HORMONAL REGULATION OF ACETYL CoA CARBOXYLASE

EFFECT OF INSULIN AND EPINEPHRINE

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SUMMARY

Acetyl CoA carboxylase in the isolated epididymal fat tissue is activated by insulin and inactivated by epinephrine and dibutyryl cAMP. Insulin activation of the enzyme occurs in the presence of inhibitors of protein synthesis and can be observed as early as 15 min. A time dependent inactivation of the enzyme occurs in the presence of ATP and Mg^{++} in vitro.

INTRODUCTION

Recently, we reported evidence in support of the theory that rat liver acetyl CoA carboxylase (EC 6.4.1.2) is regulated by phosphorylation and dephosphorylation of the protein (1). The phosphorylated form of the carboxylase is inactive or partially active, whereas the dephosphorylated form is fully active. The establishment of the two forms of acetyl CoA carboxylase and consideration of the strategic position which the enzyme occupies in the metabolic pathway suggested to us that the transformation between the two forms of acetyl CoA carboxylase would be controlled by hormones; activation by insulin and inactivation by epinephrine.

Studies of the mode of insulin stimulation of fatty acid synthesis from glucose in adipose tissue indicate that insulin affects one or more steps beyond the facilitation of glucose uptake (2). One of these insulin-stimulated steps has been identified as the activation of pyruvate dehydrogenase (3-5). This activation appears to be a result of dephosphorylation of pyruvate dehydrogenase.

In this communication, we present evidence that the rate limiting enzyme in the synthesis of long chain fatty acids, acetyl CoA carboxylase,

is activated by insulin and inactivated by epinephrine treatment of epididymal fat tissue *in vitro*.

MATERIALS AND METHODS

Insulin (24.1 units/mg), epinephrine, ATP, and crystalline bovine serum albumin were purchased from Sigma. Acetyl CoA was from Nutritional Biochemicals; $NaH^{14}CO_3$ (54.5 mCi/mM) from International Chemicals and Nuclear. Glass fiber filters were the product of Wilkens-Anderson Co., Clifton, N. J.

Male Wistar albino rats from the rat colony of the Biochemistry Department (Purdue University), weighed about 100-150 g and were fed a standard laboratory diet. In certain experiments, tissues from animals fasted for 48 to 72 hours were used.

Treatment and incubation of epididymal fat tissue were carried out as described by Taylor *et al.* (5), with a slight modification. Insulin treatment was carried out in the presence of 1.2% bovine serum albumin while epinephrine treatment was carried out in the presence of 3% bovine serum albumin.

After the incubation, the tissues were washed with Krebs-Ringer solution and homogenized in cold buffer (3 ml per g of tissue) with 20 passes of a motor-driven glass homogenizer. The homogenization buffer contained 50 mM Tris-HCl, pH 7.4; 1 mM EDTA; 5 mM mercaptoethanol and 1 mg/ml bovine serum albumin. The infranatant fraction was obtained by centrifugation at 12,000 x g for 10 minutes at 4°. In some experiments the protein fraction precipitated at 45% ammonium sulfate saturation (6) was used.

Acetyl CoA carboxylase was assayed by measuring $^{14}CO_2$ incorporation into malonyl CoA by the method of Majerus *et al.* (7), except that, after removal of protein, an aliquot of inactivated reaction mixture was plated on a glass-fiber filter, dried and counted for radioactivity.

The standard reaction mixture contained the following components (in μmoles) in 0.25 ml: Tris·HCl, pH 7.4, 17.5; dithiothreitol, 0.50; EDTA,

TABLE I

Effect of Insulin, Dibutyryl cAMP and Epinephrine on Acetyl CoA Carboxylase

Exp.	Treatment	Acetyl CoA Carboxylase (nmoles $^{14}CO_2$ incorporated/min/g tissue)
1	Control	19.4
	Insulin (3mμ/ml)	28.0
	Insulin (3 mμ/ml), Puromycin (50 μg/ml)	27.2
	Insulin (3 mμ/ml), Cycloheximide (50 μg/ml)	28.2
2	Control	11.0
	Insulin (3 mμ/ml)	20.8
3	Control	30.4
	Epinephrine (10^{-7} M)	23.0
	Epinephrine (10^{-5} M)	12.8
	Epinephrine (10^{-3} M)	11.2
	Dibutyryl cAMP (10^{-3} M)	12.2

The epididymal fat tissues from the following types of animals were used: in experiment 1, 150 g ± 10 g and fasted for 72 hours; in experiment 2, 100 g ± 10 g and fasted for 72 hours; in experiment 3, 150 g ± 10 g and fed ad libitum. The tissues from 10 to 20 animals were divided into 0.3 g/4 ml incubation medium with a appropriate additions, and incubated for 60 min at 37°C. Homogenization of the tissues after incubation and assay for acetyl CoA carboxylase are described in the Methods.

0.05; potassium citrate, 0.98; $MgCl_2$, 0.98; ATP, 0.98; $MnCl_2$, 0.245; acetyl CoA, 0.039; $KH^{14}CO_3$, 1.98 (6.65×10^5 cpm); bovine serum albumin, 0.15 mg and an appropriate amount of enzyme preparation to give about 1000 to 2000 cpm of $^{14}CO_2$ incorporation. The reaction was carried out at 30°C for 2 mins. Under these conditions, the reaction was a linear function of protein concentration and time up to 15 min.

RESULTS AND DISCUSSION

Since the complex interplay of various hormones which takes place in

the whole animal can be eliminated in studies of the isolated epididymal fat pad, the effects of insulin and epinephrine on acetyl CoA carboxylase can be independently analysed. As shown in Table I, the changes in activity of the enzyme are mediated directly by treatment with insulin or epinephrine *in vitro*. Insulin activation of acetyl CoA carboxylase (3 munits/ml) is observed as early as 15 minutes after the addition of hormone. However, full activation of the enzyme required about 60 minutes. The activation of the enzyme is independent of protein synthesis, since neither puromycin nor cycloheximide exerts any significant effect on insulin activation. However, the degree of the insulin effect depends on whether the tissue was taken from fed or starved animals. The maximum effect of insulin, i.e. up to 2-fold stimulation, was observed in tissue taken from smaller rats (100 to 150 g) which had been starved for 72 hours. When larger and normally fed rats were used, the effect of insulin was minimal and stimulation of the enzyme was less than 20%. This suggests that acetyl CoA carboxylase in the normally fed animals occurs mostly in the activated state, whereas the inactivated state of the enzyme predominates in the starved animals. Apparently, the enzyme is transformed to the inactive form in starved animals under the influence of increased amounts of epinephrine or glucagon. This conclusion is supported by studies of the effect of epinephrine on the tissues from the normally fed animals *in vitro*.

Incubation of fatty tissue with epinephrine (1 mM) resulted in a 65 to 75% inactivation of acetyl CoA carboxylase in 60 minutes (Table 1). Inactivation of the enzyme is also effected by dibutyryl cyclic AMP, as shown in Table 1. Maximum inactivation was observed in the presence of 1 mM dibutyryl cyclic AMP in 60 minutes. Similar observations have been reported recently with the rat liver enzyme (8).

As in the case of the rat liver system, cell-free enzyme preparations from normal tissue can be readily inactivated by the addition of ATP and

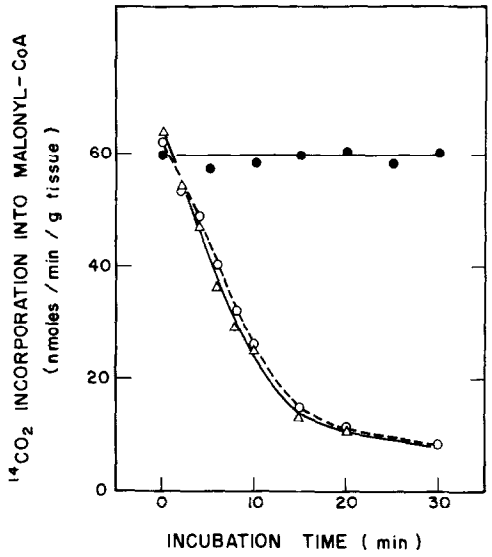

Fig. 1. Effect of ATP and Magnesium on Acetyl CoA Carboxylase Activity *in vitro*.
The cell-free enzyme preparation from the normally fed animals was passed through a column of Sephadex G-25. The enzyme fraction was preincubated at 30° for 15 min, and then incubated in the presence of 4 mM ATP and 4 mM $MgCl_2$ with 1 mM cAMP (Δ——Δ) or without cAMP (0----0). Control had no additions (●——●). 50 μl enzyme was taken at the times indicated and assayed for acetyl CoA carboxylase activity as described in the Methods.

Mg^{++}, as shown in Fig. 1. Since acetyl CoA carboxylase is modulated by temperature (10,11), in all experiments using cell-free enzyme preparation there was a preincubation period of 15 min at 30° before the actual experiment. Such preincubation increases the activity about 2-fold. The extent of inactivation is closely correlated to that observed with the activities obtained in the tissues treated with epinephrine. However, this inactivation in cell-free extracts is independent of cAMP and the presence of cAMP made no difference in the inactivation kinetics (Fig. 1). The same situation was observed with the rat liver enzyme (1). As shown in Table I, dibutyryl cAMP alone can inactivate the enzyme when tissues are treated. Therefore the lack of the cAMP effect *in vitro* is not expected. Although the amount of catalytically active cAMP dependent protein kinase (9) in this tissue may explain such an observation, this lack of a cAMP effect on the inactivation of acetyl CoA carboxylase by ATP and Mg^{++} is reminiscent

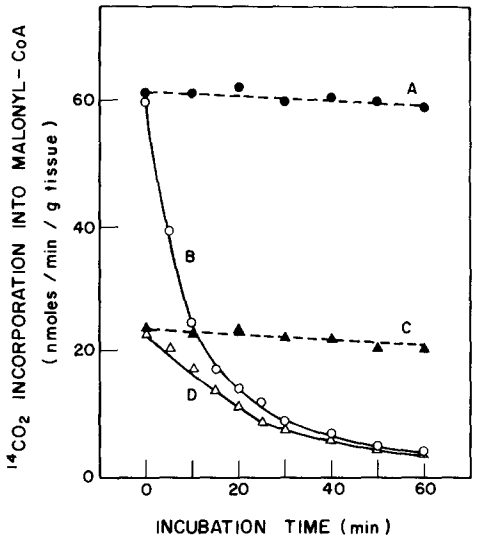

Fig. 2. The infranatant fractions were brought to 45% saturation of ammonium sulfate. The precipitate was dissolved in a small amount of buffer and passed through a column of Sephadex G-25. The enzyme preparations was preincubated for 15 min at 30°. The assay was carried out as for Fig. 1. A (●---●), enzyme from control tissue with no additions; B (○——○), enzyme from control tissue with the addition of 4 mM ATP and 4 mM $MgCl_2$; C (▲---▲), enzyme from epinephrine treated tissue without any additions; D (△——△), enzyme from epinephrine treated tissue with 4 mM ATP and 4 mM $MgCl_2$.

of the pyruvate dehydrogenase system. Pyruvate dehydrogenase is affected similarly by epinephrine (5), however, the role of cAMP in the inactivation of pyruvate dehydrogenase *in vitro* is not apparent (11).

When the tissues from the normal animals were preincubated with epinephrine for various periods, the enzyme prepared from this tissue was inactivated by ATP and Mg^{++} only to the extent that the preparation from the normal tissue can be inactivated by ATP and Mg^{++} (Fig. 2). In this experiment, the pretreatment of tissues with epinephrine resulted in an inactivation of about 60%. The enzyme preparation from tissues pretreated with epinephrine was inactivated further by ATP and Mg^{++} addition, to the level which is reached by the control preparation. Such an observation suggests that inactivation mediated by epinephrine may involve the same mechanism as does the ATP and Mg^{++} mediated inactivation. Also, this *in vitro* inactivation is clearly not the result of formation of long chain

fatty acyl CoA (8) since the system is not sufficient to generate such a metabolite.

Although *in vitro* inactivation by ATP and Mg^{++}, in accordance with phosphorylation of the protein, can readily be demonstrated, it has not yet been possible to consistently demonstrate the reverse reaction, the activation expected in the presence of a high concentration of magnesium (up to 20 mM). The failure of high concentrations of Mg^{++} to activate the enzyme in crude preparations suggests that the protein phosphatase may become very labile when the cells are broken. The lability of the phosphatase was previously observed in the case of rat liver preparations (1). Data presented in this communication establish that acetyl CoA carboxylase activity is directly regulated by insulin and epinephrine without involving protein synthesis. Further experiment must be carried out to determine whether the hormones regulate the transformation between the phosphorylated and dephosphorylated forms of the enzyme.

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REFERENCES

1. Carlson, C. A., and Kim, K.-H. (1973) J. Biol. Chem. 248, 378-380.
2. Jungas, R. L. (1970) Endocrinology 86, 1368-1375.
3. Jungas, R. L. (1971) Metabolism 20, 43-51.
4. Denton, R. M., Coore, H. G., Martin, B. R., and Randle, P. J. (1971) Nature New Biol. 231, 115-116.
5. Taylor, S. I., Mukherzee, C., and Jungus, R. L. (1973) J. Biol. Chem. 248, 73-81.

6. Vagelos, P. R., Alberts, A. W., and Martin, D. B. (1963) J. Biol. Chem. 238, 533-540.
7. Majerus, P. W., and Kilburn, E. (1969) J. Biol. Chem. 244, 6254-6262.
8. Allred, J. B., and Roehrig, K. L. (1973) J. Biol. Chem. 248, 4131-4133.
9. Corbin, J. D., Soderling, T. R., and Park, C. R. (1973) J. Biol. Chem. 248, 1813-1821.
10. Halestrap, A. P. and Denton, R. M. (1973) Biochem. J. 132, 509-517.
11. Linn, T. C., Pettit, F. H., and Reed, L. J. (1969) Proc. Nat. Acad. Sci. U.S.A. 62, 234-241.