Modulation of the activity of acetyl-CoA carboxylase and other lipogenic enzymes by growth hormone, insulin and dexamethasone in sheep adipose tissue and relationship to adaptations to lactation

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The mechanisms whereby growth hormone and dexamethasone modulate the stimulation of fatty acid synthesis by insulin in adipose tissue from lactating and non-lactating sheep have been investigated. Maintenance of adipose tissue from wethers (castrated male sheep) in tissue culture for 24 or 48 h with insulin resulted in an increased proportion of acetyl-CoA carboxylase being present in the active state; this effect was enhanced by dexamethasone and was antagonized by growth hormone. Lactation results in a decrease in both the total acetyl-CoA carboxylase of sheep adipose tissue and the proportion of the enzyme in the active state. Maintenance of adipose tissue from lactating sheep in tissue culture for 48 h in the presence of insulin plus dexamethasone increased markedly the proportion of acetyl-CoA carboxylase in the active state and increased slightly the total activity of the enzyme. Both of these effects were prevented by actinomycin D, and the change in activation status was prevented by growth hormone. Tissue culture for 6 days showed that growth hormone could also prevent the ability of insulin plus dexamethasone to increase the total activity of the enzyme. Analogous studies showed that insulin, dexamethasone and growth hormone modulated the activities of other lipogenic enzymes, but the effects were proportionately smaller than for acetyl-CoA carboxylase. Insulin also increased total protein synthesis in adipose tissue, but this was not antagonized by growth hormone. The results suggest that the fall in fatty acid synthesis in sheep adipose tissue during lactation is due to a decrease in both the total acetyl-CoA carboxylase activity and the proportion of the enzyme in the active state; these changes are probably induced by known changes in the serum concentrations of insulin and growth hormone. Lactation appears to result in the loss of a protein that is required for activation of acetyl-CoA carboxylase by insulin; production of this component appears to be prevented by growth hormone.

INTRODUCTION

Lactation results in a decrease in the rate of fatty acid synthesis in adipose tissue (Vernon & Flint, 1983). This adaptation occurs in a variety of species, including rats and ruminants, and is thought to be a device for facilitating the preferential use of lipogenic precursors by the mammary gland. The hormones responsible for the adaptation do, however, appear to differ between species; prolactin has a key role in the rat (Agius *et al.*, 1979; Flint *et al.*, 1981), whereas growth hormone rather than prolactin appears to be a major factor in sheep (Vernon & Finley, 1988). In addition, the hypoinsulinaemia of lactation probably contributes to the decrease in fatty acid synthesis in both species (Williamson, 1980; Vernon & Flint, 1983).

The mechanisms whereby growth hormone decreases fatty acid synthesis in adipose tissue have not been resolved. We have found that sheep adipose tissue survives well in culture and have used this system to show that growth hormone acts directly on the tissue to inhibit insulin-stimulated fatty acid synthesis; this effect of growth hormone is mimicked by actinomycin D, an inhibitor of gene transcription. In the present study we have used this system to determine if growth hormone affects the total activity or the activation status of acetyl-CoA carboxylase (EC 6.4.1.2), a key enzyme in the control of fatty acid synthesis (Hardie, 1989). Previous studies have shown that the total activity of this enzyme is markedly decreased by lactation in sheep adipose tissue (Vernon *et al.*, 1987). The results suggest that the diminished rate of fatty acid synthesis in adipose tissue during lactation in sheep is due to a decrease in the activation status as well as in the total activity of acetyl-CoA carboxylase, and that these adaptations are the result of changes in the serum growth hormone/insulin ratio. Furthermore, restoration of the

ability of insulin to activate acetyl-CoA carboxylase in adipose tissue from lactating sheep is dependant on gene transcription.

EXPERIMENTAL

Sheep were Finn × Dorset Horn cross-bred wethers (castrated males, about 9 months old) and ewes (3–4 years old). They were fed on cereals and hay for at least 4 weeks before slaughter, as described previously (Vernon *et al.*, 1981). Lactating ewes were suckling at least two lambs and were at about day 18 of lactation. Ewes and wethers were anaesthetized at about 10.00 h and exsanguinated, and samples of subcutaneous adipose tissue were removed aseptically as described previously (Vernon & Finley, 1988).

Explants of subcutaneous adipose tissue were prepared and maintained in culture in Medium 199 containing Earles salts, L-glutamine and 25 mM-Hepes (pH 7.5) (Gibco Biocult Ltd., Paisley, Scotland, U.K.) supplemented with 2 mM-acetate (final concentration 2.6 mM) and antibiotics as described previously (Robertson *et al.*, 1982). Cultures were usually terminated after 24 or 48 h, but when they were continued for 6 days the culture medium was replaced with fresh medium after 3 days. Insulin (17 nM), dexamethasone (10 nM) (Sigma) and sheep growth hormone (4.5 nM) (a gift from NIADDK, Bethesda, MD, U.S.A.) and actinomycin D (80 nM) were added singly or in combination to the culture medium as indicated in the text and Tables.

Fresh explants of adipose tissue and explants after culture were frozen and stored in liquid N₂ until assayed for enzyme activities. Preparation of homogenates and supernatants and assay of acetyl-CoA carboxylase were performed as described previously for sheep adipose tissue (Vernon & Taylor, 1986).

Table 1. Effects of tissue culture for 24 and 48 h in the presence of hormones and other agents on the activity of acetyl-CoA carboxylase in sheep adipose tissue

Pieces of adipose tissue from wethers (castrated male sheep) were maintained in culture for 24 or 48 h in the presence or the absence of various combinations of insulin (ins, 17 nM), dexamethasone (dex, 10 nM) and growth hormone (GH, 4.5 nM); the activity of acetyl-CoA carboxylase was then assessed. Total activity is the activity found after preincubation with citrate and $MgCl_2$. Activity in the active state was measured in the presence and absence of 20 mM-citrate; Δ citrate is the difference between these two activities. K_a for citrate was determined from citrate dose-response curves of Fig. 1. Results are means of five observations and were analysed by analysis of variance; S.E.D. is standard error of the difference. Values in a column for the given time period without a common superscript (a, b, c) differ significantly ($P < 0.05$).

Culture conditions	Acetyl-CoA carboxylase				
	Total activity (nmol/min per 10^6 cells)	Activity in active state (% of total activity)			K_a for citrate (mM)
		– citrate	+ 20 mM-Citrate	Δ Citrate	
Pre-culture	22.3	6.1	21.5	15.5	8.8
24 h culture					
No additions	23.0 ^{a,b}	11.4 ^a	32.3 ^{a,c}	20.9 ^{a,c}	7.3 ^a
Ins	25.0 ^a	11.9 ^a	68.6 ^b	56.7 ^b	4.3 ^a
Dex	21.0 ^b	3.8 ^b	16.9 ^a	13.5 ^a	5.0 ^a
Ins + dex	22.5 ^{a,b}	9.4 ^{a,b}	66.8 ^{b,c}	57.4 ^b	6.0 ^a
Ins + dex + GH	23.5 ^{a,b}	7.4 ^{a,b}	47.6 ^c	40.2 ^{b,c}	3.7 ^a
S.E.D.	1.65	2.84	9.63	9.63	1.88
48 h culture					
No additions	22.3 ^{a,b}	10.4 ^a	36.6 ^a	26.2 ^a	5.3 ^a
Ins	25.0 ^b	19.8 ^b	64.5 ^b	44.7 ^b	5.2 ^a
Dex	18.6 ^a	14.0 ^{a,b}	29.7 ^a	17.4 ^a	5.6 ^a
Ins + dex	25.0 ^b	19.2 ^b	82.4 ^c	63.2 ^c	4.7 ^a
Ins + dex + GH	22.9 ^b	13.0 ^{a,b}	30.9 ^a	17.9 ^a	4.0 ^a
S.E.D.	1.88	4.19	8.00	6.88	1.15

Expressed (initial) activities were measured within 3 min of preparation of homogenates from frozen tissue and were measured in the presence and absence of 20 mM-citrate; these activities were assumed to reflect the activity of the enzyme in the active state. Samples of supernatant were also preincubated with 20 mM-citrate and 20 mM- $MgCl_2$ before assay to activate any inactive enzyme (Vernon & Taylor, 1986). Details of the preparation of homogenates and supernatants for the assay of other enzymes have been described (Vernon *et al.*, 1987), and enzymes were assayed as described previously (Vernon, 1976; Vernon *et al.*, 1987).

Rates of fatty acid synthesis in tissue pieces before and after culture were determined in tissue explants as described previously (Vernon & Finley, 1988). For measurement of the rate of protein synthesis, fresh and post-culture explants were incubated for 4 h in culture medium with L-[4,5- 3H]leucine. The amount of 3H incorporated into protein was determined as described previously (Vernon, 1977). In some studies [3H]leucine was added at the beginning of the period of tissue culture and the amount of 3H incorporated into protein over the 48 h culture period was determined. Protein concentration was determined as described by Wang & Smith (1975). The number of adipocytes per g of tissue was determined as described previously (Robertson *et al.*, 1982). Results were analysed by analysis of variance.

RESULTS AND DISCUSSION

Studies with wethers

Maintenance of adipose tissue pieces from wethers (castrated male sheep) in culture for 48 h with various combinations of hormones showed that insulin and dexamethasone acted synergistically to increase the rate of fatty acid synthesis and that this effect was antagonized by growth hormone (results not shown). In addition, culture with dexamethasone alone decreased the rate

of fatty acid synthesis (results not shown). These observations are in full agreement with our previous studies with adipose tissue from mature ewes (Vernon & Finley, 1988).

Maintenance of adipose tissue pieces from wethers in tissue culture for up to 48 h in the absence or presence of various combinations of insulin, dexamethasone and growth hormone had no marked effect on the total activity of acetyl-CoA carboxylase (Table 1) (activity measured after preincubation with citrate and $MgCl_2$ to activate any inactive enzyme) (McNeillie & Zammit, 1982). Total activity after culture in the presence of insulin was higher than that found after culture with dexamethasone ($P < 0.05$). The activity of acetyl-CoA carboxylase in the active state was assayed in the presence and absence of various concentrations of citrate (Fig. 1, Table 1). Some citrate-independent activity is most probably due to pyruvate carboxylase activity in sheep adipose tissue (Vernon & Taylor, 1986), as in rat adipocytes (Haystead & Hardie, 1986a). There was little correlation between citrate-independent acetyl-CoA carboxylase activity after various hormone treatments in the present study (Table 1) and the rates of fatty acid synthesis after such treatments (Vernon & Finley, 1988). In contrast, changes in the proportion (and hence activity) of acetyl-CoA carboxylase in the active state, when measured in the presence of 20 mM-citrate (Table 1), correlated well with changes in the rate of fatty acid synthesis in response to various hormones. An even better correlation is apparent when the citrate-dependent activity (i.e. activity in the presence of 20 mM-citrate minus activity in the absence of citrate) (Table 1) is considered. Thus culture in the absence of exogenous hormones for 24 or 48 h had no effect on the expressed activity of the enzyme, whereas the presence of insulin in the culture medium induced a significant increase in the proportion of the enzyme in the active state after 24 h, and this was maintained over the next 24 h (Table 1). When dexamethasone was included along with insulin there was a further

Fig. 1. Effects of maintenance of sheep adipose tissue in culture with insulin, dexamethasone and growth hormone on acetyl-CoA carboxylase activity in the active state as a function of citrate concentration

Pieces of adipose tissue from wethers (castrated male sheep) were maintained in culture for (a) 24 h or (b) 48 h in the absence of exogenous agents (Δ), in the presence of 17 nM-insulin ($\circ$), in the presence of 10 nM-dexamethasone ($\blacktriangle$), in the presence of insulin plus dexamethasone ($\bullet$), or in the presence of insulin plus dexamethasone and 4.5 nM-growth hormone ($\blacksquare$). Results are also presented in (a) for pieces of tissue before culture ($\square$). Tissue pieces were then frozen and stored in liquid N_2 before assay of acetyl-CoA carboxylase activity in the active state in the presence and absence of various concentrations of citrate. Total acetyl-CoA carboxylase activity was determined by preincubation of supernatants with citrate and $MgCl_2$. Results are means of five observations. For further details see the text and the legend to Table 1.

increase in activity over the second 24 h period (Table 1). By itself, dexamethasone had no significant effect on the proportion of the enzyme in the active state, although the proportion did tend to decrease; this, coupled with the trend for the total activity to decrease during culture with dexamethasone, resulted in a consistent decrease in the amount of the enzyme in the active state per cell. The addition of growth hormone partly prevented the stimulatory effect of insulin plus dexamethasone over the first 24 h period and completely blocked their effects over 48 h (Table

1). These effects of the hormones on the proportion of acetyl-CoA carboxylase in the active state over 24 and 48 h are almost exactly the same as the effects of the hormones on the rate of fatty acid synthesis in wethers described above and found previously in studies with ewes (Vernon & Finley, 1988). Furthermore, the activity of acetyl-CoA carboxylase in the active state in the present study is very similar to the lipogenic flux of sheep adipose tissue found previously (Vernon & Finley, 1988). The ability of insulin to increase the proportion of acetyl-CoA carboxylase in the active state in rat adipocytes is well established (see Hardie, 1989). The present study shows that insulin also causes activation of this key enzyme in sheep adipose tissue. In addition, it appears that both dexamethasone and growth hormone exert their effects on the rate of fatty acid synthesis in sheep adipose tissue, at least in the short term, by altering the activation status of acetyl-CoA carboxylase.

We noted previously that, although insulin has similar effects on adipose tissue metabolism in rats and sheep, the time courses are different; clear consistent effects of insulin on sheep adipose tissue metabolism are not usually seen during short-term incubations (2–3 h) of fresh tissue. However, effects of insulin on sheep adipose tissue do become apparent during incubations of 24 h or longer, and, in contrast with analogous but more rapid effects on rat adipose tissue, are blocked by actinomycin D, an inhibitor of gene transcription (Plested *et al.*, 1987; Vernon & Finley, 1988). Again we have been unable to demonstrate a clear and consistent effect of insulin on the proportion of acetyl-CoA carboxylase in the active state in sheep adipose tissue during incubations of 2–3 h of fresh tissue from adult sheep (results not shown). Furthermore, in two experiments we found that activation of the enzyme by insulin and dexamethasone was partly blocked by actinomycin D; mean citrate-dependent activities (% of total activity) after 48 h in the presence of insulin and dexamethasone with and without actinomycin D were 62.8 and 35.2% respectively. In these same two experiments, addition of actinomycin D after 24 h of culture in the presence of insulin plus dexamethasone (i.e. actinomycin D was present over the second 24 h period of culture) had no effect on the proportion of acetyl-CoA carboxylase in the active state; the mean citrate-dependent activity was 54.6% of total activity. Similarly, the effects of insulin on a variety of metabolic processes in sheep adipose tissue over a 48 h period were prevented by addition of actinomycin D at the beginning of the culture period, but not if the transcription inhibitor was added after 24 h of culture (Plested *et al.*, 1987), although addition after 24 h of culture with insulin plus dexamethasone did diminish slightly the rate of fatty acid synthesis (Vernon & Finley, 1988).

Effects of lactation

Lactation decreases the total acetyl-CoA carboxylase activity in sheep adipose tissue to about 6% of that in non-lactating sheep (Vernon *et al.*, 1987). A similar change also occurs in rat adipose tissue (Sinnott-Smith *et al.*, 1980). In addition, further studies showed that the proportion of the enzyme in the active state was decreased by about 80% by lactation in sheep adipose tissue (R. G. Vernon & D. J. Mellor, unpublished work). The combination of decreases in total activity and activation status is sufficient to account for the 98% decrease in the rate of fatty acid synthesis with lactation in sheep adipose tissue (Vernon *et al.*, 1981; Vernon & Finley, 1988).

Our previous studies suggested that changes in the rate of fatty acid synthesis with lactation were due to changes in serum concentrations of insulin and growth hormone (Vernon & Finley, 1988). These studies showed that maintenance of adipose tissue from lactating sheep in culture for 48 h with insulin and dexamethasone resulted in a partial restoration of the rate of

Table 2. Effect of tissue culture for 48 h in the presence of hormones and other agents on the acetyl-CoA carboxylase activity of adipose tissue from lactating ewes

Pieces of adipose tissue from lactating sheep were maintained in culture as detailed in the legend to Table 1, after which the total acetyl-CoA carboxylase activity and the activity of the enzyme in the active state were assessed as in Table 1, except that the latter was measured in the presence of 20 mM-citrate only. Act D, 80 nM-actinomycin D; other abbreviations are as in Table 1. Results were analysed by analysis of variance: S.E.D., standard error of the difference; *n*, number of observations. Values in a column without a common superscript differ significantly ($P < 0.05$).

Additions to culture medium	Acetyl-CoA carboxylase (nmol/min per 10 ⁶ cells)	
	Active state	Total activity
Pre-culture	0.00 ^a	0.59 ^a
No additions	0.03 ^a	1.04 ^{a,b}
Ins	0.29 ^a	2.06 ^{b,c}
Dex	0.00 ^a	0.73 ^a
Ins + dex	0.85 ^b	2.33 ^c
Ins + dex + GH	0.18 ^a	1.70 ^{b,c}
Ins + dex + act D	0.00 ^a	0.60 ^a
S.E.D.	0.22	0.46
<i>n</i>	5	4

fatty acid synthesis; furthermore, this was prevented by the addition of either growth hormone or actinomycin D. Maintenance of adipose tissue from lactating sheep in culture for 48 h with insulin plus dexamethasone increased the total acetyl-CoA carboxylase activity 4-fold ($P < 0.05$) (Table 2) and increased the expressed activity by about 80-fold ($P < 0.02$) (Table 2). Insulin alone also increased the total acetyl-CoA carboxylase activity ($P < 0.05$). Growth hormone prevented the increase in expressed acetyl-CoA carboxylase activity induced by insulin and dexamethasone, but only had a marginal effect on the increase in total activity induced by these agents (Table 2), whereas actinomycin D prevented the increase in both expressed and total activity (Table 2). Thus changes in expressed activity in tissue from lactating sheep in response to insulin, growth hormone and other agents are qualitatively the same as noted for wether sheep, and also parallel changes in fatty acid synthesis observed when adipose tissue from lactating sheep is maintained in culture for 48 h (Vernon & Finley, 1988). However, in contrast with studies with wether sheep, the increase in expressed acetyl-CoA carboxylase activity found in response to insulin and dexamethasone is due to a combination of a major increase in activation status of the enzyme plus a proportionately smaller increase in total activity. The latter is probably due to the synthesis of new enzyme protein, as it is prevented by actinomycin D. What is particularly interesting is that the ability of insulin and dexamethasone to activate the enzyme is also completely blocked by actinomycin D, indicating a requirement for some seemingly unrecognized protein, involved in signal transduction, which is lost during lactation. Previous studies on the effect of actinomycin D on the restoration of the rate of fatty acid synthesis in adipose tissue from lactating sheep also indicated a requirement for transcription; the present study suggests that there is both synthesis of acetyl-CoA carboxylase itself and also synthesis of some component required for the activation of the enzyme.

Whereas 48 h of culture was adequate for investigating the effects of hormones on the activation status of acetyl-CoA carboxylase, it was not really sufficient to investigate the control of total activity of this enzyme. Further studies with tissue from

lactating sheep had shown that during culture with insulin and dexamethasone the rate of fatty acid synthesis increased dramatically over 24–72 h of culture and then increased more slowly for at least a further 3 days (Vernon & Sasaki, 1990). We therefore maintained such tissue in culture for 6 days in the presence or the absence of hormones. Before culture, the total acetyl-CoA carboxylase activity was 0.82 nmol/min per 10⁶ cells. After 6 days of culture in the absence of exogenous hormones the activity was still only 0.67 nmol/min per 10⁶ cells, but after 6 days of culture with insulin plus dexamethasone the activity had increased ($P < 0.001$) to 5.38 nmol/min per 10⁶ cells. Inclusion of growth hormone prevented this increase ($P < 0.001$), the total acetyl-CoA carboxylase activity after 6 days with insulin, dexamethasone and growth hormone being 0.61 nmol/min per 10⁶ cells. All values are means of four observations (each from separate sheep); the standard error of the difference was 0.93.

Other lipogenic enzymes

Although the effects of lactation are most marked for acetyl-CoA carboxylase of sheep adipose tissue, activities of several other enzymes involved in lipogenesis are also decreased (Vernon *et al.*, 1987). Hence the effects of culture with various hormones and agents were tested on several other enzymes. As found previously, lactation decreased ($P < 0.01$) (Table 3) the activity of glucose-6-phosphate dehydrogenase in sheep adipose tissue (Vernon *et al.*, 1987). Lactation also decreased the activity of fatty acid synthetase ($P < 0.01$) and of 6-phosphogluconate dehydrogenase ($P < 0.05$), but not that of isocitrate dehydrogenase (Table 3). The effect of insulin and dexamethasone on the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and fatty acid synthetase of tissue from lactating sheep were similar to their effects on acetyl-CoA carboxylase, with highest activities being found after culture with insulin plus dexamethasone (Table 3). The presence of actinomycin D again resulted in significantly lower ($P < 0.05$) activities of all three enzymes, whereas the addition of growth hormone resulted in a lower glucose-6-phosphate dehydrogenase activity ($P < 0.05$) but had no significant effect on the other two enzymes. The effects of the hormones on these enzyme activities in adipose tissue from non-lactating sheep were similar to those in tissue from lactating sheep, but, in general, were proportionately smaller. The various hormones had little or no effect on isocitrate dehydrogenase activity in tissue from both lactating and non-lactating sheep (Table 3). These observations support the view that the changes in the rate of fatty acid synthesis induced by insulin, growth hormone and dexamethasone in sheep adipose tissue are mainly due to changes in acetyl-CoA carboxylase activity, and over the initial 48 h of culture they are due primarily to changes in the activation status of this key enzyme. Furthermore, the synergistic effect of dexamethasone on insulin-induced fatty acid synthesis also seems to be due primarily to an effect on the activation status of acetyl-CoA carboxylase.

Protein synthesis

The effects of growth hormone on the insulin-plus-dexamethasone-induced increase in lipogenesis (Vernon & Finley, 1988) and acetyl-CoA carboxylase and glucose-6-phosphate dehydrogenase activities (present study) are paralleled by actinomycin D. This suggests that growth hormone inhibits the synthesis of key proteins. The minimal effect of growth hormone on fatty acid synthetase and also on total acetyl-CoA carboxylase in non-lactating sheep may be due to the relatively long half-lives of these enzymes (over 48 h in rat tissues; Volpe & Vagelos, 1976). Any effects of growth hormone on protein synthesis, however, are specific for certain proteins and are not due to a general decrease in the rate of protein synthesis. Thus, whereas

Table 3. Effects of tissue culture for 48 h in the presence of hormones and other agents on enzyme activities of adipose tissue from lactating (L) and non-lactating (NL) ewes

Pieces of adipose tissue were maintained in culture for 48 h as detailed in the legends to Tables 1 and 2, after which enzyme activities were determined as described in the text. Results were analysed by analysis of variance: S.E.D., standard error of the difference; *n*, number of observations. Values in a column without the same superscript differ significantly ($P < 0.05$). Abbreviations are as in Tables 1 and 2.

Additions to culture medium	State...	Activity (nmol/min per 10 ⁶ cells)							
		Fatty acid synthetase		Glucose-6-phosphate dehydrogenase		6-Phosphogluconate dehydrogenase		Isocitrate dehydrogenase	
		NL	L	NL	L	NL	L	NL	L
Pre-culture		26.6 ^a	6.2 ^a	163 ^a	29 ^{a,c}	65 ^a	35 ^{a,b}	326 ^{a,b}	334 ^a
No additions		27.1 ^a	7.7 ^a	186 ^a	33 ^a	82 ^{a,b}	37 ^a	345 ^{a,b}	336 ^a
Ins		32.3 ^{a,b}	10.1 ^b	231 ^{a,b}	54 ^b	77 ^{a,b}	37 ^a	375 ^a	440 ^b
Dex		30.4 ^a	7.8 ^a	193 ^a	28 ^a	67 ^a	26 ^b	347 ^b	317 ^a
Ins + dex		34.9 ^b	10.7 ^b	300 ^b	55 ^b	101 ^b	48 ^c	352 ^a	336 ^a
Ins + dex + GH		32.3 ^{a,b}	9.4 ^b	167 ^a	40 ^a	82 ^{a,b}	48 ^c	336 ^{a,b}	353 ^{a,b}
Ins + dex + act D		29.6 ^{a,b}	7.3 ^a	—	18 ^c	78 ^{a,b}	26 ^b	284 ^b	284 ^a
S.E.D.		2.8	0.7	39	6	12	5	32	47
<i>n</i>		4	5	5	7	4	4	4	6

Table 4. Effect of tissue culture for 48 h in the presence of hormones and other agents on the rate of protein synthesis in adipose tissue from ewes

Pieces of adipose tissue from lactating and non-lactating sheep were maintained in culture for 48 h as detailed in the legends to Tables 1 and 2, after which the protein concentration of the tissue and the rate of protein synthesis were measured as described in the text. The rate of incorporation of [³H]leucine into protein was also measured over the 48 h culture period. Results for lactating and non-lactating sheep did not differ significantly and so were pooled. Results were analysed by analysis of variance: S.E.D., standard error of difference; *n*, number of observations. Values in a column without the same superscript (a, b, c, d) differ significantly ($P < 0.05$). Abbreviations are as in Tables 1 and 2.

Additions to culture medium	Protein synthesis ([³ H]leucine incorporation into protein)		
	During culture (nmol/48 h per 10 ⁶ cells)	Before/after culture (nmol/4 h per 10 ⁶ cells)	Protein concentration (mg/10 ⁶ cells)
Pre-culture	—	6.3 ^a	2.01 ^a
No additions	112 ^{a,b}	21.8 ^b	2.03 ^a
Ins	151 ^{a,c}	34.8 ^c	2.05 ^a
Dex	88 ^b	17.0 ^b	1.74 ^{b,c}
Ins + dex	155 ^c	31.7 ^c	1.93 ^{a,b}
Ins + dex + GH	138 ^{a,c}	32.5 ^c	1.95 ^{a,b}
Ins + dex + act D	24 ^d	2.0 ^a	1.56 ^c
S.E.D.	20	3.5	0.12
<i>n</i>	7	7	9

culture with insulin plus dexamethasone increased the rate of incorporation of [³H]leucine into protein over the 48 h of culture in tissue from both lactating and non-lactating sheep (Table 4) (values were pooled as lactation had no effect on the rate of incorporation), addition of growth hormone had no significant effect on the rate of incorporation. Addition of actinomycin D, however, decreased ($P < 0.001$) the rate of incorporation of [³H]leucine to a very low level (Table 4). In addition, the presence of growth hormone during culture had no effect on the protein content of adipose tissue, whereas actinomycin A caused a small, but significant ($P < 0.02$), decrease (Table 4). Further evidence

for a lack of effect of growth hormone on protein synthesis in general was derived from measurement of the rate of protein synthesis in adipose tissue pieces after culture with combinations of insulin, dexamethasone and growth hormone. In these experiments the same hormone combinations were included in the incubation media as in the culture media. Preparatory experiments showed that the rate of ³H incorporation into protein was linear with respect to time for at least 4 h, regardless of previous culture conditions (results not shown). Again, the presence of insulin during culture resulted in an increased ($P < 0.05$) rate of protein synthesis which was unaffected by the presence of either growth hormone or dexamethasone (Table 4). The presence of actinomycin D during culture, however, markedly decreased ($P < 0.001$) the subsequent rate of protein synthesis (Table 4).

General discussion

Although the adaptation of fatty acid synthesis in sheep adipose tissue to meet the demands of lactation is associated with changes in activities of several lipogenic enzymes, it seems probable that the major factor is a decline in the amount and activation status of acetyl-CoA carboxylase. This is consistent with what might be inferred from our knowledge of the role and regulation of this enzyme in rat adipose tissue. The decline in serum insulin concentration with lactation in sheep (Vernon *et al.*, 1981) is likely to be partly responsible for these changes in both flux and enzyme activity, and this is exacerbated by the rise in serum growth hormone concentration (Vernon *et al.*, 1981).

The ability of growth hormone to decrease adiposity in animals and act as an insulin antagonist has been known for a long time (Goodman & Schwartz, 1974; Vernon & Flint, 1989), but the mechanisms involved have not been fully resolved. The present study thus provides further clarification by showing that growth hormone can both block the ability of insulin to activate acetyl-CoA carboxylase in the short term and impair the ability of insulin to alter the total activity (and presumably the amount and synthesis) of the enzyme in the longer term. The study also reveals a novel synergistic effect of dexamethasone on acetyl-CoA carboxylase activity. The mechanisms whereby growth hormone and dexamethasone modulate the ability of insulin to activate acetyl-CoA carboxylase are not known. Indeed, the mechanism by which insulin activates the enzyme in adipocytes

is still unclear. Insulin increases the phosphorylation of certain serine residues of acetyl-CoA carboxylase, but the relevance of this to the effects of the hormone on enzyme activity is controversial (Hardie, 1989); however, a recent study lends further support for this idea (Borthwick *et al.*, 1990). On the other hand, there is also evidence for the involvement of a low- M_r effector mediating the effects of insulin on acetyl-CoA carboxylase in rat adipocytes (Haystead & Hardie, 1986b). In any case, the recovery of the ability of insulin to activate the enzyme in adipose tissue from lactating sheep is prevented by an inhibitor of gene transcription. This implies that some component of the signal transduction cascade involved in the activation of the enzyme is lost during lactation. The present results are consistent with growth hormone exerting its effects by impairing the production of this putative mediator.

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