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EFFECT OF GLUCAGON ON ALANINE 2-OXOGLUTARATE AMINOTRANSFERASE

Ratna B Banerjee, Ranjan Bhadra and Asoke G. Datta*

Indian Institute of Chemical Biology
4 Raja S. C. Mullick Road, Calcutta-700032, INDIA

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Alanine 2-oxoglutarate aminotransferase activity in mouse liver is stimulated by the intravenous injection of glucagon. The stimulation is abolished by pretreatment with actinomycin D indicating that the increased activity is probably due to new enzyme formation. Administration of dibutyryl cyclic AMP, isoproterenol, an activator of adenyl cyclase and theophylline, an inhibitor of phosphodiesterase also increases the enzyme activity suggesting the involvement of cyclic AMP in glucagon-mediated increase of enzyme activity.

Gluconeogenesis from alanine (1) is stimulated by glucagon in the perfused liver (2,3) as well as in isolated hepatocytes (4,5). The transport of alanine across the plasma membrane is also stimulated by glucagon (6). The conversion of alanine to pyruvate, one of the precursors of gluconeogenesis, is catalysed by alanine 2-oxoglutarate aminotransferase (EC 2.6.1.2) (7). The activity of this enzyme has been reported to be increased during starvation, treatment with glucocorticoids, feeding of high protein diet (8) and in alloxan-induced diabetes (9). The effect of glucagon on this enzyme *in vivo* has, however, not been reported.

The mode of action of glucagon is not clear. Previously we have reported that glucagon stimulates fructose 1,6-bisphosphatase and that this stimulation is insensitive to actinomycin D or cycloheximide (10). Claus *et al* (11) have also reported that glucagon causes activation of fructose 1,6-bisphosphatase by phosphorylating the enzyme. On the other hand, administration of glucagon has been shown to stimulate

*To whom correspondence should be sent.

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certain enzymes in rat liver (12-15) and administration of actinomycin D prior to glucagon administration inhibits RNA synthesis, responsible for the formation of serine dehydratase (15). In another publication, Claus et al (16) have reported that glucagon brings about an inhibition of phosphofructokinase due to changes in the level of an allosteric effector. This effector was subsequently shown to be fructose 2,6-bisphosphate (17). In a more recent publication, Hers (18) showed that glucagon causes a decrease in the intracellular concentration of fructose 2,6-bisphosphate, an inhibitor of fructose 1,6-bisphosphatase and as a result, gluconeogenesis is increased.

The present communication reports that intravenous administration of glucagon stimulates alanine 2-oxoglutarate aminotransferase in mouse liver and that this effect is probably mediated via cyclic 3' 5'-adenosine monophosphate.

Materials and Methods

Glucagon, dibutyryl cyclic 3',5'-adenosine monophosphate, isoproterenol, actinomycin D, theophylline were obtained from Sigma Chemical Co., St. Louis, Missouri. Glucagon (40 µg/100 g body weight except for the dose response curve), dibutyryl cyclic 3',5'-adenosine monophosphate (0.05 mM/Kg in 0.1 ml of 0.9% NaCl) or isoproterenol (40 µg/100 g) were injected via the tail vein of Swiss albino mice, IICB inbred strain (25-28 g body weight). The inhibitors, actinomycin D (100 µg/100 g) or theophylline (2,4 mg/100 g) when used, were injected intraperitoneally two hours before the administration of the stimulating agents. The control and experimental group of animals were sacrificed 15 min after the administration of the stimulators. The livers were homogenised in a medium containing 0.225 M mannitol, 0.075 M sucrose, 0.01 M Tris-HCl buffer of pH 7.8, and 0.05 mM EDTA (19). After centrifugation for 40 min at 10,000 x g, the supernatant was used for the determination of alanine aminotransferase activity. The assay system was similar to that described by Segal and Matsuzawa (20). The reaction was started by the addition of L-alanine (17 mM) and the decrease in absorbance due to oxidation of NADH was measured at 340 nm. The definition of one unit of enzyme activity was as defined by Segal and Matsuzawa (20). Protein was measured by the method of Lowry et al (21), using bovine serum albumin as standard.

Results and Discussion

The results presented in Table 1 show that the administration of glucagon increased the activity of alanine 2-oxoglutarate aminotransferase significantly. The stimulation by glucagon was abolished by

Table 1. The effect of glucagon on the activity of L-alanine 2-oxoglutarate aminotransferase in vivo

Treatment	No. of observations	Units/g liver	% above control value	P value
Control	13	17.74 ± 4.20	-	-
Glucagon	11	30.13 ± 4.81	69.8	<0.001
Control + actinomycin D	3	21.34*	-	-
Glucagon + actinomycin D	3	21.4*	-	-

Results expressed as means ± S.D.

*Mean of three experiments

actinomycin D suggesting that glucagon might have exerted its effect through increased enzyme synthesis. In a different set of experiment cycloheximide, another inhibitor of protein synthesis, was also found to abolish the glucagon effect (data not shown here). A similar effect has been reported for tyrosine transaminase (22), serine dehydratase (15,22) and phosphoenol pyruvate carboxykinase (22).

The increase of enzyme activity was found to be maximal at 40 μg of glucagon per 100 gm body weight and at that dose, maximum enzyme activity was observed at 15 min after glucagon administration (Fig. 1). Hence, in all subsequent experiments these conditions were maintained. Kinetic studies indicate that the apparent K_m of the enzyme preparations from control and glucagon treated animals for both the substrates remained unchanged (L-ala =14.3 mM : α-ketoglutarate = 0.208 mM). However, the V_{max} of the glucagon-stimulated enzyme for both alanine and α-ketoglutarate (α-KG) were increased as compared to the control (Fig. 2).

The action of glucagon is known to be mediated via increased intracellular concentration of cyclic AMP (23). As shown in Table 2, intravenous administration of dibutyryl cyclic AMP (db-cAMP) mimics the glucagon effect. The stimulation in both the cases was approximately 70% over the control value. Theophylline, a phosphodiesterase inhibitor (24) was also found to increase alanine aminotransferase.

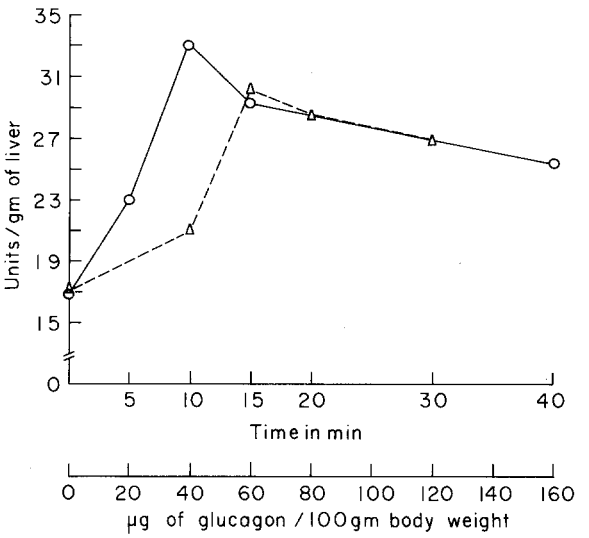

Fig. 1 : Time-course (dotted line) and dose-response (solid line) curve of alanine-2-oxoglutarate aminotransferase. a) Glucagon (20 to 160 µg/100 g body weight) was administered to mice. Enzyme was prepared as described in the text. b) After administering glucagon (40 µg/100 g body weight), the mice were sacrificed at various time intervals and enzyme activity was determined.

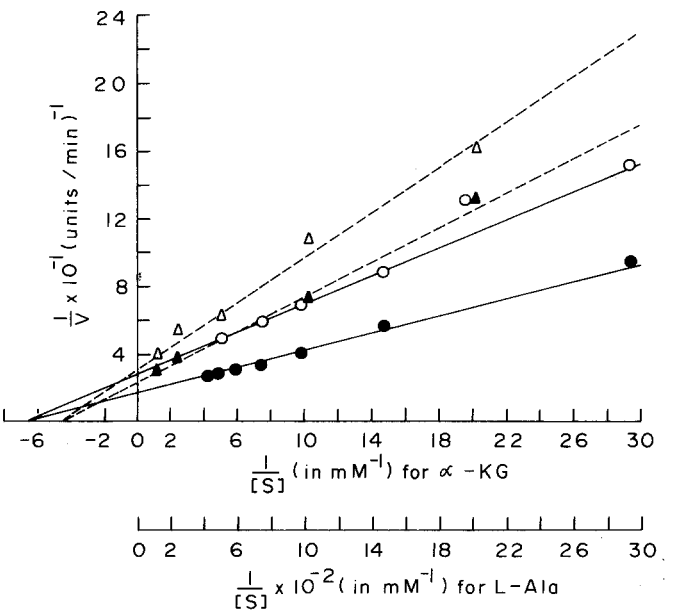

Fig. 2 : Lineweaver-Burke Plot of alanine 2-oxoglutarate aminotransferase. a) Control, in presence of α-KG (Δ) and L-ala (O). b) Glucagon-treated, in presence of α-KG (▲) and L-ala (●). c) App. V_{max} [α-KG] = 0.031 units/min (control) and 0.042 units/min (glucagon). d) App. V_{max} [L-ala] = 0.034 units/min (control), 0.056 units/min (glucagon).

Table 2. Stimulation of alanine 2-oxoglutarate aminotransferase by various agents *in vivo*.

Treatment	No.of observations	Units/g liver	% above control value	P value
Control	8	16.52 ± 3.50		
Dibutyryl C-AMP	4	28.31 ± 5.84	71.4	<0.02
Theophylline	8	28.97 ± 7.48	75.4	<0.001
Isoproterenol	4	31.12 ± 3.33	88.4	<0.001

Results expressed as means ± S.D.

If the increase in alanine aminotransferase by glucagon is mediated by cyclic AMP, one would expect that isoproterenol, an activator of adenyl cyclase (25) should also enhance the activity of the enzyme. Table 2 shows that isoproterenol did enhance the enzyme activity. However, the administration of theophylline together with db-cAMP or isoproterenol and glucagon with isoproterenol did not elicit a further increase (data not shown). This may be due to the accumulation of excess cyclic AMP which has been reported to have an inhibitory effect above optimal concentrations (26).

From these results it may be premature to decide whether the increased activity after glucagon administration is due to modification of the enzyme or to increased enzyme synthesis. Immunoprecipitation experiments with antibodies raised against the purified enzyme should help to settle this question.

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