ACTION OF EPINEPHRINE AND NOREPINEPHRINE IN BROKEN CELL PREPARATIONS[1]

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The hyperglycemic action of epinephrine is known to be due in part to the increased breakdown of glycogen in liver and muscle which occurs after the administration of this agent. This increased glycogenolysis has been shown to be the result of increased phosphorylase activity in these tissues after exposure to epinephrine either *in vitro* or *in vivo*.

The realization that phosphorylase activity of liver and muscle was subject to rapid and marked changes led to the investigation of the mechanism of these changes. Two enzymes were found in extracts of liver: one (inactivating enzyme) removed protein-bound phosphate residues from phosphorylase resulting in an essentially inactive enzyme and releasing inorganic phosphate; the other (reactivating enzyme or kinase) catalyzed the transfer of phosphate residues from adenosinetriphosphate (ATP) to the inactive phosphorylase with the restoration of enzymatic activity. A comparable situation exists in heart muscle.

Crude broken cell preparations of liver, suitably fortified with ATP and inactive phosphorylase, were found to catalyze the transformation from inactive to active phosphorylase relatively slowly unless epinephrine (or glucagon) was added. Centrifugation of homogenates at low speed resulted in preparations which no longer displayed increased active phosphorylase formation in the presence of epinephrine. Incubation of the low speed precipitate with ATP and epinephrine (or glucagon) gave rise to a heat-stable material which, in turn, stimulated active phosphorylase formation in supernatant fractions of liver homogenates.

The heat-stable factor was isolated and identified as a new cyclic mononucleotide, adenosine-3′,5′-phosphoric acid (3,5-AMP). The formation of 3,5-AMP by tissue preparations appeared to involve the cyclization of ATP, the fate of the remaining two phosphate residues of ATP being unknown.

The ability to form 3,5-AMP did not appear to reside in any of the classical subcellular organelles, as prepared by differential centrifugation. Although most of the activity appeared in the so-called nuclear fraction, active preparations could be obtained which contained no discernible intact nuclei. Cell membranes, which fractionate in an unknown fashion, were considered one possible locus.

Particulate fractions from homogenates of many tissues were capable of forming 3,5-AMP from ATP. In addition to liver, these included brain, heart and skeletal muscle, intestinal muscle and mucosa, aorta and uterus. As yet, the accumulation of 3,5-AMP has been shown to be increased by the addition of epinephrine only in preparations from liver, heart and skeletal muscle. Caffeine

[1] The subject matter of this discussion will be incorporated in a forthcoming publication to appear in a later issue of *Pharmacological Reviews*. Therefore, only a brief summary of the presentation at THIS SYMPOSIUM is included here.

increased the accumulation of the nucleotide in all tissues tested, apparently by inhibiting the hydrolysis of the compound to 5′-AMP by a very active diesterase which was also inhibited by theophylline.

Thus the glycogenolytic action of epinephrine in liver, heart, and skeletal muscle appears to be a result of the following sequence of events: epinephrine interacts with some insoluble component of the cell, giving rise to an increased accumulation of 3,5-AMP, which, in turn, interacts with more dispersed material, resulting in an increased conversion of inactive to active phosphorylase. The relation of one or more of the events in this sequence to other effects of epinephrine has not been clarified. It is interesting to note that particulate fractions from dog heart were most sensitive to isopropylnorepinephrine, less but equally sensitive to *l*-epinephrine and *l*-norepinephrine, and even less sensitive to *d*-epinephrine, as judged by the ability of these agents to increase 3,5-AMP accumulation. Furthermore, the heart phosphodiesterase which hydrolyzes and inactivates 3,5-AMP is even more sensitive to theophylline than to caffeine.

DISCUSSION

ACTIONS OF SEROTONIN AND EPINEPHRINE ON INTACT AND BROKEN CELL PREPARATIONS FROM THE LIVER FLUKE, *FASCIOLA HEPATICA*

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I would like to discuss briefly some aspects of the effects of epinephrine and of serotonin on the carbohydrate metabolism of a trematode parasite, the liver fluke, *Fasciola hepatica*. This organism metabolizes carbohydrate at a high rate. Production of propionic and acetic acids in an approximate ratio of 3:1 accounts for almost all of the carbohydrate utilized anaerobically. Only 4 to 8% of the metabolized carbohydrate is converted to lactic acid. Contrary to its effect on higher organisms, neither epinephrine nor norepinephrine has any action on the carbohydrate metabolism of these trematodes. On the other hand, serotonin and other indolalkylamines at low concentrations cause an increase in glucose utilization, glycogen breakdown and lactic acid production (1). Production of volatile fatty acids is not affected to a significant degree by serotonin. Since serotonin, but not epinephrine, stimulates glycogenolysis in the flukes, the possibility was considered that serotonin might increase the activity of phosphorylase in these organisms. In order to prove this hypothesis, phosphorylase activity was determined in cell-free homogenates of flukes which had been cultured with serotonin as well as in control flukes cultured in the absence of this indolalkylamine. It was found that flukes which have been cultured with serotonin, either in the presence or in the absence of glucose, showed higher phosphorylase activity than the controls. The increase in phosphorylase activity produced by serotonin was observed in the presence or absence of adenosinemonophosphate. This has shown that by either assay method phosphorylase activity is increased in flukes cultured with serotonin (2).

Since it was shown by Sutherland and Rall that activation of phosphorylase in cell-free