Preliminary Notes

The action of adenosine 3′,5′-monophosphate on the incorporation of leucine into liver proteins

Glucagon and adrenalin are known to exert several important effects on the metabolism of the liver besides their well-known action on glycogenolysis. To mention only the effects observed on isolated liver preparations, both hormones have been found to inhibit the incorporation of acetate and other precursors into fatty acids[1,2] and cholesterol[3], and to stimulate the production of ketone bodies[2,4] and of urea[5]. More recently, we have reported an inhibitory influence of the glycogenolytic agents on the incorporation of amino acids into the proteins of liver slices[6].

According to the investigations of SUTHERLAND and his co-workers[7,8], the earliest known metabolic event involved in the glycogenolytic effect of glucagon or adrenalin is the formation from ATP of the cyclic nucleotide adenosine 3′,5′-monophosphate. This nucleotide then promotes the conversion of inactive dephospho-phosphorylase into the phosphorylated active form of the enzyme, which in turn is responsible for the increased rate of phosphorolysis of glycogen.

It has been shown[9] that 3′,5′-AMP, when added in small amounts to liver slices incubated in a phosphate–saline medium, stimulates glucose production and glycogen breakdown. Earlier experiments[4] from this laboratory have demonstrated that the nucleotide also exerts a ketogenic effect under the same conditions. The results re-

TABLE I

ACTION OF GLUCAGON AND 3′,5′-AMP ON LEUCINE INCORPORATION

About 170 mg of rabbit-liver slices were incubated in 3 ml 0.12 *M* NaCl, 0.02 *M* potassium phosphate, pH 7.4, and 10^{-5} *M* L-leucine uniformly labelled with ^{14}C and corresponding to a radioactivity of 0.05 μC/ml. When added, glucagon was present at a concentration of 33 μg/ml and the nucleotides were $3.3 \cdot 10^{-4}$ *M*. The flasks were incubated with shaking for 45 min at 37° under pure O_2. The proteins were isolated by a conventional procedure involving precipitation and extensive washing with trichloroacetic acid, removal of nucleic acids and lipids, and treatment with performic acid.

Expt.	$\mu C \times 10^6$ *incorporated/mg protein*		
	Control	*Glucagon*	*3′,5′-AMP*
78	122	82 (— 33 %)	77 (— 37 %)
79	370	270 (— 27 %)	230 (— 38 %)
80	208	178 (— 15 %)	155 (— 25 %)
81	89	74 (— 17 %)	70 (— 21 %)
86	100	86 (— 14 %)	82 (— 18 %)
3′-AMP	101 (+ 1 %)		
87	166		119 (— 28 %)
3′-AMP	167 (+ 1 %)		
5′-AMP	160 (— 4 %)		

Abbreviations: ATP, adenosine triphosphate; 3′,5′-AMP, adenosine 3′,5′-monophosphate; 3′-AMP, adenosine 3′-monophosphate; 5′-AMP, adenosine 5′-monophosphate.

ported here and summarized in Table I indicate that 3′,5′-AMP likewise inhibits the incorporation of amino acids into the proteins of rat-liver slices incubated in a phosphate–saline medium, to at least the same extent as does glucagon.

In other experiments not described here, little or no effect of 3′,5′-AMP on the incorporation of amino acids into proteins was observed when either rat- or rabbit-liver slices were incubated in a Krebs–bicarbonate medium, whereas glucagon and adrenalin were inhibitory under these conditions[6]. Neither has it been possible to reproduce with the nucleotide the effects of the glycogenolytic agents on the incorporation of acetate into fatty acids or cholesterol in liver slices incubated in a Krebs–bicarbonate medium. However, 3′,5′-AMP also does not stimulate glucogenolysis in this medium, and it seems very probable, therefore, that the nucleotide does not penetrate sufficiently rapidly within the cells or is too rapidly inactivated to be effective in a Krebs–bicarbonate medium.

In view of the results obtained in phosphate–saline, it is reasonable to conclude that 3′,5′-AMP is a common mediator in the various metabolic effects exerted by glucagon and adrenalin on the liver. On the other hand, it seems unlikely that the actions of the cyclic nucleotide on lipid metabolism could be secondary consequences of the activation of phosphorylase[3]. Various experiments have led to the same conclusion with respect to the effect of the glycogenolytic agents on protein synthesis. It appears, therefore, that 3′,5′-AMP must influence one or more additional enzyme systems besides that involved in the activation of phosphorylase. The nature of these systems raises interesting problems.

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The nature of the fluorescence of an enzyme-reduced coenzyme-reduced substrate complex

This paper presents evidence that L-glutamate decreases the dissociation of the glutamic dehydrogenase–TPNH complex, and that the increase in the intensity of fluorescence of that complex caused by the addition of L-glutamate is due solely to the increase in the amount of TPNH bound rigidly to the enzyme surface.

Abbreviations: TPNH, DPNH, reduced tri- and diphosphopyridine nucleotides; GAD, glutamic dehydrogenase.