extracts is related to the concentration of cyclic 3,5-AMP, attempts were made to demonstrate the effects of serotonin on the synthesis of this compound. In collaboration with Drs. Sutherland and Rall, we have been able to demonstrate that particulate preparations from the liver fluke, when incubated with ATP and Mg, formed no significant amount of cyclic 3,5-AMP. However, addition of serotonin (1×10^{-5} M) to the reaction mixture stimulated the synthesis of the nucleotide. Epinephrine, when tested under the same conditions, did not have this effect. When NaF was added to the reaction mixture, serotonin (1×10^{-5} M) also caused a marked increase in the synthesis of the nucleotide, while epinephrine had no effect.

The effects of serotonin, in addition to our previous finding that serotonin is present in these organisms (3), suggest that this compound might stimulate both glycogenolysis and glycolysis. It is possible that serotonin, or a related compound, plays the same role in the carbohydrate metabolism of this invertebrate that epinephrine plays in that of higher organisms.

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DISCUSSION

THE EFFECT OF SYMPATHOMIMETIC AMINES ON PHOSPHORYLASE ACTIVITY OF THE ISOLATED RAT HEART[1]

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From the beautiful work of C. F. Cori, G. Cori, Sutherland and his co-workers, Krebs and Fischer and other investigators in this field we have learned much about the metabolic action of epinephrine (3) and about the role of phosphorylase in the regulation of blood sugar and its possible roles in muscular contraction (1, 2, 3).

We would like to report briefly experiments concerned with the action of epinephrine and other sympathomimetic amines on contraction of the isolated rat heart and on the state of the phosphorylase enzymes in this tissue. We believe that these experiments provide some support for the view discussed by Ellis this morning that at least some of the physiological actions of epinephrine and similar compounds are related to an effect at the early stages of glycogenolysis, or more specifically on the state of the phosphorylase enzymes.

Isolated rat hearts were perfused with Locke solution in a Langendorff apparatus and the contractions recorded on a smoked drum. At the end of an experiment the hearts were quickly frozen in a dry ice-alcohol slush. When drugs with a stimulating effect were administered, the hearts were frozen at the time of maximal response. If no stimulation occurred, the hearts were frozen about one minute after drug administration. Extracts were made from the frozen hearts and phosphorylase activity determined in the presence and absence of adenylic acid as described by Cori and Illingworth (2).

In previous experiments we had demonstrated that in hearts stimulated by the addition

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of aminophylline or epinephrine to the perfusion fluid there was an increased phosphorylase *a* activity and a decreased phosphorylase *b* activity compared to control hearts (5). We have since restudied the effect of epinephrine and also the action of other sympathomimetic amines.

The results of a series of experiments with known potent cardiac stimulating drugs are given in Figure 1.

In control experiments the phosphorylase ratio, *i.e.*, the units of phosphorylase activity without adenylic acid in % of the activity with adenylic acid, was 56. This ratio was significantly increased in hearts stimulated by *l*-epinephrine, *l*-norepinephrine or *dl*-isoproterenol, which indicated that a transformation of phosphorylase *b* to *a* had occurred in response to these drugs. The doses given in the figure are the total μg of base administered

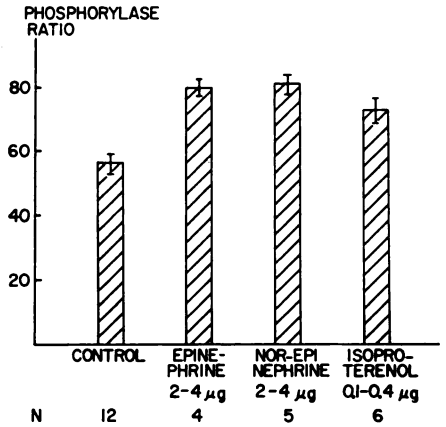

FIG. 1. Effect of cardiac-stimulating sympathomimetic amines on phosphorylase activity of the perfused rat heart.

For methods see the text. The doses are given as total amounts of free base. The bars represent the phosphorylase activity of a heart extract measured in the absence of adenylic acid in % of the activity in the presence of adenylic acid. The values for the SEM are ndicated on the bars.

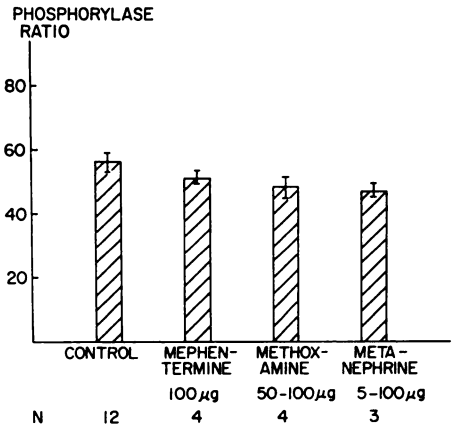

FIG. 2. Effect of sympathomimetic amines with weak cardiac stimulating action on the phosphorylase activity of the perfused rat heart. See text and legend to Figure 1.

in 0.2 ml saline solution by an infusion pump over a period of 12 seconds and delivered close to the orifices of the coronary arteries. The amounts of drugs used produced somewhat less than the maximal stimulation of the heart. It should be noted particularly that isoproterenol, which was about ten times as potent as epinephrine in stimulating the heart, was also about ten times as potent as epinephrine in its effect on phosphorylase activity. In studies with the rat diaphragm Ellis *et al.* (4) observed that isoproterenol was more active than epinephrine in stimulating glycogenolysis and also in improving contraction of the potassium-depressed diaphragm.

In a second series of experiments we studied the action of some sympathomimetic amines which are known to have only a weak or no stimulating action on the heart. The results of these experiments are shown in Figure 2.

Mephentermine, at the dose level used in these experiments (100 μg), stimulated the heart slightly and had no significant effect on the phosphorylase ratio. At 50 to 100 μg *dl*-methoxamine gave an inconsistent positive inotropic response, but had no effect on phosphorylase activity. Metanephrine, the product of O-methylation of epinephrine, used here in the racemic form, had no cardiac stimulating action and no effect on phosphorylase activity even at a dose level of 100 μg.

These experiments show that there is a rough parallelism between the ability of sympathomimetic amines to stimulate the heart and to increase phosphorylase *a* activity. Further quantitative studies at wider dose ranges of these and other drugs are needed to answer two important questions: 1) Is sympathomimetic stimulation of the heart always associated with an increased phosphorylase *a* to *b* ratio, and 2) is it possible for the phosphorylase ratio to increase without a mechanical effect on the heart?

We believe that the importance of this type of investigation lies in the simultaneous study of two parameters of the heart, mechanical activity and the activity of an enzyme system. The fact that there appears to be a parallelism between the action of certain drugs on cardiac activity and on phosphorylase indicates that these are related. An understanding of the nature of such an interrelationship must await further investigation.

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