Growth-hormone–prolactin interactions in the regulation of mammary and adipose-tissue acetyl-CoA carboxylase activity and gene expression in lactating rats

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The factors and mechanisms responsible for the reciprocal changes in lipogenesis in rat mammary gland and adipose tissue during the lactation cycle have been investigated. Lactation decreased the activation status and mRNA concentration of acetyl-CoA carboxylase in adipose tissue. Litter removal decreased the mRNA concentration of acetyl-CoA carboxylase in the mammary gland and increased the enzyme's mRNA concentration and activation status in adipose tissue. Lowering serum prolactin concentration in lactating rats decreased the amount of mammary acetyl-CoA carboxylase mRNA and increased that of adipose tissue, and increased the activation status of the enzyme in adipose tissue. Decreasing serum growth hormone (GH) alone had little effect on acetyl-CoA carboxylase in lactating rats, although it did lower pup growth rate and serum concentration of insulin-like growth factor-I. Lowering serum GH concentration exacerbated the effects of decreasing serum prolactin on mammary-gland (but not adipose-tissue) acetyl-CoA carboxylase mRNA and further increased the rise in activation status of the adipose-tissue enzyme induced by decreasing serum prolactin. Changes in acetyl-CoA carboxylase mRNA in both mammary and adipose tissue were paralleled by changes in total enzyme activity except after litter removal, when there was a disproportionately large decrease in total enzyme activity of the mammary gland. Thus prolactin has a major and GH a minor role in the regulation of acetyl-CoA carboxylase activity during lactation. Changes in mammary activity in response to prolactin and GH are primarily due to alterations in gene transcription, whereas adaptation in adipose tissue involves both changes in gene transcription and activation status.

INTRODUCTION

Lactation results in a re-adjustment of lipid metabolism to meet the demands of the mammary gland for milk fat synthesis. This includes a major increase in the rate of fatty acid synthesis in the mammary gland and a concomitant decrease in adipose tissue (Vernon & Flint, 1983; Williamson, 1986). Acetyl-CoA carboxylase is probably the most important enzyme regulating the lipogenic flux and is subject to both acute and chronic control by hormones (Clegg, 1988; Hardie, 1989). Acute control is achieved, at least in part, by a change in phosphorylation status, with a concomitant change in activation status (Clegg, 1988; Hardie, 1989). Lactation results in a decrease in adipose-tissue acetyl-CoA carboxylase activity (Sinnott-Smith *et al.*, 1980) and a rise in both the activity and amount of mammary acetyl-CoA carboxylase activity (Mackall & Lane, 1977). A very recent study has shown that there are corresponding changes in the amount of acetyl-CoA carboxylase mRNA in the two tissues during lactation (Lopez-Casillas *et al.*, 1991).

Prolactin is the major lactogenic hormone in the rat; lowering its serum concentration with drugs such as bromocriptine decreases milk yield markedly (Cowie *et al.*, 1980) and decreases and increases the rate of lipogenesis in the mammary gland and adipose tissue (Williamson, 1980; Vernon & Flint, 1983) respectively. For the mammary gland at least, the fall in lipogenesis is paralleled by a decrease in the activity of acetyl-CoA carboxylase (McNeillie & Zammit, 1982; Munday & Williamson, 1982).

Although suppressing serum prolactin decreases the milk yield in rats, it does not decrease it completely (Madon *et al.*, 1986). Also, suppressing serum prolactin concentration does not decrease the rate of lipogenesis (Agius *et al.*, 1979; Oller do Nascimento *et al.*, 1989) or acetyl-CoA carboxylase activity (McNeillie & Zammit, 1982) of the mammary gland to the very low levels found on litter removal for the same length of time.

This suggests that other factors are involved in maintaining acetyl-CoA carboxylase activity of the mammary gland. Growth hormone (GH) is a possible candidate; its importance in maintaining and stimulating milk production in domestic ruminants is well documented (Tucker, 1974; Peel & Bauman, 1987; Chilliard, 1989), whereas in rats lowering both serum prolactin and GH decreases milk yield to very low levels indeed (Madon *et al.*, 1986). We have therefore investigated the roles and interactions of GH and prolactin in regulating acetyl-CoA carboxylase activity and gene expression in lactating rats.

EXPERIMENTAL

Animals and treatments

Wistar rats (A. Tuck and Son, Rayleigh, Essex, U.K.) were fed on Labsure irradiated CRM diet (Labsure, Poole, Dorset, U.K.) and water *ad libitum*. They were mated at 2–3 months of age, and the number of pups per mother was adjusted to ten within 24 h after birth. Litters were removed and/or injections were begun on days 12–14 of lactation. All injections were subcutaneous and were administered twice daily, at 09:00 and 17:00 h for 2 days. Rats were not injected at 09:00 h on day 3, but were anaesthetized with a combination of Hypnorm and Hypnoid (Jansen Pharmaceutical, Oxford, U.K.) at about 10:30 h. Rats received the following, either alone or in combinations as given in the text and Tables: bromocriptine (a gift from Sandoz), 500 µg/injection; γ-globulin fraction of an antiserum to rat GH (AGS), 220 mg/injection, equivalent to 4.5 ml of antiserum (see below); sheep prolactin, 1.5 mg/injection; sheep GH, 1 mg/injection; or carrier solutions (Madon *et al.*, 1986). Sheep prolactin and GH (pituitary derived) were gifts from NIADDK, Bethesda, MD, U.S.A. Details of the preparation and characterization of AGS and its γ-globulin fraction have been described previously (Madon *et al.*, 1986); for the present study it is pertinent to note

that the antiserum does not cross-react significantly with prolactin and does not alter serum prolactin concentration in lactating rats (Madon *et al.*, 1986). In some experiments rats and their litters were weighed daily between 09:00 and 10:00 h, beginning 24 h before the start of injections. Virgin rats were the same age as the lactating rats.

Once rats were anaesthetized, samples of mammary and adipose tissues were removed and frozen immediately in liquid nitrogen before assay for acetyl-CoA carboxylase activity and mRNA. Samples of blood were taken for hormone assay and further samples of adipose tissue for cell volume determination.

Enzyme assay

Total and initial (expressed) acetyl-CoA carboxylase activities were determined as described previously (Barber *et al.*, 1991). NaF (100 mM) was included in the assay when measuring initial activity, and in some experiments 2 units of protein phosphatase (prepared as described by Easom & Zammit, 1984) was included during the preincubation before assay for total activity. Inclusion of protein phosphatase increased total activity of both mammary and adipose-tissue preparations by about 10% regardless of the physiological state of the animal. AMP-stimulated protein kinase was assayed as described by Davies *et al.* (1989).

RNA isolation

Total cellular RNA was isolated from mammary gland and adipose tissue by using guanidinium thiocyanate and CsCl centrifugation (Chirgwin *et al.*, 1979). All preparations of RNA were resuspended in water and their RNA concentration was determined by the A_{260} , where 1 A_{260} unit corresponds to 40 μ g of RNA.

Amplification of sequences corresponding to rat acetyl-CoA carboxylase from cDNA prepared from lactating-rat mammary-gland RNA

Polyadenylated RNA was prepared from total cellular RNA by oligo (dT)-cellulose chromatography (Aviv & Leder, 1972). For the preparation of first-stand cDNA, 1 μ g of polyadenylated RNA was denatured and then annealed to 5 μ g of random hexamers (Pharmacia). The final reaction conditions were 50 μ g of polyadenylated RNA/ml, 250 μ g of random hexamers/ml, 50 mM-Tris/HCl, pH 8.3, 50 mM-KCl, 10 mM-MgCl₂, 1 mM-EDTA, 10 μ g of BSA/ml, 1 mM-dithiothreitol, 0.5 mM-spermidine, 1.5 mM of each deoxynucleoside triphosphate, 1000 units of RNAGuard (Pharmacia)/ml, 150 μ Ci of [32 P]dCTP/ml (3000 Ci/mmol; Amersham) and 250 units of avian myeloblastosis virus reverse transcriptase/ml (Pharmacia) in a total volume of 20 μ l. The reaction was incubated at 42 °C for 90 min. EDTA and NaOH were added to give final concentrations of 20 mM and 0.2 M respectively, and the mixture was incubated at 60 °C for 1 h. The cDNA was precipitated by addition of 0.1 vol. of 3 M-sodium acetate, pH 6.0, and 2 vol. of ethanol. The efficiency of the reaction was determined by the incorporation of the [32 P]dCTP into the cDNA and was generally in the range 25–30% of input RNA.

For amplification of a cDNA sequence corresponding to amino acids 512–789 of rat acetyl-CoA carboxylase (Lopez-Casillas *et al.*, 1988), 50 ng of cDNA was added to 100 μ l of PCR buffer [10% (v/v) dimethyl sulphoxide, 67 mM-Tris/HCl, pH 8.8, 7.6 mM-MgCl₂, 16.6 mM-(NH₄)₂SO₄, 1 mM-2-mercaptoethanol, 67 μ M-EDTA, 170 μ g of BSA/ml and 1.5 mM of each deoxynucleoside triphosphate (Frohman *et al.*, 1988), containing 100 pmol each of the upstream and downstream primer]. The sequence of the upstream primer was 5'-GAAAACCCAGATGAGGGGTTTAAGCCCAG3' and that of the downstream primer 5'-AGTCATTACCATCTTCATTACCTCAATCTCTGCATA3'.

The mixture was denatured at 95 °C for 5 min and, after cooling to 72 °C, 2.5 units of *Thermus aquaticus* (Taq) DNA polymerase (Anglian Biotec.) were added. The reaction mixture was then subjected to 45 cycles of denaturing at 92 °C for 30 s, annealing of the primers at 55 °C for 30 s and extending the cDNA at 72 °C for 1 min. The resulting PCR product was cloned into the pGEM 3Zf (+) phagemid (Promega). A single colony (pR1⁺) shown to contain the expected 834 bp insert was sequenced by the dideoxy chain-termination method (Sanger *et al.*, 1977). The sequence of the cloned PCR product was 99.6% similar to the expected sequence (Lopez-Casillas *et al.*, 1988). Three base changes were noted; two direct substitutions and one frameshift mutation. The magnitude of these mutations is consistent with the reported accuracy of Taq DNA polymerase (Tindall & Kunkel, 1988).

Northern-blot analysis of RNA

Samples (30 or 40 μ g) of total cellular RNA were separated by electrophoresis in 1.2%-agarose gels containing 2.2 M-formaldehyde and then transferred to Hybond N nylon membranes (Amersham) (Davis *et al.*, 1986). A labelled antisense RNA probe was generated from pR1⁺ by using SP6 RNA polymerase (Promega) (Melton *et al.*, 1984). Membranes were prehybridized at 50 °C overnight in hybridization solution [50% formamide, 0.75 M-NaCl, 50 mM-NaH₂PO₄, pH 7.4, 5 mM-EDTA, and Ficoll 400, BSA, polyvinylpyrrolidone and SDS each at 0.1% (w/v), and 200 μ g of denatured salmon testes DNA/ml] and then hybridized overnight at 50 °C in hybridization solution containing antisense RNA probe at 2×10^6 d.p.m./ml. After hybridization membranes were rinsed in 0.3 M-NaCl/30 mM-sodium citrate, digested with RNAase and washed at 50 °C in 0.1% SDS in 15 mM-NaCl/1.5 mM-sodium citrate. Membranes were exposed to Fuji XAR-5 film at -70 °C with intensifying screens. The autoradiographs were scanned with a Bio-Rad laser densitometer with one-dimensional software. Hybridization of RNA with the antisense RNA probe yielded a 10.5 kb band and a diffuse band around 3.8 kb, which has been attributed to degraded acetyl-CoA carboxylase mRNA (Bai *et al.*, 1986). Results have been expressed as amount of 10.5 kb species and also as total acetyl-CoA carboxylase mRNA. To facilitate comparison of results obtained from different membranes, a control sample of 14-day-lactating rat mammary-gland RNA was included on all the membranes. The control sample on each filter was assigned an arbitrary value of 100 with respect to each of these parameters, and all other values were calculated with respect to the control samples.

Other assays

Serum was prepared from blood samples and assayed for insulin, prolactin (Flint *et al.*, 1979) and insulin-like growth factor-I (IGF-I) (Madon *et al.*, 1986). Adipocyte mean cell size and number was determined as described previously (Aitchison *et al.*, 1982).

Statistical analysis

Results are expressed as means $\pm$ S.E.M. and were compared by Student's *t* test. Conclusions were also checked by analysis of variance of untransformed and log-transformed data.

RESULTS

Pup growth rate and serum hormone concentrations

Pup growth rate did not vary significantly in non-treated rats over the 2-day experimental period (Table 1). Both bromocriptine and AGS alone decreased ($P < 0.01$) pup growth rate to a similar extent over the first 24 h period of treatment, but bromocriptine

Table 1. Effects of varying serum prolactin and GH concentrations in lactating rats on pup growth rate and serum insulin and IGF-I concentrations

Lactating rats were injected twice daily for 2 days with combinations of bromocriptine (BrC) (to decrease serum prolactin), AGS, sheep prolactin (PRL) and sheep GH. Rats were anaesthetized on the morning after the cessation of treatment and blood was obtained for assay of serum hormone concentrations. Pups were weighed daily at about 09:00 h. Mean pup weight gain (all groups) on the day before commencement of injections was 17.3 g/day. Results are means $\pm$ S.E.M. of 5–7 observations.

State of rat	Treatments	Pup wt. gain (g/day)		Serum hormones (ng/ml)	
		0–24 h	24–48 h	IGF-I	Insulin
Virgin	–	–	–	1611 $\pm$ 181	2.96 $\pm$ 1.10
Lactating	–	17.2 $\pm$ 1.6	14.3 $\pm$ 0.8	1081 $\pm$ 51	0.62 $\pm$ 0.01
	BrC	7.0 $\pm$ 1.1	3.9 $\pm$ 1.4	1150 $\pm$ 86	1.16 $\pm$ 0.30
	AGS	8.8 $\pm$ 0.9	9.2 $\pm$ 0.9	617 $\pm$ 70	0.50 $\pm$ 0.17
	BrC + AGS	6.0 $\pm$ 0.9	–5.0 $\pm$ 2.6	605 $\pm$ 86	3.44 $\pm$ 1.88
	BrC + AGS + PRL + GH	16.8 $\pm$ 1.1	9.3 $\pm$ 2.1	1316 $\pm$ 157	1.14 $\pm$ 0.54

Table 2. Effects of varying serum prolactin and GH concentrations on acetyl-CoA carboxylase mRNA concentration and total enzyme activity of mammary tissue in lactating rats

Lactating rats were injected twice daily for 2 days with combinations of bromocriptine (BrC), AGS and prolactin (PRL), after which they were anaesthetized and samples of mammary tissue removed and frozen immediately in liquid N₂. Litter-removed rats had their litters removed 2 days before sampling; virgin rats were the same age as the lactating rats. The experiment was performed in November/December. The concentrations of RNA, acetyl-CoA carboxylase mRNA and total activity were determined as described in the text. For determination of the amount of acetyl-CoA carboxylase mRNA, 40 μ g samples of RNA were run on agarose gels and then hybridized with an antisense probe to acetyl-CoA carboxylase mRNA. This indicated a 10.5 kb band and a diffuse band around 3.8 kb. In the Table, 10.5 kb mRNA and total mRNA refer to the amount of acetyl-CoA carboxylase mRNA in the 10.5 kb band and in the whole lane respectively. An RNA standard (a sample of mammary RNA) was included on all gels, and the amount of 10.5 kb and total hybridization were assigned arbitrary values of 100; values for experimental samples are expressed relative to the standard values. Results are means $\pm$ S.E.M. of 5 or 6 observations.

State	Treatment	Total RNA (mg/g tissue)	Acetyl-CoA carboxylase				Total activity (μ mol/min per g of tissue)
			10.5 kb mRNA		Total mRNA		
			(units/40 μ g of RNA)	(units/mg of tissue)	(units/40 μ g of RNA)	(units/mg of tissue)	
Lactating	—	4.41 $\pm$ 0.18	69 $\pm$ 14	7.66 $\pm$ 1.65	94 $\pm$ 16	10.42 $\pm$ 1.93	4.04 $\pm$ 0.16
Lactating	BrC	3.98 $\pm$ 0.21	18 $\pm$ 9	1.71 $\pm$ 0.89	26 $\pm$ 8	2.55 $\pm$ 0.79	1.71 $\pm$ 0.14
Lactating	BrC + PRL	4.89 $\pm$ 0.39	84 $\pm$ 24	10.30 $\pm$ 3.52	79 $\pm$ 18	9.59 $\pm$ 2.18	3.57 $\pm$ 0.52
Lactating	AGS	5.40 $\pm$ 0.56	72 $\pm$ 16	10.23 $\pm$ 3.21	75 $\pm$ 10	10.40 $\pm$ 2.18	3.97 $\pm$ 0.21
Lactating	BrC + AGS	3.17 $\pm$ 0.26	11 $\pm$ 5	0.72 $\pm$ 0.25	19 $\pm$ 8	1.65 $\pm$ 0.77	0.73 $\pm$ 0.02
Lactating	Litter removed	1.52 $\pm$ 0.12	19 $\pm$ 7	0.70 $\pm$ 0.23	22 $\pm$ 6	0.83 $\pm$ 0.20	0.09 $\pm$ 0.01

Table 3. Effects of lactation and varying serum prolactin and GH concentrations during lactation on acetyl-CoA carboxylase mRNA concentration and total activity in rat adipose tissue

Lactating rats were injected twice daily for 2 days with combinations of bromocriptine (BrC), AGS and prolactin (PRL), after which they were anaesthetized and samples of adipose tissue were removed and immediately frozen in liquid N₂. Litter-removed rats had their litters taken away 2 days before sampling. Virgin rats were the same age as the lactating rats. The experiment was performed in November/December. The concentrations of RNA, acetyl-CoA carboxylase mRNA (total and 10.5 kb species) and total enzyme activity were determined as described in the text and the legend to Table 2. Amounts and activity/10⁶ cell units were calculated by dividing the amount or activity/g of tissue by number of adipocytes/g of tissue. Results are means $\pm$ S.E.M. of 5 or 6 observations.

State	Treatment	Total RNA ($\mu\text{g}/10^6$ cell unit)	Acetyl-CoA carboxylase				Total activity (nmol/min per 10^6 cell units)
			10.5 kb mRNA		Total mRNA		
			(units/30 μg of RNA)	(units/ 10^6 cell units)	(units/30 μg of RNA)	(units/ 10^6 cell units)	
Virgin	—	36.6 ± 4.5	198 ± 131	279 ± 197	115 ± 32	140 ± 29	17.8 ± 3.5
Lactating	—	31.6 ± 7.8	153 ± 113	74 ± 44	71 ± 14	55 ± 8	11.2 ± 4.1
Lactating	BrC	43.0 ± 7.0	604 ± 140	708 ± 158	340 ± 73	471 ± 94	47.5 ± 7.3
Lactating	BrC + PRL	38.3 ± 3.5	84 ± 52	84 ± 40	49 ± 25	51 ± 19	16.2 ± 3.4
Lactating	AGS	34.2 ± 4.2	138 ± 98	118 ± 70	65 ± 32	58 ± 22	14.2 ± 2.8
Lactating	BrC + AGS	42.4 ± 4.5	544 ± 177	840 ± 343	344 ± 81	499 ± 132	49.4 ± 12.0
Lactating	Litter removed	36.6 ± 4.5	760 ± 239	1095 ± 230	230 ± 28	386 ± 86	35.4 ± 7.4

Fig. 1. Northern analysis of acetyl-CoA carboxylase mRNA in lactating rat mammary gland

Lactating rats were injected twice daily for 2 days with combinations of bromocriptine (BrC), AGS and GH, after which they were anaesthetized and samples of mammary tissue were removed and frozen immediately in liquid N₂. The experiment was performed in June. Total cellular RNA was isolated from the mammary glands of rats treated with saline (a), AGS (b), BrC (c), AGS + BrC (d) and AGS + BrC + GH (e), and 40 µg samples were run on agarose gels, blotted and hybridized with an antisense RNA probe to acetyl-CoA carboxylase. This indicated a 10.5 kb band and a diffuse band around 3.8 kb. The RNA before blotting was stained with ethidium bromide, and the 28 s rRNA species is shown beneath each lane of the Northern blot. Representative data shown are for two animals from each treatment group of six rats.

had a greater ($P < 0.02$) effect than AGS during the second 24 h period (Table 1). The combined treatment of bromocriptine plus AGS had a greater effect than either agent alone ($P < 0.05$) and resulted in pups losing weight over the second 24 h period. The effects of AGS plus bromocriptine were reversed over the first 24 h period and partly reversed over the second 24 h period by concurrent treatment with prolactin and GH (Table 1). AGS interferes with the assay for GH, so serum IGF-I concentrations were measured to assess effectiveness of AGS treatment. AGS, but not bromocriptine, significantly decreased ($P < 0.001$) the serum IGF-I concentration (Table 1). Lactation itself decreased ($P < 0.05$) the serum IGF-I concentration and tended to decrease the serum insulin concentration (Table 1). Treatment with bromocriptine plus AGS increased serum insulin ($P < 0.05$, analysis of variance). Treatment with bromocriptine alone tended to increase serum insulin (the value was intermediate between that of untreated lactating rats and rats given the combined treatment) (Table 1). Treatment with bromocriptine decreased serum prolactin concentration from over 400 ng/ml to 11 ± 1 ng/ml; AGS had no effect on the ability of bromocriptine to decrease serum prolactin concentration (results not shown).

Acetyl-CoA carboxylase total activity

Total acetyl-CoA carboxylase activity and mRNA concentrations were measured in winter (Table 2) and summer (Fig. 1, and results not shown) and one experiment for adipose tissue (Table 3) was performed in the winter.

Bromocriptine treatment decreased total acetyl-CoA carboxylase activity of the mammary gland ($P < 0.01$) (Table 2), and this effect was prevented by concurrent administration of prolactin (Table 2). Treatment with AGS had no effect by itself, but exacerbated ($P < 0.001$) the decrease in total acetyl-CoA carboxylase activity induced by bromocriptine administration (Table 2). In a further experiment (results not shown), the effect of AGS in the presence of bromocriptine was partly prevented by concurrent treatment with GH ($P < 0.01$). However, even treat-

ment with AGS plus bromocriptine did not decrease total acetyl-CoA carboxylase activity to the very low levels found 2 days after litter removal (Table 2). Injections of either prolactin or prolactin plus GH did not even partly prevent the effects of litter removal (results not shown).

Results for adipose tissue are expressed per 10^6 cell units (Table 3) to allow for differences in cellularity arising from changes in adipocyte mean cell volume which occur during lactation (Flint *et al.*, 1979). Activity/g of tissue was divided by the number of adipocytes/g of tissue, but, as adipocytes are not the only cell type present in adipose tissue, results are expressed per cell unit. Lactation tended to decrease the total acetyl-CoA carboxylase activity of adipose tissue (Table 3). In contrast with mammary tissue, bromocriptine treatment increased adipose-tissue total acetyl-CoA carboxylase activity ($P < 0.01$) (Table 3), and this was prevented by concurrent administration of prolactin (Table 3). AGS either alone or in combination with bromocriptine had no effect on total acetyl-CoA carboxylase activity (Table 3). Litter removal increased acetyl-CoA carboxylase activity in adipose tissue, but, in contrast with the mammary gland, the effect was not different from that achieved by bromocriptine treatment (Table 3).

Administration of bromocriptine or AGS either alone or in combination had no effect on total hepatic acetyl-CoA carboxylase activity of lactating rats (results not shown).

Acetyl-CoA carboxylase mRNA concentration

Northern-blot analysis (Fig. 1) showed hybridization of the acetyl-CoA carboxylase probe to a 10.5 kb mRNA in lactating rat mammary tissue and to a more diffuse band of around 3.8 kb. The same pattern of hybridization is observed in adipose tissue. This lower-molecular-mass hybridization has been found previously and has been attributed to degraded acetyl-CoA carboxylase mRNA (Bai *et al.*, 1986). The 10.5 kb mRNA therefore represents the most functionally active species, and should be considered when making comparisons between total enzyme activity and functional acetyl-CoA carboxylase gene expression. Nevertheless an appreciation of total acetyl-CoA carboxylase mRNA, including the degraded mRNA species, may become important under conditions where acetyl-CoA carboxylase gene expression is turned off and where the half-life of the mRNA may be much shorter than the enzyme itself. The half-life of rat acetyl-CoA carboxylase mRNA has been estimated to be about 9 h under a variety of physiological conditions (Pape & Kim, 1989), compared with the half-life of the protein, which may be as long as 48 h (Volpe & Vagelos, 1976).

In lactating rats subjected to a variety of endocrinological manipulations, the abundance of the 10.5 kb mRNA species and of total acetyl-CoA carboxylase mRNA in mammary and adipose tissue varied in parallel with changes in total enzyme activity (Tables 2 and 3).

Treatment with bromocriptine decreased ($P < 0.001$) the amounts of both the 10.5 kb species and total enzyme mRNA (Table 2; Fig. 1) in mammary gland, and this was prevented by the concurrent administration of prolactin (Table 2). Treatment with AGS alone had no effect on either the 10.5 kb mRNA or total acetyl-CoA carboxylase mRNA in mammary gland in one experiment (Table 2; Fig. 1), but did result in a small but significant ($P < 0.02$) decrease in the 10.5 kb species/mg of tissue in the second experiment (results not shown). Treatment with AGS plus bromocriptine decreased mammary-gland acetyl-CoA carboxylase mRNA/mg of tissue (10.5 kb species and total) by a further 60% compared with bromocriptine alone (Table 2) ($P < 0.05$ from analysis of variance of log-transformed results); this observation was confirmed in the second experiment in the summer (Fig. 1). In the second experiment, concurrent treatment

Table 4. Effect of varying serum prolactin and GH concentrations on the activation status of acetyl-CoA carboxylase in mammary and adipose tissue and AMP-stimulated protein kinase activity of adipose tissue

Lactating rats were injected twice daily for 2 days with combinations of bromocriptine (BrC), AGS, prolactin (PRL) and GH, after which they were anaesthetized and samples of mammary and adipose tissue were removed and immediately frozen in liquid N₂ for enzyme assay. Litter-removed rats had their litters taken away 2 days before sampling. Virgin rats were the same age as the lactating rats. Results are means $\pm$ S.E.M. for the numbers of observations in parentheses.

State	Treatment	Tissue...	Acetyl-CoA carboxylase (proportion in active state $\times$ 100)		AMP-stimulated protein kinase (pmol/min per 10 ⁶ cell units)
			Mammary gland	Adipose tissue	Adipose tissue
Virgin	—	—	—	43.9 $\pm$ 6.9 (5)	88.7 $\pm$ 22.6 (5)
Lactating	—	—	23.3 $\pm$ 2.8 (6)	20.6 $\pm$ 2.0 (21)	104.2 $\pm$ 17.4 (5)
Lactating	BrC	—	40.8 $\pm$ 5.1 (6)	37.5 $\pm$ 2.8 (16)	97.4 $\pm$ 7.2 (6)
Lactating	BrC + PRL	—	—	22.8 $\pm$ 3.3 (5)	82.6 $\pm$ 11.7 (5)
Lactating	AGS	—	29.4 $\pm$ 3.8 (6)	19.3 $\pm$ 2.8 (11)	114.6 $\pm$ 17.0 (5)
Lactating	BrC + AGS	—	35.2 $\pm$ 1.9 (6)	45.3 $\pm$ 2.4 (17)	125.5 $\pm$ 26.1 (5)
Lactating	BrC + AGS + GH	—	36.8 $\pm$ 3.3 (6)	30.7 $\pm$ 2.8 (6)	—
Lactating	Litter removed	—	—	37.1 $\pm$ 1.9 (5)	89.2 $\pm$ 8.2 (5)

with GH partly prevented this effect of AGS on acetyl-CoA carboxylase mRNA concentration (Fig. 1). Litter removal also decreased ($P < 0.001$) the amounts of both total and 10.5 kb acetyl-CoA carboxylase mRNA in mammary gland to levels similar to those found on treatment with AGS plus bromocriptine (Table 2).

The decrease in mammary tissue acetyl-CoA carboxylase mRNA on bromocriptine treatment was specific in that there was no change in the total RNA concentration of the mammary gland compared with control animals (Table 2), whereas there was a significant ($P < 0.01$) fall in both the amount of 10.5 kb and total acetyl-CoA carboxylase mRNA per unit of total RNA. The greater decrease in acetyl-CoA carboxylase mRNA found on treatment with both AGS and bromocriptine compared with bromocriptine alone is due to a fall in total RNA/g of tissue (Table 2) and a tendency for acetyl-CoA carboxylase mRNA/unit of total RNA to decrease; this decrease in acetyl-CoA carboxylase mRNA/ μ g of total RNA was significant ($P < 0.05$) in the second experiment (Fig. 1). Similarly, litter removal decreased both the total RNA concentration ($P < 0.01$) and acetyl-CoA carboxylase mRNA/unit of total RNA of the mammary gland ($P < 0.01$) (Table 2).

For adipose tissue, the effects of lactation and of the various treatments on acetyl-CoA carboxylase mRNA were in general opposite to those on mammary gland, and again paralleled changes in total enzyme activity (Table 3). Lactation decreased ($P < 0.05$) total acetyl-CoA carboxylase mRNA/adipocyte and tended to decrease 10.5 kb mRNA (Table 3). Both litter removal and bromocriptine treatment markedly increased ($P < 0.01$) acetyl-CoA carboxylase mRNA (10.5 kb and total species) to a similar extent (Table 3). The effects of bromocriptine were prevented by concurrent administration of prolactin (Table 3). Administration of AGS, either alone or with bromocriptine, had no effect on acetyl-CoA carboxylase mRNA. The various treatments had little effect on the amount of total RNA/cell, effects being specific in that the amount of acetyl-CoA carboxylase mRNA/total RNA changed markedly in response to treatment (Table 3).

Acetyl-CoA carboxylase: activation status

The proportion of mammary acetyl-CoA carboxylase in the active state was increased by bromocriptine treatment in the summer (Table 4). This observation was confirmed in June of the

following year (results not shown). Treatment with bromocriptine or bromocriptine plus AGS in November through to February, however, had no effect on the proportion of mammary acetyl-CoA carboxylase in the active state (results not shown). Administration of AGS in the presence or absence of bromocriptine had no effect on the proportion of the enzyme in the active state in either summer (Table 4) or winter (results not shown). Injections of GH (Table 4) to rats concurrently given AGS plus bromocriptine had no effect on the proportion of mammary acetyl-CoA carboxylase in the active state.

The response of adipose-tissue acetyl-CoA carboxylase activation status to bromocriptine and other treatments did not show seasonal variation, so results for several experiments have been pooled (Table 4). Lactation decreased the activation status ($P < 0.001$), and this effect was reversed by bromocriptine ($P < 0.001$). The effect of bromocriptine was, in turn, prevented by the concurrent administration of sheep prolactin ($P < 0.02$) (Table 4). Treatment with AGS alone had no effect on activation status, but AGS acted synergistically with bromocriptine to increase activation status further ($P < 0.05$) (Table 4). The effect of AGS in the presence of bromocriptine was prevented by concurrent administration of GH ($P < 0.01$). Litter removal increased ($P < 0.001$) the activation status of acetyl-CoA carboxylase to the same extent as prolactin (Table 4).

AMP-stimulated protein kinase

Lactation, litter removal or manipulation of either serum prolactin or GH concentration did not alter the activity of AMP-stimulated protein kinase (Table 4).

DISCUSSION

The key role of prolactin in the reciprocal changes in lipogenesis which occur in mammary and adipose tissue during lactation in rats is well established (Agius *et al.*, 1979; Flint *et al.*, 1981), but the mechanism has not been fully defined. The present study extends previous observations that prolactin increases mammary acetyl-CoA carboxylase activity (McNeillie & Zammit, 1982; Munday & Williamson, 1982), by showing that the hormone decreases adipose-tissue acetyl-CoA carboxylase activity and that, for both tissues, changes in total enzyme activity are paralleled by changes in the amount of acetyl-CoA carboxylase mRNA. Thus effects appear to be achieved by a reciprocal

control of gene expression in the two tissues. A very recent study (Lopez-Casillas *et al.*, 1991) shows that the acetyl-CoA carboxylase gene contains two promoters, one of which is active in mammary tissue and the other in adipose tissue. It would thus appear that prolactin is capable of switching on the mammary promoter and switching off the adipose-tissue promoter; the latter effect must be indirect, as adipocytes appear to lack prolactin receptors (Vernon & Flint, 1983).

In addition to its effect on total acetyl-CoA carboxylase, prolactin also modulates acetyl-CoA carboxylase activation status, particularly in adipose tissue. Lactation results in a loss of the ability of insulin to activate adipose-tissue acetyl-CoA carboxylase *in vivo*, and this is relieved by decreasing serum prolactin with bromocriptine (Graham *et al.*, 1990). In addition, decreasing serum prolactin also causes a rise in serum insulin concentration (Agius *et al.*, 1979). Thus the increase in activation status of acetyl-CoA carboxylase of adipose tissue after treatment with bromocriptine is most probably due to an increase in serum insulin coupled with a restoration of response to insulin; the overall effect is to augment the consequence of decreasing prolactin on total acetyl-CoA carboxylase of the tissue. This contrasts with effects of varying serum prolactin concentration on the mammary enzyme. Although previous studies (McNeillie & Zammit, 1982; Munday & Williamson, 1982) have shown that bromocriptine decreases the total amount of mammary acetyl-CoA carboxylase enzyme, those studies showed no effect on activation status as found in the winter in the present study. Furthermore, in the studies by McNeillie & Zammit (1982) and Munday & Williamson (1982) the mammary tissue was freeze-clamped, excluding possible rapid changes in activation status during tissue removal. In the summer, bromocriptine treatment did increase the activation status of the enzyme, thus partially attenuating the effect of the drug on total acetyl-CoA carboxylase activity. This might explain the observation by Oller do Nascimento *et al.* (1989) that bromocriptine treatment did not decrease mammary lipogenesis, whereas previous studies reported a decrease (Agius *et al.*, 1979; Flint *et al.*, 1981); unfortunately the times of the year when those experiments were conducted were not reported.

AMP-stimulated protein kinase is thought to have a key role in phosphorylation and inactivation of acetyl-CoA carboxylase *in vivo* (Hardie, 1989; Cohen & Hardie, 1991). However, no changes in activation of this enzyme were apparent in adipose tissue after the various manipulations. Thus changes in activation status are being achieved either through changes in phosphatase activity or through subtle changes in AMP-stimulated kinase activity which are lost *in vitro*.

Despite its important role in ruminant animals, the role of GH in lactation in rats has not been clearly defined. Madon *et al.* (1986) found that, before peak lactation (about 7 days *post partum*), decreasing serum GH had no effect on milk yield (as indicated by pup growth rate) provided that serum prolactin concentration was normal. In the present study with rats at peak lactation, decreasing serum GH alone was sufficient to cause some fall in milk yield, inferred by a fall in pup growth rate. This may reflect a greater requirement for nutrients at peak lactation than at 7 days *post partum*. The fall in milk yield owing to AGS treatment was not due to an increase in adipose-tissue acetyl-CoA carboxylase activity, and hence a diversion of nutrients from the mammary gland to the adipose tissue, possibly owing to the dominant role of prolactin. Decreasing serum GH in lactating rats does impair lipolysis (Vernon *et al.*, 1987), so a decreased release of stored fatty acids may be partly responsible for the decreased milk yield in AGS-treated rats.

When both serum GH and prolactin concentrations are decreased, milk yield falls to the point that pups lose weight.

Mammary acetyl-CoA carboxylase activity and mRNA concentration fall further than when prolactin alone is decreased, indicating synergy between GH and prolactin in the control of the amount of mRNA and total activity of the enzyme in the mammary gland. No such synergy is apparent in adipose tissue with respect to total acetyl-CoA carboxylase activity, and the further increase in activation status is probably due to a greater increase in serum insulin than when prolactin alone is decreased. Also, although decreasing serum prolactin increases adipose-tissue acetyl-CoA carboxylase to the same level as seen on litter removal, decreasing both prolactin and GH does not decrease mammary enzyme activity to the very low levels found on litter removal. However, litter removal and decreasing GH plus prolactin have similar effects on mammary acetyl-CoA carboxylase mRNA. Litter removal thus probably leads to either a greater decrease in translation of acetyl-CoA carboxylase mRNA or a greater rate of enzyme degradation, or perhaps both, than does treatment with AGS plus bromocriptine. That mammary acetyl-CoA carboxylase mRNA concentration does not fall to very low levels on litter removal may be due to a more constitutive expression of this mRNA in the gland (Luo & Kim, 1990).

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