0·025	9	17·1 $\pm$ 4·94	<0·001
0·20	7	32·9 $\pm$ 1·43	<0·025	7	63·4 $\pm$ 14·29	<0·001
0·30	8	33·9 $\pm$ 2·39	<0·005	8	59·8 $\pm$ 12·91	<0·001
0·50	6	37·6 $\pm$ 2·40	<0·001	7	90·1 $\pm$ 13·26	<0·001
1·0	6	43·6 $\pm$ 3·44	<0·001	6	92·4 $\pm$ 11·96	<0·001

* Student's *t*-test was applied for differences between two means with different numbers of individuals. (For calculation see G. W. Snedecor, "Statistical Methods", the Iowa State College Press, 5th ed., 1956, p. 91.)

It is seen that each dose of epinephrine produced a significant increase in isometric tension. With the exception of the 0·06-μg dose, epinephrine also caused a statistically significant increase in the activity of phosphorylase *a*. A graphic representation of these results is given in Fig. 1.

The open circles indicate the mean per cent increases in isometric tension; closed circles indicate the mean values for the activity of phospho·ylase *a*. Ordinates were chosen so that the increase in tension and the phosphorylase *a* activity at the highest dose of epinephrine (1 μg) coincided. The curve was drawn to illustrate the mechanical response of the heart to increasing doses of epinephrine (open circles). Simultaneously with the change in tension there is a progressive increase in enzyme activity. It is difficult to measure the small alterations in enzyme activity produced by minute amounts of epinephrine. Nevertheless, at two doses of the drug (0·05 and 0·1 μg) which caused only minimal increases in tension, there was a statistically significant increase

in the activity of phosphorylase *a*. At higher doses of epinephrine both the enzymatic and mechanical effects are greatly increased and highly significant statistically. The experiments therefore support the conclusion that when the heart is stimulated by epinephrine the increase in force of contraction is accompanied by a rise in the activity of phosphorylase *a*. Significant increases in activity of phosphorylase *a* in heart have

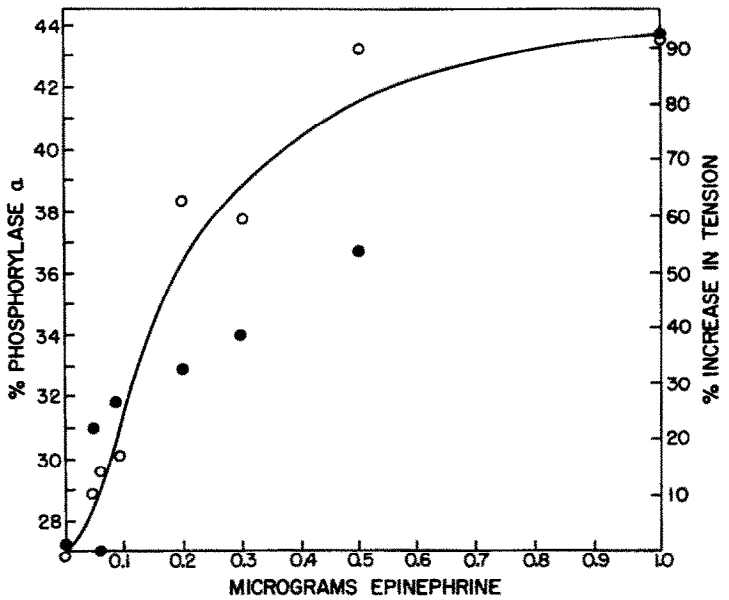

FIG. 1. The effect of epinephrine on isometric tension and phosphorylase activity of the isolated perfused rat heart.

been demonstrated after administration of ganglion-stimulating drugs[15] or electrical stimulation of the cardiac sympathetic nerves.[10] Both of these procedures produce an endogenous release of small amounts of catecholamines. These observations strengthen the view that phosphorylase activation plays a role in the action of sympathomimetic amines on heart function.

The problem of whether the activation of phosphorylase is the cause of the increased force of contraction or whether other biochemical or biophysical changes produced by epinephrine are more directly concerned with alterations of cardiac function remains to be decided by future experiments. Whatever the exact sequence of events may be that leads to cardiac stimulation by epinephrine, phosphorylase activation appears to play a part, possibly by making more energy available from the glycogen stores of the heart.

The experimental procedures used by Mayer *et al.*[13] differed in several respects from those employed by us.[9] The former investigators used open-chest dogs, and samples of heart tissue were taken for phosphorylase determinations. Therefore, there may be several reasons for the different results obtained by the two laboratories. Apart from the differences in experimental technique, it is possible that in the intact animal cardiovascular reflexes and humoral factors influence the action of epinephrine on

heart phosphorylase. Finally, it should be pointed out that the small increases in phosphorylase *a* activity produced by low doses of epinephrine are difficult to establish experimentally and that the two groups of investigators are in agreement that the positive inotropic effects of epinephrine at higher dose levels are associated with increases in the activity of phosphorylase *a*.

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