GLUCAGON EFFECT ON RAT LIVER PROTEIN SYNTHESIS IN VIVO

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SUMMARY. The in vivo effect of glucagon administration on hepatic polyribosomal profiles has been studied. Glucagon did not change significantly total, free or bound polyribosomal fractions 30-45 minutes after its administration. The combined administration of glucagon plus antiinsulin serum failed to show any significant effect of glucagon over the antiinsulin serum treated control. Glucagon increased valine production in the perfused isolated liver. These results suggest that the well known amino acid catabolic action of glucagon may be preferentially mediated through an increased proteolysis. Since it is known that glucagon increases considerably in vivo the liver cyclic AMP levels then its lack of effect on polyribosomal profiles might indicate that the postulated role for the cyclic nucleotide on liver protein synthesis must be taken cautiously.

INTRODUCTION

Glucagon administration is known to increase urea levels in vivo as well as in the perfused isolated liver preparation (1,2). This catabolic action has been teleologically interpreted as to favor gluconeogenic demands increased during fasting (3). At present is not clear whether or not this glucagon effect on amino acid catabolism is an expression of a decreased protein synthesis, an increased proteolysis or a combination of both. Furthermore, glucagon increases liver cyclic AMP (4) and this has been suggested as to play a role in protein synthesis regulation through its effect on ribosomal protein phosphorylation (5,6). On these grounds, it was deem desirable to study the effect of glucagon administration on hepatic protein synthesis in vivo. The present report deals with the study of rat liver polyribosomes profiles after glucagon administration under different metabolic conditions. Polyribosomal profiles have been taken as a true reflection of the rate of protein synthesis in vivo (7).

MATERIAL AND METHODS

200 g. male Wistar rats, fed or fasted for 10-12 hrs., were used in these experiments. When indicated 200 µg. glucagon were injected 30-45 minutes before the rats were sacrifized by decapitation. Livers were extracted and rapidly sumerged in ice cold buffer A (buffer A: 0.1 M Tris, pH 7.5; 0.15 M sucrose; 0.025 M KCl and 0.008 M $MgCl_2$).

The livers of six rats were pooled and homogeneized with 2 volumes of buffer A. Nuclei, mitochondria and lysosomes were removed by centrifugation at 20,000 x g for 15 minutes and the upper 4/5 of the supernatant was retained as the cytoplasmic fraction. The cell sap was obtained by centrifugation of the cytoplasmic fraction at 150,000 x g for 2 hours.

The different polyribosomal populations were separated following the procedure of Andrews and Tata (8) by layering 4 ml. of the cytoplasmic fraction on a discontinuous sucrose gradient, contining liver cell sap, made up as described by VanVenrooij et al. (9).

For the determination of the polyribosomal profiles 20 A_{260} of total, membrane bound and free polyribosomes were layered on a 20-40 per cent sucrose gradient, made up in tubes for the Beckman type SW41 rotor, in medium A and centrifuged for 90 minutes at 41,000 rpm. After centrifugation the polyribosomal profiles were analyzed at 260 mμ with a continuous flow monitoring system with a disc integrator attached to it.

The polyribosomal fraction was taken to be the area of the polyribosomes region of the recorded absorbance pattern divided by the area represented by the monomers plus dimers peaks.

Glucose was measured in deproteinized blood samples with hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (10).

Livers isolated from 200 g. male rats were perfused with a hemoglobin-free non-recirculating medium according to Miller et al. (11).

Valine was measured by an isotopic dilution method involving aminoacylation of transfer RNA (12).

RESULTS AND DISCUSSION

The total polyribosomal fractions from control or glucagon treated rat livers were determined (Table I and fig. 1, A and B). Glucagon did not significantly affect liver polyribosomal fractions of either fed or fasted rats.

Liver polyribosomes occur either attached to endoplasmic reticulum membranes or free in the cytoplasm. The membrane bound polyribosomes are supposed to be engaged in serum protein synthesis, while hepatic cell proteins would be built up by free polyribosomes (13,14). Since glucagon has been described to induce an autophagic process of hepatic ergastoplasmic membranes (15) as well as to induce the synthesis of certain enzymes such as tyrosine transaminase (16) and serine dehydratase (17), the question was raised on the possibility of a selective glucagon effect upon the two polyribosomes populations, remaining the total polyribosomal profiles almost unchanged. Table II shows that glucagon treatment did not change significantly the polyribosomal

TABLE I. Glucagon effect on total polyribosomal fractions from liver of fed and fasted rats.

		Polyribosomes / monomers + dimers
Fed	Control	1.6
	Glucagon	1.3
Fasted	Control	1.5
	Glucagon	1.6

Total polyribosomes were extracted after glucagon treatment and the polyribosomal fractions determined as described in methods.

TABLE II. Glucagon effect on total, membrane bound and free polyribosomal fractions from livers of fed rats.

	Polyribosomes / monomers + dimers		
	Total	Free	Bound
Control	2.78	2.20	2.07
Glucagon	2.50	1.99	1.79

The conditions of the experiment were as described in methods.

TABLE III. Antiinsulin serum and glucagon effect on hepatic total polyribosomal fractions and blood glucose levels of fed rats.

	Polyribosomes / monomers + dimers	Blood glucose concentration (mM).
Control	2.78	4.45 ± 0.5
AIS	1.50	7.85 ± 0.7
AIS plus glucagon	1.26	8.70 ± 0.3

Antiinsulin serum was injected either alone or with glucagon to fed rats. Polyribosomal fractions and blood glucose were determined as described in methods. Results are mean values of six experiments ± SEM.

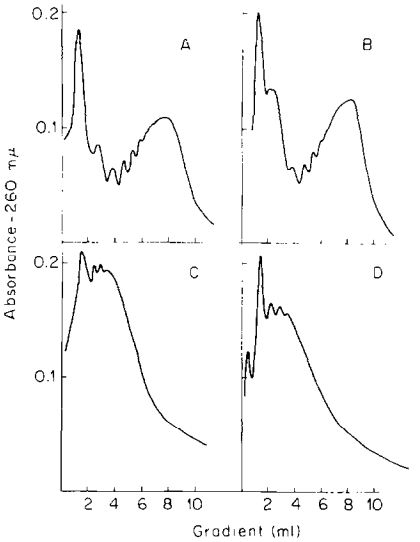

Figure 1. Glucagon and antiinsulin serum effect on hepatic total polyribosomal profiles from fed rats.

When indicated antiinsulin serum was injected to fed rats without or inmediately before glucagon. Total polyribosomal profiles were obtained as described in methods. (A): control; (B): glucagon; (C): antiinsulin serum; (D): antiinsulin serum plus glucagon.

fractions of any of the different polyribosomes populations in liver of fed rats.

Glucagon is a well known stimulant of insulin release in vivo (18) as well as in vitro (19). In order to eliminate the possibility of a lack of glucagon effect due to an increased insulin secretion resulting in glucagon/insulin ratios that could mask the glucagon induced catabolism (20), experiments were carried out to study the glucagon effect in the presence of antiinsulin serum (AIS). Lack of insulin brought about by AIS treatment produced an intense shift from polyribosomes tp monomeric ribosomes (Fig. 1,C).

Glucagon, although it increased blood glucose values over the AIS treated animals (Table III), did neither significantly increase nor prevent this AIS catabolic effect (Fig. 1, D). Table III shows the calculated polyribosomal fractions of the control, AIS, and AIS plus glucagon treated animals. These results suggested that glucagon effect on amino acid catabolism might not be the consequence of a decreased protein synthesis.

In order to study the glucagon effect on liver proteolysis isolated rat livers were perfused with or without added glucagon. Rates of release of a poorly oxidized amino acid, such as valine, were determined as an index of the rates of proteolysis. Results on Table IV shows an increase of 380 percent of valine release in the presence of 1.7×10^{-9} M glucagon, indicating

TABLE IV. Glucagon effect on the rate of valine production by the perfused isolated liver.

	Valine released µmol/g. L.W./hr.	Per cent stimulation.
Control	1.3 ± 0.2	___
Glucagon	3.4 ± 0.7	380

Rat liver was perfused as described in methods with and without 1.7×10^{-9} M glucagon added. Results are mean values of six experiments ± SEM.

a net proteolytic effect of the hormone. Similar increase in the rate of valine production induced by glucagon has been recently reported (21).

The above described results indicate that glucagon induced urea production may be facilitated by an enhanced proteolysis, rather than by a decreased protein synthesis.

Glucagon is known to increase hepatic cyclic AMP levels (4). In liver two cyclic AMP dependent protein kinases have been described catalyzing the phosphorylation of some of the ribosomal proteins (22). On the other hand it has been postulated that the ribosomal proteins phosphorylation may play a regulatory role on protein synthesis (5,6,24). If glucagon mediated increase in liver cyclic AMP levels results in an activation of ribosomal protein phosphorylation in vivo (24), the above reported data seems to suggest that the regulatory role of this protein phosphorylation would bring about qualitative rather than quantitative changes in protein synthesis.

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