EFFECTS OF STARVATION, INSULIN OR PROLACTIN DEFICIENCY ON THE ACTIVITY OF ACETYL-CoA CARBOXYLASE IN MAMMARY GLAND AND LIVER OF LACTATING RATS

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1. Introduction

During lactation in the rat the rate of lipogenesis in vivo in mammary gland is several-fold higher than that in liver and adipose tissue and it alters with the nutritional and hormonal state of the animal [1]. Starvation (24 h) decreases the rate in the lactating gland by 98% and this is reversed by refeeding for 2 h [1,2] or by injection of insulin [2]. Conversely, short-term insulin deficiency induced with streptozotocin [3] inhibits mammary gland lipogenesis [1]. These changes in the rates of mammary gland lipogenesis correlate with the alterations in the activation state of pyruvate dehydrogenase in vivo [4–7]. In starvation, inactivation of pyruvate dehydrogenase appears to be the major factor in the control of mammary gland lipogenesis, but there is evidence for an insulin-sensitive step which is after the formation of acetyl-CoA [2].

In the other lipogenic tissues (adipose tissue and liver) it is now well established that acetyl-CoA carboxylase is a regulatory enzyme and that its activity can be altered by hormones [8]. In epididymal fat pads, unlike liver, there appears to be coordinate control between the activation state of pyruvate dehydrogenase and acetyl-CoA carboxylase in response to alterations of the plasma insulin concentration [9].

Phosphorylation and inactivation of purified acetyl-CoA carboxylase from lactating rabbit [10] and rat mammary gland [11] by endogenous cAMP-dependent and independent protein kinases has been demonstrated. Dephosphorylation of the acetyl-CoA carboxylase isolated from rabbit mammary gland with an exogenous phosphatase results in activation of the protein [12]. The physiological importance of this mode of regulation within the gland in vivo is not known. The present paper describes changes in the activation state of acetyl-CoA carboxylase in mammary gland of lactating rats which occur in parallel with the known alterations in the rate of lipogenesis and activation state of pyruvate dehydrogenase.

2. Materials and methods

2.1. *Treatment of tissues*

Female rats of the Wistar strain (200–250 g) were used. Lactating rats with 9 or 10 pups were used at the peak of lactation 10–14 days post-partum. All rats were anaesthetized at 10:30 h (13:00 h for 24 h starved rats refed for 5 h) by the i.p. injection of sodium pentobarbitol (60 mg/kg body wt). Tissues were immediately excised, freeze-clamped, ground to a fine powder and stored under liquid nitrogen. Frozen powder (100 mg) was homogenized by hand in 1 ml 0.3 M mannitol, 50 mM Tris-acetate, 2 mM EDTA buffer (pH 7.4). After twice centrifuging in an Eppendorf centrifuge at 12 000 rev./min for 30 s, the supernatant was diluted 1 in 2 with the same buffer containing 20 mg/ml defatted BSA. This final homogenate (1 in 20) was assayed for enzyme activity within 3 min of commencing its preparation and within 3–4 h of removal of tissue.

2.2. *Acetyl-CoA carboxylase assay*

Acetyl-CoA carboxylase activity was determined

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by measuring the incorporation of [^{14}C]bicarbonate into acid-stable material [13,14] at 30°C for 90 s (since the reaction is only linear for the first 3 min). The assay incubation (final vol. 0.5 ml) routinely contained 100 mM Tris–acetate (pH 7.4), 0.5 mM EDTA, 10 mg/ml defatted BSA, 1 mM glutathione, 20 mM magnesium acetate, 0.3 mM acetyl-CoA, 5 mM ATP, 20 mM sodium [^{14}C]bicarbonate. Samples of homogenate (50 μl) were assayed immediately for initial activity in the presence and absence of 20 mM sodium citrate. A sample was also assayed after a 30 min preincubation at 30°C in the presence of 20 mM sodium citrate to measure total activity of the enzyme. Reaction blanks which contained no acetyl-CoA were also performed.

Each assay was stopped by the addition of 5 M HCl (100 μl) and after centrifugation at 12 000 rev./min for 30 s, a sample of supernatant (0.5 ml) was removed and dried down in a scintillation vial. The residue was redissolved in 0.2 ml H_2O, scintillation fluid was added and dpm were obtained using a 1215 Rackbeta liquid scintillation counter.

The reaction was shown to be linear for the range of protein concentrations used. One unit of enzyme activity represents 1 μmol substrate incorporated/min at 30°C.

3. Results

3.1. *Acetyl-CoA carboxylase activity in mammary gland*

The transition from the virgin state to peak lactation was accompanied by a 50-fold increase in total activity of acetyl-CoA carboxylase in the mammary gland (table 1): this finding is in general agreement with [15]. There was a significant increase in the percentage of enzyme in the active form in the lactating mammary gland of fed rats.

Starvation (24 h with pups) decreased the total activity of acetyl-CoA carboxylase in the lactating gland which presumably represents a loss of enzyme protein (table 1). This loss was not restored by refeeding for 2.5 h or 5 h. Starvation also resulted in a decrease in the percentage of the enzyme in its active form and this was accompanied by a decrease in the ability of citrate to activate the enzyme. However, refeeding chow (2.5 h or 5 h) increased the propor-

Table 1
Acetyl-CoA carboxylase activity in the mammary gland of virgin and lactating rats under different nutritional and hormonal conditions

State of rats	No. obs.	Initial activity as % of total		Total activity	
		0 mM Citrate	20 mM Citrate	mUnits/mg protein	Units/g wet wt of tissue
Virgin fed	(11)	24.7 ± 3.4[a]	76.8 ± 4.6	6.3 ± 1.2[c]	0.09 ± 0.02[c]
Lactating:					
fed	(10)	32.0 ± 1.7	77.4 ± 1.6	45.7 ± 3.8	4.63 ± 0.47
starved (24 h)	(11)	18.1 ± 2.7[c]	58.7 ± 5.6[b]	21.8 ± 2.2[c]	2.39 ± 0.23[c]
starved (24 h) refed 2.5 h	(4)	36.7 ± 3.4[e]	†85.7 ± 2.2[a,d]	17.7 ± 2.5[c]	2.03 ± 0.28[c]
starved (24 h) refed 5 h	(3)	40.2 ± 3.2[a,e]	†88.0 ± 3.8[a,d]	26.9 ± 5.3[a]	2.98 ± 0.38[a]
fed + streptozotocin (2 h)	(6)	20.4 ± 1.7[c]	65.1 ± 1.9[c]	45.7 ± 3.7	3.99 ± 0.42
fed + bromocryptine	(3)	27.9 ± 3.1	78.6 ± 6.2	39.8 ± 5.8	3.87 ± 0.67

[a] $P < 0.05$ (Student's t-test); [b] $P < 0.005$; [c] $P < 0.0005$; values significantly different from lactating fed control values
[d] $P < 0.05$; [e] $P < 0.005$; values for refed rats significantly different from corresponding starved control values

Results are mean values ± SEM

Streptozotocin (50 mg/kg body wt; solution in 0.01 M sodium citrate (pH 4.5)) was injected into the tail vein 2 h before experiments to induce short-term insulin deficiency [3]. Prolactin deficiency was induced by a subcutaneous injection of bromocryptine (10 mg/kg body wt; solution in 10% (v/v) ethanol containing 1% (w/v) tartaric acid) 24 h before the experiments [18]

tion of active enzyme by 100% and restored the activation by citrate (table 1).

Like starvation, short-term insulin-deficiency (2 h) induced by streptozotocin treatment decreased the percentage of enzyme in the active form and the ability of citrate to activate it. However, in this situation the total activity remained unchanged.

Prolactin deficiency (24 h with pups) induced by bromocryptine treatment had no significant effect on initial activation state, activation by citrate or total enzyme activity. Bromocryptine treatment does decrease lipogenesis by ~46% [16] but this appears to occur without any change in the activation state of acetyl-CoA carboxylase.

3.2. *Acetyl-CoA carboxylase activity in liver*

Total acetyl-CoA carboxylase activity increased by ~100% in livers of fed lactating rats compared to livers of virgin rats (table 2). Starvation (24 h) of lactating rats resulted in ~50% decrease in total activity of the enzyme and this was not restored by refeeding for 5 h (table 2). These changes are similar to those observed in the mammary gland. However, in none of the situations examined was there any significant change in the percentage of hepatic enzyme in its activated form or in the ability of citrate to activate the enzyme.

4. Discussion

The large increase in total acetyl-CoA carboxylase activity during lactation correlates with the increase in mammary gland lipogenesis [1,16] and confirms [15].

As is the case in adipose tissue [9,14,17] short-term regulation is via changes in the proportion of acetyl-CoA carboxylase in its active form and the ability of citrate to activate the enzyme. In situations where insulin availability is greatly decreased, i.e., streptozotocin treatment and 24 h starvation [1] initial activity and citrate activation are both diminished. When plasma insulin returns to normal on refeeding [1] mammary gland acetyl-CoA carboxylase is reactivated. Thus insulin may be implicated in this short-term regulation and since it has been shown to regulate acetyl-CoA carboxylase activity in adipose tissue [9,14,17] it may well have the same function in the lactating mammary gland. This regulation could be the result of the phosphorylation–dephosphorylation cycle demonstrated for rat mammary gland acetyl-CoA carboxylase especially since phosphorylation is known to inhibit the citrate activation of the enzyme [11].

The decrease in mammary gland lipogenesis observed after 24 h starvation is accompanied by a diminution of total acetyl-CoA carboxylase activity and this relatively long-term response is unaffected by short-term changes in insulin availability. This form of regulation via a decrease in enzyme concentration is not exhibited by pyruvate dehydrogenase in the lactating mammary gland [6].

The only change in hepatic acetyl-CoA carboxylase under the conditions studied was a decrease in total activity in response to starvation. Thus it appears that the enzyme in this tissue is not susceptible to short-term changes in activation state. Others have observed in non-lactating rats that hepatic acetyl-CoA carboxylase activity does not change in vivo in response

Table 2
Acetyl-CoA carboxylase activity in livers of virgin and lactating rats under different nutritional conditions

State of rats	No. obs.	Initial activity as % of total		Total activity	
		0 mM Citrate	20 mM Citrate	mUnits/mg protein	Units/g wet wt of tissue
Virgin fed	(12)	18.0 ± 1.8	50.7 ± 2.0	2.4 ± 0.4[a]	0.31 ± 0.05[a]
Lactating:					
fed	(9)	19.4 ± 1.0	61.6 ± 5.5	4.8 ± 0.6	0.67 ± 0.11
starved (24 h)	(8)	23.0 ± 4.6	58.9 ± 4.5	2.1 ± 0.2[b]	0.34 ± 0.05[b]
starved (24 h) refed (5 h)	(3)	25.1 ± 2.8	64.6 ± 3.4	1.8 ± 0.4[a]	0.29 ± 0.06[a]

[a] $P < 0.005$ (Student's t-test); [b] $P < 0.0005$; values significantly different from lactating fed control values

For experimental details see text and table 1

to changes in plasma insulin whereas the enzyme in adipose tissue does [9].

The present findings indicate that acetyl-CoA carboxylase activity in the lactating mammary gland changes in parallel with rates of lipogenesis and pyruvate dehydrogenase activity in response to various experimental manipulations. It remains to be established whether such changes are the result of altered insulin levels per se or whether antagonistic hormone(s) are involved. The phosphorylation and inactivation of mammary gland acetyl-CoA carboxylase can occur with a cAMP-dependent protein kinase [10,11]. However, we have been unable to show inhibition of mammary gland lipogenesis by hormones that would lead to increased intracellular levels of cAMP. Thus, there is a need to correlate the molecular mechanism of acetyl-CoA carboxylase regulation with events occurring in vivo and to define the role of this enzyme in the control of lipogenesis in the lactating mammary gland.

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