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RAPID CHANGES IN CHICK LIVER ACETYL-CoA CARBOXYLASE INDICATIVE OF PHOSPHORYLATION CONTROL*

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SUMMARY: Liver fatty acid synthesis was suppressed 75,95 and 90% within 1, 2 and 4 hrs respectively of depriving chicks of food. Accompanying this rapid drop in lipogenesis was a marked reduction in acetyl-CoA carboxylase activity, i.e., 40 and 75% decrease after 2 and 4 hrs of fasting. Adding 10mM citrate to the crude liver supernatant, or incubating the supernatant at 37°, 30 min increased activity of the briefly fasted birds, but neither method restored carboxylase activity to fed level. Heat and citrate activation were additive and together resulted in an activity comparable to the fed condition. The heat-dependent activation was accelerated by exogenous phosphoprotein phosphatase, and completely blocked by 100 mM NaF. Thus, enhancement of carboxylase activity from liver of briefly fasted chicks appears to be a dephosphorylation process. This is the first report indicating acute changes in chick carboxylase activity may involve a phosphorylation-dephosphorylation mechanism.

INTRODUCTION: The amount of liver acetyl-CoA carboxylase (EC 6.4.1.2) and/or fatty acid synthetase, both of which vary with dietary conditions, appear to dictate the maximal rate of fatty acid synthesis (1,2). However, the rate of enzyme protein turnover of these enzymes is too slow to explain large and rapid fluctuations in hepatic lipogenic rate (5,6,8,11). In this respect marked changes in malonyl-CoA production for fatty acid synthesis can be affected rapidly via modulation of the conformation of acetyl-CoA carboxylase, i.e., formation of the catalytically active polymeric state is promoted by high citrate, while disaggregation to inactive protomer is induced by long chain acyl-CoAs (7-9). The polymer-protomer transition of carboxylase occurs with the purified enzyme as well as with the enzyme in intact cells and liver tissue of rats and chicks (7-10).

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The amount of polymer in intact tissue was highly correlated to the rate of fatty acid synthesis (7-10), but depolymerization of carboxylase was too slow to fully explain acute fluctuations in fatty acid synthesis (8,9, 10). Furthermore, rat and chick liver fatty acid synthesis from acetate is often inhibited without reduced cellular citrate concentrations (11,12). A solution to this dilemma appears to lie in the fact that the activity of rat liver acetyl-CoA carboxylase is modified by phosphorylation and dephosphorylation (13,14). For example, glucagon treatment of rat hepatocytes or injection into rats significantly reduced carboxylase activity (13,15). However, Lane and associates have strongly argued that chick liver acetyl-CoA carboxylase is not regulated via a phosphorylation-dephosphorylation mechanism (7,16). In this report we have examined the response of chick liver acetyl-CoA carboxylase to brief periods of food deprivation. Our data provide the first evidence that liver acetyl-CoA carboxylase in briefly fasted (2-6 hrs) chicks displays many characteristics which are consistent with the regulation of carboxylase activity by a phosphorylation-dephosphorylation mechanism.

METHODS: The objective of these studies has been to ascertain the mechanism by which brief food deprivation impairs fatty acid synthesis in liver of chicks. One-day-old male Leghorn chicks were grown to 300-350g on a high carbohydrate commercial diet. The chicks were fed ad libitum. The influence of short-term periods of food deprivation on chick liver fatty acid synthesis and acetyl-CoA carboxylase activity was achieved by denying chicks access to food for the duration of time (usually < 6 hrs) cited in Tables. In vivo fatty acid synthesis rates were determined by measuring $^{3}H_2O$ incorporation into saponifiable lipid as previously described (2). $^{3}H_2O$ (2mCi/bird) was injected intraperitoneally 15 min before killing. Plasma $^{3}H_2O$ specific activity was used as an estimate of body water specific activity (2).

Acetyl-CoA carboxylase activity was determined in supernatants (10 min, Eppendorf microfuge, 4°) of liver homogenate using $H^{14}CO_3$ fixation into stable product during a 60 sec assay at 37° (8). The liver homogenate was prepared by quickly removing and chilling the liver in ice-cold water, and homogenizing in cold buffer (1g/2ml) containing Tris/acetate, 50mM (pH 7.4); mannitol, 300mM; and dithiothreitol, 5mM. Where indicated NaF (100mM) was added to the homogenization buffer to inhibit endogenous phosphatase activity. Activation of acetyl-CoA carboxylase via the action of phosphoprotein phosphatase was examined by addition of partially purified (post DEAE-cellulose) rat liver phosphorylase phosphatase (17), which was generously provided by Dr. J. Allred, Ohio State University, to the liver homogenate used for determination of carboxylase activity (see Table 3). Except where noted, acetyl-CoA carboxylase activity was determined in the supernatants of liver extract after the following treatments: (a) 4°, no citrate; (b) 4°, + 10 mM citrate; (c) 45 min, 37° no citrate, and (d) 45 min, 37° + 10 mM citrate. Exceptions to these treatments are cited in the Tables. A unit of acetyl-

CoA carboxylase activity is defined as micromoles of $H^{14}CO_3$ fixed into acid-stable product/min at 37°.

RESULTS AND DISCUSSION: In terms of lipogenesis the chick rapidly enters the fasting state, i.e., a marked reduction in fatty acid synthesis within 1-2 hrs (Table 1; refs 5,8). Concomitantly the activity of acetyl-CoA carboxylase is suppressed by brief periods of fasting (Table 1). A 40 and 75% decrease in carboxylase activity occurred within 2 and 4 hrs of food deprivation, respectively (Table 1). Refeeding for 1.5 hr restored enzymatic activity and lipogenic rate to a point comparable to the fed condition (Table 1). The half-life of acetyl-CoA carboxylase protein is 36-48 hrs (3). Thus the rapid 3-4 fold fluctuations in enzymatic activity are unlikely caused by changes in enzyme content, but rather represent a modification of the catalytic efficiency of the enzyme. In this respect the catalytic functionality of acetyl-CoA carboxylase in chick liver cells is at least in part dependent on the citrate concentration of the cytosol (7,18). Citrate is essential to maintaining acetyl-CoA carboxylase in its catalytically active polymeric conformation (18). Consequently a lowering of liver citrate concentrations during brief fasting could induce disaggregation and impair functionality of carboxylase. However, homogenizing the liver samples in the presence of 10 mM citrate did not reverse the reduction in

Table 1. Response of chick liver acetyl-CoA carboxylase to short-term food deprivation and glucagon injection.

Hours food deprivation	AcCoA-carboxylase - citrate	AcCoA-carboxylase +10mM citrate	Fatty acid synthesis
	(units per g liver)		
0	2.07	3.10	3.2
1	1.57	2.68	0.8
2	1.29	2.30	0.2
4	0.54	1.34	0.3
6	0.47	1.54	0.3
6 + 1.5 refed	1.79	2.61	3.4

Exactly at each time point 2 chicks were killed for determination of acetyl-CoA carboxylase activity and fatty acid synthesis rate. Variation between chicks at each time was < 15%.

carboxylase activity associated with food deprivation even though the citrate did enhance enzymatic activity at all times (Table 1). The ineffectiveness of citrate was consistent with our previous observation that fatty acid synthesis from acetate in chick hepatocytes could be inhibited by glucagon or cyclic AMP without an a priori reduction in cellular citrate (11).

The rapid diminution of carboxylase activity occurring in chick liver with short-term fasting (Tables 1 and 2) was remarkably similar to the response of acetyl-CoA carboxylase in rats or isolated rat liver cells acutely treated with glucagon (13,15). The mode of action of glucagon is to enhance the extent of phosphorylation of rat liver carboxylase and hence suppress its activity (13,14). Similarly dephosphorylation of the enzyme by a phosphatase enhances catalytic function (19). However, chick liver acetyl-CoA carboxylase reportedly is not dependent on such a covalent-modification process (16). Nevertheless the data of Tables 2 and 3 indicate this may not be an accurate conclusion.

In addition to being stimulated by citrate, chick acetyl-CoA carboxylase activity in the supernatant of a crude liver homogenate underwent a heat dependent increase in enzymatic activity (Tables 2 and 3). This phenomenon is comparable to that which occurs with rat liver and adipose carboxylase (13,20). The extent of the heat activation was most pronounced in chicks deprived of food (Table 2). Interestingly carboxylase activity in the liver

Table 2. Activation of latent chick liver acetyl-CoA carboxylase activity by heat and citrate.

Hours food deprivation	Acetyl-CoA carboxylase activity - citrate		+ 10mM citrate	
	4°	37° - 30 min	4°	37° - 30 min
0	2.80	4.44 (158)	3.26 (116)	4.66 (166)
4	0.98	2.60 (265)	2.15 (220)	3.97 (405)
6	1.15	2.58 (224)	2.00 (174)	3.95 (343)

Exactly at each time point 3 chicks were killed and liver samples homogenized in cold buffer ± 10mM citrate. Values in parentheses are percentages of the 4°, -citrate values. Standard errors were < 15% of mean.

Table 3. Inhibition of the activation of chick liver acetyl-CoA carboxylase by fluoride.

Minutes at 37°	Acetyl-CoA carboxylase activity			
	No additions	+ phosphatase	+ fluoride	+ phosphatase + fluoride
	(units per g liver)			
Fed state				
0	1.85	1.85	1.65	1.68
2	2.21	2.08	1.57	1.39
4	2.10	2.11	1.30	1.26
6	2.03	2.16	1.15	1.20
10	2.21	2.09	1.10	1.29
30	2.64	3.22	1.34	1.32
Six hr food deprivation				
0	0.46	0.46	0.38	0.48
2	0.55	0.76	0.37	0.43
4	0.69	0.80	0.42	0.54
6	0.75	1.19	0.39	0.48
10	1.02	1.64	0.36	0.52
30	1.29	2.38	0.42	0.39

Liver extracts from fed or fasted chicks were prepared in a cold buffer (4°) ± 100 nM NaF and no added citrate (refer METHODS). Acetyl-CoA carboxylase activity was determined exactly at each time point using the $HC^{14}O_3^-$-fixation assay described in METHODS. Phosphatase was added as a partially purified preparation (post-DEAE sephadex) from rat liver (17).

from 4 and 6 hr fasted birds was nearly equivalent to the activity in the fed chicks when the liver supernatants were incubated 30 min, 37° in presence of 10 mM citrate (Table 2). The less extensive citrate stimulation of carboxylase activity in the fed chicks was consistent with the fact that a greater proportion of the carboxylase enzyme exists in the active polymeric conformation in fed chicks (8). Finally, the rise in enzyme activity induced by heating (30 min, 37°) was additive with the citrate stimulatory effect. This suggests the two activation mechanisms are independent processes.

Previous studies with rat liver and adipose carboxylase have revealed that the heat activation phenomenon represents loss of regulatory phosphate via (a) a phosphatase mediated dephosphorylation and/or (b) proteolytic clip-

ping of a regulatory peptide (19-21). The data of Table 3 strongly indicate that activation of latent carboxylase activity in the chick is a phosphatase mediated dephosphorylation process. For example the rise in enzyme activity caused by incubating at 37° was completely blocked by inclusion of 100 mM NaF in the homogenization buffer (Table 3). Furthermore the addition of partially purified phosphoprotein phosphatase increased the extent of carboxylase activation, and accelerated the rate of carboxylase activation in the fasted chick (Table 3). Thus the rapid decrease in carboxylase activity accompanying food deprivation of chicks clearly has characteristics which are consistent with a phosphorylation-dephosphorylation control mechanism. The existence of such a covalent regulatory process for chick carboxylase has not been previously identified, but its occurrence would explain the suppression of chick hepatocyte fatty acid synthesis from acetate by glucagon in the presence of high cellular citrate concentrations (11).

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