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INDUCTION OF ACETYL-CoA CARBOXYLASE IN PRIMARY RAT HEPATOCYTE CULTURES BY GLUCOSE AND INSULIN

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SUMMARY

Acetyl-CoA carboxylase, which catalyzes the initial step of fatty acid synthesis, was studied in primary cultures of hepatocytes from adult rats. The enzyme activity could be enhanced between 24 h and 48 h in culture about twofold by elevation of the insulin concentration from 10^{-9} to 10^{-7} mol/l and about threefold by increasing the glucose concentration from 5 to 20 mmol/l. Both effects were additive. The increase of activity was linear with time over 24 h and could be blocked by either cordycepin or cycloheximide, which suggests that the activity enhancement was due to induction rather than due to allosteric effects or interconversion.

INTRODUCTION

Acetyl-coenzyme A carboxylase (EC 6.4.1.2), the first enzyme in fatty acid biosynthesis, is of fundamental significance for this pathway (1-3). Short term control of the enzyme activity can be effected by allosteric regulation (4-6) as well as by hormone dependent interconversion (7, 8). Long term control is based on hormone and diet dependent enzyme induction (9, 10) and degradation (11, 12). Insulin therapy of diabetic rats as well as refeeding of starved animals with carbohydrates lead to a marked increase of the acetyl-CoA carboxylase level. Yet, unequivocal information does not seem to be available whether carbohydrates increase the enzyme level by itself or by an elevation of the insulin concentration. This may be due to the difficulty to differentiate primary and secondary effects of an inducing agent in the complex system of the intact animal. On the other hand less complex systems like incubated liver slices, isolated perfused liver or suspensions of isolated hepatocytes are not suited for

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Dedicated to Prof.Dr.H.Holzer on the occasion of his 60th birthday

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longer lasting induction experiments because of their short survival time. However, primary cultures of hepatocytes, which are prepared from adult animals and can be maintained for at least a few days without loss of liver specific functions (13-16), represent a clear system for the study of enzyme induction. In the present investigation the long term regulation of acetyl-CoA carboxylase by insulin and glucose was studied in primary hepatocyte cultures. The enzyme activity could be enhanced about twofold by high insulin and about threefold by high glucose concentrations. In either case the increase was linear with time and could be prevented by inhibitors of protein synthesis which indicates enzyme induction rather than activation. Some of the results have been reported in a preliminary abstract (17).

MATERIAL AND METHODS

Fetal calf serum, culture medium and coenzymes were obtained from Boehringer GmbH, D-6800 Mannheim. Collagenase type II, penicillin, streptomycin sulfate were from Sigma, D-8021 Taufkirchen. Bovine insulin, dexamethasone, cycloheximide, cordycepin and bovine serum albumin were supplied by Serva, D-6900 Heidelberg. [^{14}C] bicarbonate was from Amersham Buchler, D-3300 Braunschweig.

Hepatocytes were isolated from fed male Wistar rats (150-180 g, Winkelmann, D-4790 Paderborn) as described earlier (16). The cells were cultured in Medium 199 with Earle's salts (18) containing defatted serum albumin 2g/l (19), $NaHCO_3$ 18 mmol/l, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid 15 mmol/l, streptomycin sulfate 117 mg/l and penicillin 60 mg/l. The osmolarity was adjusted to 285 mosm/l, the pH of the medium gassed with Carbogen was 7.40. The standard medium contained 10^{-9} mol/l insulin and 10^{-9} mol/l dexamethasone. About 2 x 10^6 cells in 3 ml standard medium containing 5 % fetal calf serum were inoculated into 60 mm culture dishes, which were coated with a collagen film. Incubation was carried out at 37°C in an incubator gassed with humidified 13 % O_2, 5 % CO_2 and 82 % N_2. After 4 and 24 h the standard medium was changed and fetal calf serum was then omitted. The enhancement of acetyl-CoA carboxylase was initiated after 24 h by elevation of the insulin or/and glucose concentration as indicated. In order to avoid a lactate acidosis the medium was changed twice within 24 h in cultures with high glucose concentrations.

After washing with 0.9 % NaCl the cell monolayers were collected with a rubber policeman and homogenized at 23°C in 0.6 ml NaCl 120 mmol/l, KCl 25 mmol/l, 2-mercaptoethanol 2 mmol/l, ethylenediaminetetraacetic acid 0.1 mmol/l, KH_2PO_4 20 mmol/l, $KHCO_3$ 6 mmol/l, pH 7.2 using a Potter homogenizer. The acetyl-CoA carboxylase activity was determined according to (20, 21) with the following modification: a 12 000 $\bar{g}$ x 15 min supernatant was preincubated in presence of potassium citrate 5 mmol/l, $MgCl_2$ 5 mmol/l, albumin 1 g/l at 37°C for 30 min. The assay of enzyme activity was immediately commenced as described (21). DNA was determined according to (15).

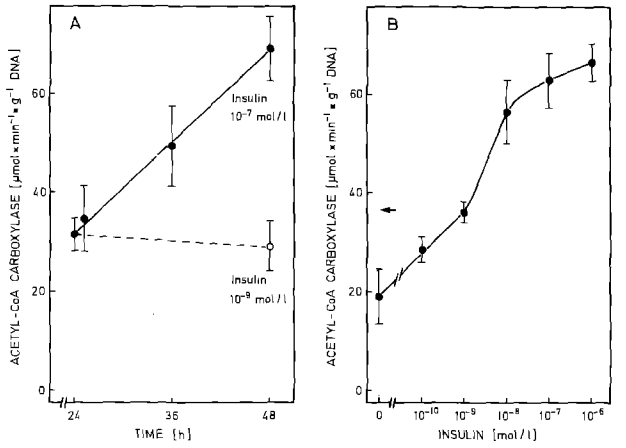

Fig. 1. Induction of acetyl-CoA carboxylase in primary cultures of hepatocytes by insulin: A) Time dependence, B) Insulin dependence. Liver parenchymal cells of male rats were cultured under standard conditions. The induction period between 24 h and 48 h in culture was initiated by medium change and addition of insulin. Values are means ± SEM of 6 to 12 cultures. The initial value is indicated by an arrow.

RESULTS

Within the first day of culture a rapid decrease of acetyl-CoA carboxylase activity occurred. However, after this initial period only a small further loss in enzyme activity was observed under standard culture conditions. Thus the long term influence of insulin and glucose on the enzyme was studied during the second day of culture. The enhancement of the insulin concentration to 10^{-7} mol/l led to a significant increase of acetyl-CoA carboxylase activity to about 200%, which was linear with time for at least 24 h (Fig. 1A). The acetyl-CoA carboxylase activity was decreased without insulin to about 60% during 24 h, it was nearly maintained in presence of 10^{-9} mol/l insulin and could be enhanced to 160 % and more by 10^{-8} mol/l or higher concentrations of insulin (Fig. 1B). A simultaneous elevation of the dexamethasone concentration to 10^{-7} mol/l resulted in a slight reduction of this increase.

An elevation of the basic glucose concentration to 20 mmol/l led to a significant increase of the acetyl-CoA carboxylase activity, which was linear with time and reached more than 300 % within 24 h (Fig. 2A). The enhancement of carboxylase activity showed a similar dependence on glucose concentration in the ab-

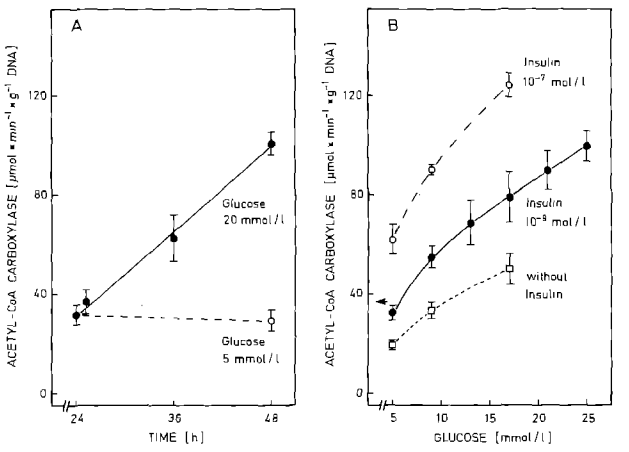

Fig. 2. Induction of acetyl-CoA carboxylase in primary cultures of hepatocytes by glucose: A) Time dependence, B) Glucose dependence. Liver parenchymal cells of male rats were cultured under standard conditions. The induction period between 24 h and 48 h in culture was initiated by medium change and addition of glucose. The insulin concentration was 10^{-9} mol/l, if nothing else is mentioned. Values are means ± SEM of 6 to 12 cultures. The initial value is indicated by an arrow. For comparison: physiological insulin levels in the portal vein: $\leq 3 \times 10^{-9}$ mol/l (29).

sence or presence of low or even high insulin concentrations (Fig. 2B). Since insulin and glucose dependent enhancements were additive (Fig. 2B), maximal absolute enzyme activity was obtained in presence of high insulin and glucose concentrations. High levels of other carbohydrates e.g. mannose and galactose up to 20 mmol/l as well as lactate up to 10 mmol/l led to a slight increase of acetyl-CoA carboxylase activity only which corresponded to the slight rise of the glucose concentration due to glucose formation from mannose, galactose or lactate. 2-Deoxyglucose was completely ineffective.

Both the insulin as well as the glucose dependent increase of carboxylase activity were inhibited by either cordycepin or cycloheximide which specifically block the protein synthesis on the transscriptional or translational level (Tab. 1). During periods up to 8 h these inhibitors did not produce any dramatic changes of the cell morphology. Thus it could be concluded that the enhancement of acetyl-CoA carboxylase activity was due to induction. With regard to the relatively slow carboxylase induction it would be desirable to study the induction and its inhibition over a longer

Table 1 Inhibition of insulin and glucose dependent enhancement of the acetyl-CoA carboxylase level by cycloheximide and cordycepin

	Acetyl-CoA carboxylase [μmol x min^{-1} x g^{-1} DNA]		
Addition:	-	Insulin	Glucose
--	52.5 ± 3.6	71.8 ± 5.7*	70.3 ± 2.3*
cordycepin	53.2 ± 5.7	59.5 ± 4.0	57.2 ± 0.3
cycloheximide	51.9 ± 5.7	47.1 ± 7.6	45.5 ± 2.1

Values are means ± SEM of three determinations with each of two or three parallel cultures. 24 h old cultures of rat hepatocytes were incubated for 8 h under standard conditions with or without addition of insulin 10^{-7} mol/l, glucose 10 mmol/l (final concentration: 15 mmol/l), cordycepin 10 mg/l, cycloheximide 10 mg/l. Significant differences compared with the corresponding culture without addition of insulin or glucose are indicated by asterisks:* $p < 0.025$

period. Yet this was not possible, since the survival of cultured hepatocytes was impaired by a longer lasting inhibition of protein synthesis.

DISCUSSION

In the present communication it is shown that acetyl-CoA carboxylase activity could be enhanced in primary cultures of hepatocytes from adult rats by insulin as well as by glucose. In order to study the long term regulation of this enzyme, it was necessary to determine the enzyme activity under conditions which exclude the measurement of short term regulation effects e.g. allosteric activation or interconversion. This could be achieved by preparation of the liver extract at 23°C (22) and preincubation of the enzyme in presence of citrate (21). Under these conditions apparently the total carboxylase activity is measured (23, 24), which correlates well with the immunologically determined enzyme level under different metabolic conditions (10, 11).Furthermore the linear enhancement of carboxylase activity within 24 h as well as its inhibition by either cordycepin or cycloheximide indicate that the insulin and the glucose dependent increase of acetyl-CoA carboxylase activity was due to induction rather than allosteric regulation or interconversion. Beyond that it is unlikely that the enhancement

was due to the transition of an "apoenzyme" to a "holoenzyme" form, that may occur as a short time regulation effect in isolated hepatocytes (25).

In contrast to a previous investigation with diabetic rats, by which an indirect effect of carbohydrates on acetyl-CoA carboxylase induction was suggested (9), the present study demonstrates that glucose can induce the enzyme directly rather than indirectly via insulin. This could be explained by the fact that intact animals are too complex systems in order to differentiate conclusively primary or secondary effects of an inducing substance. Similar to the induction of other enzymes by insulin in liver cell cultures (16, 26) initial hormone levels were necessary in order to obtain a significant enhancement of acetyl-CoA carboxylase level, which were slightly higher than the physiological levels in the portal vein. That may be due to the degradation of more than 80% of the initially added insulin during 24 h as well as to the fact that insulin receptors are partially degraded by collagenase during preparation of single hepatocytes. In contrast to the insulin dependent induction of glucokinase (16, 26) or the epinephrine dependent induction of tyrosine aminotransferase (27) no permissive action of glucocorticoids was observed neither on the insulin nor on the glucose dependent enhancement of the carboxylase level. This is in accord with the finding that no change of acetyl-CoA carboxylase activity was observed after glucocorticoid treatment or adrenalectomy of normal rats (28).

Since glucose and mean insulin concentrations required for induction were in the physiological range observed in the portal vein (29, 30) the two effects seem to be of physiological importance for the regulation of carbohydrate dependent liponeogenesis in liver.

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