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EFFECT OF GLUCAGON ADMINISTRATION ON MICE LIVER FRUCTOSE-1, 6-BISPHOSPHATASE

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SUMMARY

Intravenous administration of glucagon in mouse (200 ug/ 100 gm body wt), stimulated liver fructose-1,6-bisphosphatase at physiological pH by approximately 100% within 15 minutes. The stimulation was not due to protein synthesis. Similar stimulation was also observed on administration of cyclic AMP. Removal of the adrenal gland abolished the stimulatory effect of glucagon but not of cyclic AMP.

INTRODUCTION

Previous studies indicate that the hormonal regulation of gluconeogenesis is mediated at the steps catalyzed by certain key enzymes (1-6). One site lies between phosphoenolpyruvate and pyruvate and the second between fructose 6-phosphate and fructose 1,6-bisphosphate. Recently, Taunton et al (7,8) reported that the fructose 1:6-bisphosphatase(Fru-P_2ase)[1] activity of rat liver enzyme significantly increases within a short period after the intravenous infusion of glucagon whereas the activities of phosphofructokinase and pyruvate kinase are decreased. They further showed that insulin antagonizes the action of gluca-

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Abbreviations : Fru-P_2ase, Fructose-1,6-bisphosphatase; Fru-P_2, Fructose-1, 6-bisphosphate.

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gon at the level of these enzymes. Current trend of research on glucagon action indicates that the hormone acts through the mediation of cyclic AMP(9). We now show that the activity of mouse liver Fru-P_2ase measured at physiological pH is enhanced after the administration of glucagon or cyclic AMP, and that the effect of glucagon and not cyclic AMP is abolished by adrenalectomy, which of itself does not significantly alter the activity of Fru-P_2ase.

MATERIALS AND METHODS

Glucagon, 200 µg/100 gm body wt or dibutyryl cyclic AMP (0.05 mM/kg in 0.1 ml of 0.9% NaCL) was injected into the tail vein of mice (14gm-18gm wt) and the control group received the same volume of normal saline. Both experimental and control groups were sacrificed after 15 mins of glucagon or cyclic AMP injection and the liver was homogenized with 0.25M sucrose containing 1 mM Tris-HCl pH-7.4. The supernatant, after centrifugation for 40 min at 12000g, was used for determination of Fru-P_2ase activity (10). The assay system in a final volume of 1 ml consisted of triethanolamine-diethanolamine buffer (pH-7.2) 200 mM, EDTA 0.1 mM, $MgCl_2$ 2.0 mM, NADP 0.4 mM; ammonium sulfate 40 mM, Fru-P_2 0.1 mM and 2 µg each of purified phosphofructose isomerase and glucose-6-phosphate dehydrogenase. The reaction was started by the addition of Fru-P_2 and the reduction of NADP was measured at 340 mµ in a Carl Zeiss Spectrophotometer (model PM Q II). One unit of the enzyme is defined as that amount that hydrolyses 1 µmol of Fru-P_2 per minute. Protein was measured by the method of Lowry *et al* (11) using bovine serum albumin as the standard.

RESULTS

The results presented in table I show that the administration of glucagon (200 µg/100 gm body weight) increases the Fru-P_2ase activity significantly. The intravenous administration of dibutyryl cyclic AMP(0.05 mM/kg in 0.1 ml of 0.9% NaCl) mimics the glucagon effect on Fru-P_2ase in mice. The stimulation in both the cases was approximately 100% over the control. However, glucagon had no additive stimulatory effect on Fru-P_2ase activity when injected 2 mins after dibutyryl cyclic AMP administration.

The activity of Fru-P_2ase increased at all pH values, the maximum increase (about 115%) being at pH 7.2 (Table II).

TABLE - I

FRUCTOSE -1,6-BISPHOSPHATASE ACTIVITY IN MOUSE LIVER AFTER INTRAVENOUS ADMINISTRATION OF GLUCAGON AND DIBUTRYL CYCLIC 3'-5' ADENOSINE MONOPHOSPHATE

Group	Fru-P_2ase Activity* Units/gm liver
Control	10.96 ± 0.05 (14)
Control + Glucagon	22.85 ± 0.06 (10)
Control + Dibutyryl cAMP	21.78 ± 0.10 (8)

*Values are Mean ± S.E. Figures within bracket indicate number of animals.

TABLE - II

EFFECT OF INTRAVENOUS INJECTION OF GLUCAGON ON THE ACTIVITY OF FRUCTOSE-1,6-BISPHOSPHATASE AT DIFFERENT pH IN MOUSE LIVER.

pH	Fru-P_2ase activity* Units/gm of liver		
	Control	Glucagon	% of Stimulation
6.5	7.23	10.85	50
7.2	10.16	21.84	114
8.0	7.22	12.72	70
9.0	7.59	12.77	55

*Average values from four animals.

Removal of the adrenal gland did not significantly affect Fru-P_2ase activity but it abolished the stimulatory effect of glucagon and not of cyclic AMP (Table III). Glucagon also stimulated Fru-P_2ase activity of rabbit liver and kidney (Table IV).

DISCUSSION

Involvement of Fru-P_2ase in glucagon-mediated hepatic gluconeogenesis has been suggested by Clark et al (4). They further postulated that glucagon acts through cyclic AMP at the site of Fru-P_2ase and phosphofructokinase. In this communication we present evidence that glucagon at a dose of 200 µg per 100 gm body wt stimulates Fru-P_2ase assayed at pH 7.2 by about 100%. The increase in activity was less at other pH values. The increase is not due to the synthesis of new enzyme as glucagon stimulates Fru-P_2ase activity in animals pretreated with actinomycin D or cyclohexamide (results not shown here). Our results confirm the previous findings of Taunton et al (7,8) who measured the activity only at the non-physiological pH of 8.8.

It is well known that cyclic AMP stimulates gluconeogenesis (3-5, 9). The Fru-P_2ase activity, found after dibutyryl cyclic AMP administration in our experiments, is consistent with the results obtained by Taunton et al (7,8). Glucocorticoids have permissive role which facilitate the action of glucagon in gluconeogenesis (12,13). Our results with adrenalectomized animals confirm the above hypothesis as removal of adrenal glands abolishes the stimulation of Fru-P_2ase activity after the intravenous administration of glucagon without affecting the enzyme activity significantly by itself. It is also interesting to note that unlike glucagon, cyclic AMP increases the Fru-P_2ase activity in adrenalectomized animals. This observation points to two important suggestions that :

TABLE - III

EFFECT OF ADRENALECTOMY ON THE GLUCAGON STIMULATED FRUCTOSE 1:6 BISPHOSPHATASE ACTIVITY IN MICE LIVER

Group	Fru-P_2ase activity* Units/gm of liver
Control	10.65 ± 0.03
Control + Glucagon	20.90 ± 0.04
Adrenalectomized	7.61 ± 0.02
Adrenalectomized + Glucagon	9.55 ± 0.21
Adrenalectomized + dibutryl cAMP	19.10 ± 0.370

*Values are mean ± SE from five animals.

TABLE - IV

EFFECT OF GLUCAGON ON FRU-P_2ASE ACTIVITY OF RABBIT LIVER AND KIDNEY

Tissue	System	Fru-P_2ase activity* Units/gm liver	
		pH 7.2	pH 9.2
Liver	Control	3.32	2.17
	Glucagon	5.76	3.32
Kidney	Control	2.3	-
	Glucagon	4.2	-

*Average of three experiments.

1) cyclic AMP probably activates a protein kinase which in turn activates Fru-P_2ase by phosphorylation and 2) the effect of glucagon on cyclic AMP levels is probably indirect. Very recently, Riou et al (14) have reported increased activity of liver Fru-P_2ase through phosphorylation of the enzyme by cyclic AMP dependent protein kinase. Thus, it seems the stimulation of Fru-P_2ase activity by glucagon, observed in the present communication, might have taken place by the same mechanism.

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