ACTIVATION OF PHOSPHOFRUCTOKINASE FROM THE LIVER FLUKE *FASCIOLA HEPATICA* BY SEROTONIN AND CYCLIC 3′,5′-AMP*

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THE physiological importance of serotonin in mammalian tissues has not been determined. Evidence, however, has been accumulating indicating that serotonin has a function in invertebrate tissues similar to that of epinephrine in higher animals[1,2,3]. The experiments discussed below are part of an investigation on the role of serotonin in the carbohydrate metabolism of the liver fluke, *Fasciola hepatica*.

This organism metabolizes carbohydrate at a high rate. Production of propionic and acetic acids accounts for almost all the carbohydrate utilized anaerobically. Only 4–8% of the metabolized carbohydrate is converted to lactic acid[4]. Contrary to its effect on higher organisms, neither epinephrine nor norepinephrine has any action on the carbohydrate metabolism of these trematodes. Serotonin and other indolalkylamines at low concentration caused an increase in glucose utilization, glycogen breakdown and lactic acid production[5]. Production of volatile fatty acids was not affected to a significant degree by serotonin. These changes in the rate of glycolysis and glycogenolysis by serotonin were always accompanied by an increase in the rhythmical movement of these organisms[5,6]. Therefore, the stimulant effect of serotonin on muscular activity is associated with a shift from fatty acids to lactic acid formation. This indicates that in this organism lactic acid fermentation could meet increased energy requirements more efficiently than fatty acid fermentation.

Investigations were carried out to locate the enzyme or enzymes principally affected when the rate of glycolysis was increased by serotonin. The enzymes required for the series of reactions in the Embden-Meyerhof scheme of phosphorylating glycolysis (Fig. 1) were shown to be present in the parasite. The rate of lactic acid production from dif-

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ferent substrates was measured in homogenates from control flukes as well as from flukes preincubated with serotonin[7]. As in the case of the intact organisms the rate of lactic acid formation from glucose by control homogenates from flukes preincubated with serotonin was increased. This was accompanied by an increase in glucose utilization.

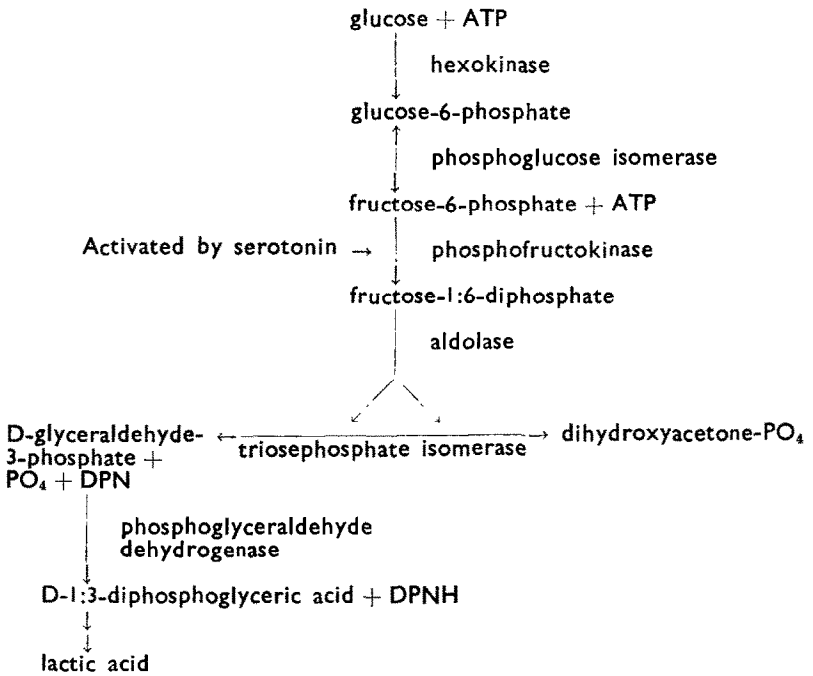

FIG. 1. Glycolytic reactions and enzymes of *Fasciola hepatica*.

The increase in lactic acid production was also observed when glucose-6-phosphate (G-6-P) or fructose-6-phosphate (F-6-P) was used as the substrate. When fructose diphosphate (FDP) was the substrate the production of lactic acid by control homogenates was markedly increased indicating that the concentration of this ester limits the rate of glycolysis. The increase in glycolysis in homogenates from flukes pretreated with serotonin was much less when FDP was the substrate. An example of these experiments is shown in Fig. 2. This indicates that the stimulation of glycolysis by preincubation with serotonin was brought about by increasing the formation of FDP from F-6-P. This reaction is catalyzed by phosphofructokinase which phosphorylates F-6-P to FDP by ATP (Fig. 1).

Further evidence concerning the serotonin-induced increase in activity of phosphofructokinase in intact organisms was obtained by determining enzymatically the concentration of the hexosephosphate esters (G-6-P, F-6-P, FDP)[7]. In control parasites which produce lactic acid at

a low rate the concentrations of G-6-P and F-6-P accumulated were much higher than that of FDP. Therefore, the reactions glucose → F-6-P are faster than those of F-6-P → lactic acid indicating that phosphofructokinase limits the rate of glycolysis in these organisms. On the other hand, in serotonin-treated parasites which produce high amounts of lactic acid the concentration of G-6-P and F-6-P accumulated was reduced while the concentration of FDP was increased. The results indicate that the hexosemonophosphates are being removed at a faster rate in serotonin-treated flukes due to the increased phosphofructokinase activity.

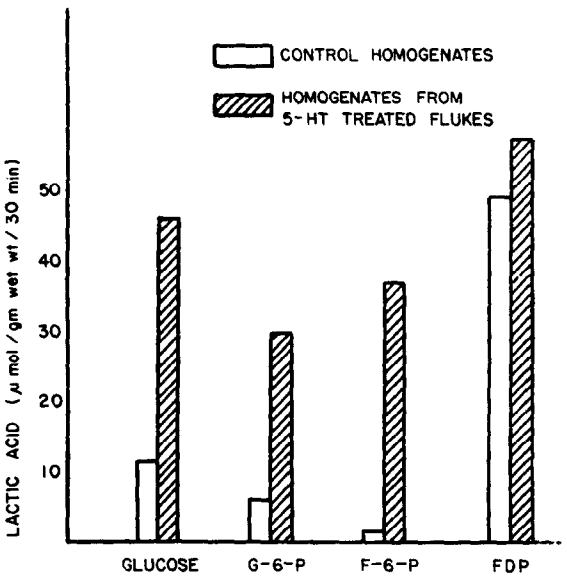

FIG. 2. Lactic acid production in homogenates from control and serotonin (5-HT)-treated flukes in the presence of different substrates. Incubation was carried out in an atmosphere of nitrogen for 30 min. Final molar concentrations of the constituents in the medium were: potassium glycylglycine buffer 5×10^{-2}M pH 7·5; $MgCl_2$ 8×10^{-3}M; ATP 1×10^{-2}M; DPN 7×10^{-3}M; nicotinamide 3×10^{-2}M; potassium arsenate 1×10^{-2}M and substrate 5×10^{-3}M.

Further evidence supporting the view that phosphofructokinase is stimulated in flukes preincubated with serotonin was obtained by assaying the enzyme in both homogenates. This was carried out by measuring the rate of FDP formation from F-6-P and ATP in the presence of Mg^{++}. The reaction in the assay mixtures was stopped by perchloric acid. The amounts of FDP in aliquots of the neutralized mixtures were determined enzymatically through the aldolase, glyceraldehyde-3-phosphate dehydrogenase system. It was found that while phosphofructo-

kinase activity in control homogenates was very low enzyme activity was markedly increased in homogenates from serotonin-treated flukes.

Since it was reported that serotonin increases the production of cyclic 3′,5′-AMP by fluke homogenates[3] the possibility was considered that activation of phosphofructokinase by serotonin might be mediated via this nucleotide. A similar mechanism was shown to occur in relation to the activating action of epinephrine on phosphorylase[8]. Both serotonin and cyclic 3′,5′-AMP, when added directly to the phosphofructokinase assay mixtures, caused marked increase in enzyme activity. The activating effect of serotonin was dependent on the presence of a heavy particulate fraction (2000 × *g*), ATP, Mg^{++}. It was, however, possible to demonstrate the effect of the cyclic nucleotide in a soluble fraction (105,000 × *g*). Since conditions for stimulation of the synthesis of cyclic nucleotide were found to be identical to those for phosphofructokinase activation by serotonin it is possible that the latter effect is mediated via the synthesis of the cyclic 3′,5′-AMP.

Investigations on a partially purified phosphofructokinase from these homogenates have revealed that activation by the cyclic nucleotide is dependent on the presence of ATP and Mg^{++}. Kinetic studies have revealed that there is an inverse relationship between substrate concentrations and the degree of activation by the cyclic nucleotide. The activation by this nucleotide was maximal when the substrate was present in suboptimal concentrations. An increase in the concentration of ATP beyond optimal concentration caused an inhibition in the activity of phosphofructokinase. Cyclic 3′,5′-AMP protected the enzyme from the inhibitory action of ATP. Mg^{++} in great excess did not appreciably inhibit the enzyme.

It is concluded that the increase in phosphofructokinase can account, at least in part, for the increase in glycolysis by serotonin in intact flukes. A mechanism that controls the activity of phosphofructokinase in mammalian tissues has not yet been elucidated. The presence of such a mechanism is strongly suggested by the recent work of Newsholme and Randle[9]. They reported that in both rat diaphragm and rat heart, anoxia caused an increase in the phosphorylation of glucose which was accompanied by a fall in the levels of G-6-P and F-6-P and a rise in the level of FDP. These changes are identical to those caused by serotonin on the liver fluke.

Evidence available suggests that the effect of serotonin is an indirect action and might be mediated via the cylic 3′, 5′-AMP. It is not yet possible to offer a definite explanation for the mechanism by which the cyclic nucleotide activates this enzyme. The fact that the activation process is maximal when the substrate is present in suboptimal concentration suggests that the cyclic nucleotide might increase the affinity of the enzyme to the substrate. The fact that cyclic 3′, 5′-AMP activates

the enzyme while an excess of ATP inhibited its activity raises the possibility that intracellular concentrations of both nucleotides might control the activity of phosphofructokinase in the liver fluke.

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