Mechanisms involved in the adaptations of the adipocyte adrenergic signal-transduction system and their modulation by growth hormone during the lactation cycle in the rat

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The mechanisms responsible for the diminished lipolytic response of adipocytes to catecholamines after litter removal from lactating rats and their modulation by growth hormone have been investigated. Lactation, litter removal and growth-hormone treatment did not alter the ability of noradrenaline to activate protein kinase A (A-kinase), showing that the defect in signal transduction in rats after litter removal is after A-kinase. Litter removal had no effect on hormone-sensitive lipase activity itself, but the proportion of the lipase associated with the fat droplet was decreased; growth-hormone treatment

increased hormone-sensitive lipase activity and the proportion associated with the fat droplet. In addition, a number of other adaptations in the β -adrenergic signal-transduction system occur during the lactation cycle and in response to growth hormone treatment, including changes in receptor number, adenylate cyclase activity and cyclic AMP phosphodiesterase activity, but a defect in the ability of hormone-sensitive lipase to associate with the lipid droplet appears to be the major reason for the diminished response to catecholamines on litter removal.

INTRODUCTION

Lactation usually results in a partial depletion of the lipid reserves of white adipose tissue, which are subsequently restored on removal of the litter or during natural weaning (Vernon and Flint, 1984). Restoration of lipid reserves in the rat is of interest because it is associated with a transient fall in the lipolytic response to catecholamines (Vernon and Finley, 1986), which may be due to a defect in growth-hormone (GH) action (Vernon et al., 1987). The molecular mechanisms involved have not been identified.

The chain of events leading from the interaction of catecholamines with the β -adrenergic receptor to phosphorylation and activation of hormone-sensitive lipase by protein kinase A (A-kinase) is well known. In addition, there is growing evidence for control at the level of interaction of hormone-sensitive lipase with its substrate, the triacylglycerols of the lipid droplet within the adipocytes. The lipid droplet is surrounded by a filamentous structure (Slavin, 1972; Franke et al., 1987), which may restrict access of lipase to triacylglycerol. For example, disruption of the cellular integrity by homogenization leads to an apparent activation of lipase activity in adipose tissue (Ninomiya et al., 1990); catecholamines may cause a translocation of hormone-sensitive lipase from the cytosol to a membrane fraction in 3T3-L1 adipocytes (Hirsch and Rosen, 1984). Recently a protein termed perilipin has been identified; this protein, which is associated with the lipid droplet, is phosphorylated by A-kinase and is postulated to be an anchor protein for hormone-sensitive lipase (Greenberg et al., 1991).

The various components of the β -adrenergic signal-transduction system of adipocytes are known to be under chronic endocrine control. For example, the number of β -receptors appears to be modulated by glucocorticoids (see Malbon et al.,

1988; Giudicelli et al., 1989), whereas the amounts and activities of the GTP-binding proteins are modulated by thyroid hormones (see Malbon et al., 1988; Houslay, 1990). In the present study we have investigated the effects of lactation and litter removal, and also manipulation of serum GH concentration, on a number of components of the β -adrenergic signal-transduction system. The study shows that during the lactation cycle several components of the signal-transduction system appear perturbed, with a key impairment at the level of hormone-sensitive lipase interaction with the lipid droplet.

MATERIALS AND METHODS

Animals

Wistar rats (A. Tuck and Son, Rayleigh, Essex, U.K.) were fed on Labsure irradiated CRM diet (57% carbohydrate, 18% protein, 2.4% fat; 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 injections were begun on days 12–14 of lactation. Some lactating rats were injected with a γ -globulin fraction of an antiserum to rat GH (220 mg/injection, equivalent to 4.5 ml of antiserum). Details of the preparation and characterization of the antiserum and its γ -globulin fraction have been described previously (Madon et al., 1986). Some rats which had their litters removed were injected with 1.5 mg of sheep GH (a gift from NIADDK, Bethesda, MD, U.S.A.)/injection, starting at the time of litter removal. Control virgin, lactating and litter-removed rats were injected with carrier solution (Madon et al., 1986). 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 and were killed by cervical dislocation (for assay of β -receptor number and

Abbreviations used: GH, growth hormone; IGF-I, insulin-like growth factor-I; PMSF, phenylmethanesulphonic acid; DTT, dithiothreitol; A-kinase, protein kinase A; PIA, N^6 -phenylisopropyladenosine.

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adenylate cyclase activity) or anaesthetized with 1 ml of Sagatal (RMB Animal Health Ltd., Dagenham, Essex, U.K.) containing 66 μ l of Hypnorm (Janssen Pharmaceuticals, Oxford, U.K.) (for all other measurements) at about 10:30 h.

Preparation of serum and adipocytes

Immediately after killing or anaesthesia, samples of blood and parametrial adipose tissue were removed. Serum was prepared and stored at -20°C for assay of insulin-like growth factor-I (IGF-I) (Madon et al., 1986). Adipocytes were prepared by collagenase digestion in Krebs–Ringer bicarbonate buffer [1.22 mM CaCl_2 (Krebs and Henseleit, 1932) containing 25 mM Hepes, pH 7.4, 5.5 mM glucose, 30 mg of BSA/ml and 1.5 mg of collagenase/ml (Sigma, type II)]. After about 60 min at 37°C , undigested material was removed by filtration through a nylon mesh. Adipocytes were separated from stromovascular cells by flotation and washed three times with the digestion medium (but with 10 mg of BSA/ml and no collagenase). Number of adipocytes/ml was determined as described previously (Vernon et al., 1987). All BSA used in this and other procedures was fatty-acid-free and dialysed prior to use (Hanson and Ballard, 1968).

Assay of β -adrenergic-receptor number and adenylate cyclase activity

Adipocytes were disrupted by suspension in 10 ml of 10 mM Tris/HCl (pH 7.4)/20 mM EDTA/0.2 mM sucrose/0.2 mM phenylmethanesulphonyl fluoride (PMSF) at room temperature and vortex-mixed for 1 min at room temperature. The preparation was centrifuged at 3500 g for 15 min at room temperature. The infranant was removed, diluted to 50 ml with ice-cold suspension medium and centrifuged at 42000 g for 30 min at 4°C . The membrane pellet was resuspended in 50 mM Tris/HCl (pH 7.4)/10 mM MgCl_2 , snap-frozen and stored in liquid N_2 .

β -Adrenergic-receptor number of the membrane fraction was determined by using (–)-[^3H]dihydroalprenolol as described previously (Watt et al., 1991), except that a range of (–)-[^3H]dihydroalprenolol concentrations (1–30 nM) were used. For measurement of adenylate cyclase activity membranes were incubated for 10 min at 37°C in 33.3 mM Tris/HCl (pH 7.4)/6.25 mM MgCl_2 /2 mM EGTA/0.5 mM dithiothreitol (DTT)/0.1 mM Ro7-2956/1 mM ATP/0.2 mM EDTA/30 mM KCl, containing 6.7 mM phosphocreatine, 30 units of creatine kinase/ml, 0.8 unit of adenosine deaminase/ml, 8 mg of BSA/ml plus other additions as indicated in the text and Tables (final volume 50 μ l). The reaction was terminated by addition of 50 μ l of 50 mM Tris/HCl (pH 7.4)/12 mM EDTA and heating in a boiling-water bath for 15 s. Samples were snap-frozen and stored in liquid N_2 before assay for cyclic AMP with kits obtained from Amersham International (Amersham, Bucks., U.K.). Ro 7-2956, a cyclic AMP phosphodiesterase inhibitor, was a gift from F. Hoffman–La Roche and Co., Basle, Switzerland. The protein concentration of membrane fractions was determined by the Bradford (1976) method, with IgG as standard. Use of IgG as standard (as recommended by the supplier) gives protein concentrations which are 3-fold higher than when BSA is used as standard.

Cyclic AMP phosphodiesterase activity

Parametrial adipose tissue from anaesthetized rats was immediately frozen in liquid N_2 and stored therein until assay. Homogenates of adipose tissue (10%, w/v) were prepared in ice-

cold 0.25 M sucrose/20 mM Tris/HCl (pH 7.4)/1 mM EDTA containing 1 μ g of leupeptin/ml and 0.1 mM PMSF (Anderson et al., 1989) and centrifuged (Eppendorf) for 1 min at 14000 rev./min (Anderson et al., 1989), and the supernatant fraction was assayed for cyclic AMP phosphodiesterase activity (Marchmont & Houslay, 1980).

A-kinase activity and glycerol release by isolated adipocytes

Adipocytes were prepared as described above by using tissue from anaesthetized animals, and were incubated for 25 min in Krebs–Ringer bicarbonate buffer containing 25 mM Hepes (pH 7.4), 5.5 mM glucose, 30 mg of BSA/ml, 0.8 μ g of adenosine deaminase/ml and various concentrations of noradrenaline as indicated in the text. After 25 min, 0.8 ml samples of cells plus medium were taken and added to 0.2 ml of ice-cold 50 mM Tris/HCl (pH 7.4)/50 mM EDTA/0.5 mM DTT containing 2.5 mM Ro7-2956, 1 mM benzamidine/HCl, 0.1 mM PMSF, 0.2 μ g of soya-bean trypsin inhibitor/ml, and cells were homogenized (Honnor et al., 1985). Total and expressed A-kinase was then assayed by using Kemptide (120 μ g/ml) as described by Clegg and Ottey (1990), except that incubation time was decreased to 2 min. Samples of incubation medium were also taken for glycerol determination as described previously (Vernon et al., 1987).

Hormone-sensitive lipase activity

Pieces of parametrial adipose tissue (about 100 mg total wt.) from anaesthetized rats were incubated for 25 min at 37°C in Krebs–Ringer bicarbonate buffer containing 25 mM Hepes (pH 7.4), 5.5 mM glucose, 30 mg of BSA/ml, 0.8 μ g of adenosine deaminase/ml and other additions as indicated in the text and Tables. At the end of the incubation, tissue was blotted, weighed and homogenized in 1.1 ml of ice-cold 0.25 M sucrose/1 mM EDTA/10 mM Tris/HCl (pH 7.4) buffer. The whole homogenate and the fat cake, obtained by centrifugation at 14000 rev./min (Eppendorf centrifuge) for 10 min and resuspended in 500 μ l of fresh homogenization buffer, were assayed for hormone-sensitive lipase activity by release of fatty acid from endogenous lipid by a modification of the method of Vaughan et al. (1964) and Itaya and Ui (1965). Samples of whole homogenate (500 μ l) or resuspended fat cake (200 μ l, plus 300 μ l of homogenization buffer) were incubated for 1 h at 37°C in a mixture comprising 20 mM sodium phosphate (pH 7.0), 40 mg of BSA/ml, 10 mM MgCl_2 and 1 mM ATP (final volume 1 ml). The reaction was terminated by addition of 1.5 ml of 0.9 M triethanolamine/0.1 M acetic acid/6.45% (w/v) $\text{Cu}(\text{NO}_3)_2$ and 4 ml of CHCl_3 . After shaking for 1.5 min, 1 ml samples of the CHCl_3 phase were transferred to glass tubes containing 100 μ l of 0.1% (w/v) sodium diethyldithiocarbamate in butan-1-ol, mixed, and the A_{440} was measured to determine fatty acid concentration. A range of palmitic acid concentrations were used as standards.

RESULTS AND DISCUSSION

Serum IGF-I

Effectiveness of the antiserum to GH and also of injections of sheep GH were checked by assaying for serum IGF-I. Lactation itself decreased serum IGF-I concentration ($P < 0.001$), whereas litter removal restored the value back to that found in virgin rats (Table 1). Treatment with the antiserum to GH decreased ($P < 0.001$), whereas injections of sheep GH increased ($P < 0.05$), serum IGF-I concentrations.

Table 1 Effects of lactation, litter removal and varying serum GH concentration on components of the β -adrenergic signal-transduction system of adipocytes and the serum IGF-I concentration

Adipose tissue was obtained from virgin rats, lactating rats, lactating rats which had been pre-treated for 2 days with an antiserum to rat GH, and lactating rats 2 days after litter removal treated with or without sheep GH or carrier solution. Adipocyte membranes were prepared and used for assessment of the number of β -adrenergic receptors and measurement of forskolin (100 μ M)-stimulated adenylate cyclase activity. Cyclic AMP phosphodiesterase activity was measured in white adipose tissue snap-frozen in liquid N₂ after removal from the animal. Maximum A-kinase activity was measured in isolated adipocytes after preincubation with various concentrations of noradrenaline for 25 min (values from each rat obtained with different concentrations of noradrenaline were meaned, and mean values were used in analysis of variance). Results are means $\pm$ S.E.M. (from analysis of variance) with number of observations in parentheses. Values within a column which do not have the same superscript (a, b, c, d) differ significantly ($P < 0.05$).

State	Treatment	β -Receptor number (fmol of ligand bound/mg of protein)	Forskolin-stimulated adenylate cyclase activity (pmol/min per mg of protein)	Cyclic AMP phosphodiesterase activity (pmol/min per 10 ⁶ cells)	A-kinase maximum activity (nmol/min per 10 ⁶ cells)	IGF-I concn. (ng/ml)
Virgin	Carrier	33.6 ^a $\pm$ 5.7 (5)	336 ^a $\pm$ 36 (10)	333 ^{ac} $\pm$ 26 (5)	4.26 ^a $\pm$ 0.60 (5)	678 ^a $\pm$ 54 (6)
Lactating	Carrier	63.5 ^{bc} $\pm$ 5.7 (5)	209 ^b $\pm$ 34 (11)	253 ^b $\pm$ 26 (5)	4.91 ^{ab} $\pm$ 0.60 (5)	508 ^b $\pm$ 54 (6)
Lactating	Anti-GH serum	46.2 ^a $\pm$ 5.7 (5)	—	—	—	146 ^c $\pm$ 54 (6)
Litter removed	Carrier	50.0 ^{ab} $\pm$ 6.4 (4)	364 ^a $\pm$ 40 (8)	392 ^c $\pm$ 26 (5)	6.21 ^b $\pm$ 0.48 (8)	716 ^a $\pm$ 54 (6)
Litter removed	GH	71.2 ^c $\pm$ 6.4 (4)	399 ^a $\pm$ 40 (8)	264 ^{ba} $\pm$ 26 (5)	5.25 ^{ab} $\pm$ 0.55 (6)	917 ^d $\pm$ 54 (6)

Table 2 Effects of lactation and litter removal with and without treatment with GH on the basal and isoprenaline-stimulated adenylate cyclase activity of adipocyte membranes

Adipocyte membranes were prepared from virgin rats, lactating rats, and lactating rats 2 days after litter removal which had received either sheep GH injections or carrier solution for 2 days before killing. Adenylate cyclase activity was measured in the presence or absence of GTP (1 μ M) and/or isoprenaline (10 μ M). Results are means of five or six observations and were analysed by analysis of variance; standard errors of differences for comparing values in rows and columns are 3.96 and 5.75 respectively. Values in a column which do not have the same superscript (a, b) differ significantly ($P < 0.05$).

State	Treatment	Additions...	Adenylate cyclase activity (pmol/min per mg of protein)			
			Basal	GTP	Isoprenaline	Isoprenaline + GTP
Virgin	Carrier		9.0	10.1	29.9	40.8 ^a
Lactating	Carrier		14.9	12.9	23.4	29.1 ^b
Litter removed	Carrier		11.5	9.5	28.7	40.8 ^a
Litter removed	GH injection		10.3	9.0	27.7	37.4 ^{ab}

β -Adrenergic-receptor number, adenylate cyclase and cyclic AMP phosphodiesterase activity

Scatchard analyses of dihydroalprenolol binding to adipocyte β -receptors were linear, indicating a single component with an affinity for the ligand of about 10 nM (results not shown). Ligand binding to β -receptors was increased ($P < 0.05$) by lactation (Table 1). This enhanced level of binding was decreased ($P < 0.05$) by treatment of rats with the antiserum to GH (Table 1) and by litter removal (2 days after litter removal, the ligand binding was intermediate between that of virgin and untreated lactating rats) (Table 1). Treatment of rats with GH prevented the fall in binding induced by litter removal (Table 1). A decrease in the number of β -adrenergic receptors of adipocytes with no change in affinity was also found by Ros et al. (1992), but those authors did not measure binding with membranes from non-lactating rats.

Addition of 100 μ M forskolin resulted in a maximum activation of adenylate cyclase activity, which was not increased further by addition of 1 μ M GTP or a range of concentrations from 0.1 nM to 100 μ M guanosine 5'-[β - γ -imido]triphosphate (results not shown). Thus the activity in the presence of 100 μ M forskolin appears to be the maximum activity of the enzyme in

the preparation. In contrast with the β -receptor, lactation caused a decrease ($P < 0.05$) in forskolin-stimulated cyclase activity (Table 1), which was completely reversed by litter removal (Table 1). Neither lactation nor litter removal had a significant effect on basal adenylate cyclase activity (Table 2). The maximum effect of isoprenaline was achieved with a concentration of 10 μ M with membranes from both virgin and lactating rats (results not shown). GTP (1 μ M) had no effect on adenylate cyclase activity by itself, but acted synergistically with isoprenaline to increase activity (Table 2). Adenylate cyclase activity in the presence of isoprenaline plus GTP was significantly lower ($P < 0.05$) in membranes from lactating rats compared with those from virgin rats and rats after litter removal (Table 2). Neither lactation nor litter removal had any effect on the ED₅₀ for isoprenaline (about 0.8 μ M). Treatment of rats with GH after litter removal had no effect on basal or stimulated adenylate cyclase activity (Tables 1 and 2) or ED₅₀ for isoprenaline.

Lactation decreased ($P < 0.05$) cyclic AMP phosphodiesterase activity of adipose tissue, and this was restored by litter removal ($P < 0.01$ compared with lactating-rat value) (Table 1). Concurrent treatment with GH prevented ($P < 0.01$) the rise in phosphodiesterase activity on litter removal (Table 1). A decrease in cyclic AMP phosphodiesterase activity during lactation was found previously by Aitchison et al. (1982).

Figure 1 Effects of lactation and litter removal with and without treatment with sheep GH on the ability of noradrenaline (a) to activate A-kinase and (b) to stimulate glycerol release in isolated adipocytes

A-kinase activity ratio is expressed as activity/total activity. Each value is the mean of three or four observations (S.E.M. values not shown): ○, virgin; ●, lactating; ■, lactating, litter removed; □, lactating, litter removed, treated with GH.

A-kinase activity

A-kinase activity was measured both in adipose tissue frozen in liquid N₂ immediately after removal from anaesthetized rats and in isolated adipocytes after incubation with various concentrations of noradrenaline or other agents. In both whole tissue (results not shown) and isolated adipocytes (Table 1), total A-kinase activity (i.e. measured in presence of 1 μM cyclic AMP) tended to be highest in litter-removed rats. Incubation of adipocytes with noradrenaline (1 nM–1 mM) had no effect on total A-kinase activity in any of the four states examined (results not shown). Incubation of isolated adipocytes with noradrenaline in the presence of adenosine deaminase increased the proportion of A-kinase in the active state from about 20% to 70% (Figure 1a) in all four states; neither maximum activation nor the concentration of noradrenaline required for half-maximum activation varied significantly with physiological state or treatment (Figure 1a). Glycerol release was also measured in this experiment (Figure 1b). The concentration of noradrenaline required for half-maximum stimulation of glycerol release (about 10 nM) did not differ between states, and was markedly lower than for activation of A-kinase (about 300 nM), but the maximum rate of glycerol release was significantly lower for adipocytes from litter-removed rats (Figure 1b) than in other states, in agreement with our earlier studies (Vernon and Finley, 1986; Vernon et al., 1987). When glycerol release was expressed as a function of either A-kinase activity ratio (Figure 2) or expressed A-kinase

Figure 2 Relationship between A-kinase activation status and rate of glycerol release by adipocytes from virgin rats (○), lactating rats (●), lactating litter-removed rats (■) and lactating litter-removed rats treated with GH (□)

Results are means of three or four values and were obtained from the experiment described in Figure 1.

activity (results not shown), the same relationship clearly applied for virgin, lactating and litter-removed rats treated with GH, but a different relationship applied for untreated litter-removed rats, with a given A-kinase activity eliciting a lower rate of glycerol release than in the other states.

Hormone-sensitive lipase

There are several possible explanations for this decreased ability of A-kinase to increase lipolysis in litter-removed rats: decreased amount of hormone-sensitive lipase; increased protein phosphatase activity; increased AMP-stimulated kinase activity; an impaired ability of hormone-sensitive lipase to interact with the lipid droplet.

The protein phosphatases (primarily 2A and, to a lesser extent, 1) which dephosphorylate hormone-sensitive lipase in adipocytes (Olsson and Belfrage, 1987) are inhibited by okadaic acid (Cohen et al., 1990), maximum inhibition being achieved with about 1 μM okadaic acid in intact adipocytes (Haystead et al., 1989; Rutter et al., 1991). Preincubation of adipocytes from either lactating or litter-removed rats with 1 μM okadaic acid increased ($P < 0.05$) the rate of glycerol release (Table 3); subsequent addition of noradrenaline plus adenosine deaminase markedly increased glycerol release, but this was not accentuated by preincubation with okadaic acid (Table 3). Similar results (stimulation of lipolysis in the absence of catecholamine, but not in the presence of 1 μM catecholamine) have been found previously in studies with okadaic acid using adipocytes from male rats (Haystead et al., 1989). In one experiment a range of okadaic acid concentrations up to 5 μM was used, but again without effect on the rate of noradrenaline-stimulated lipolysis. These results suggest that increased protein phosphatase activity is unlikely to be the reason for the diminished lipolytic response to catecholamines on litter removal.

Phosphorylation of hormone-sensitive lipase by AMP-stimulated kinase prevents phosphorylation and hence activation by A-kinase (Garton et al., 1989). Litter removal had no effect on AMP-stimulated kinase activity of adipose-tissue homogenates

Table 3 Effect of preincubation with okadaic acid on the amount of glycerol released in response to noradrenaline by adipocytes from lactating rats and litter-removed rats

Adipocytes were incubated for 25 min in the absence and presence of 1 μ M okadaic acid, after which noradrenaline (0.1 mM) and adenosine deaminase (0.8 μ g/ml) were added and the cells incubated for a further 25 min. Results are means $\pm$ S.E.M. of five (lactating) and four (litter-removed) observations.

Incubation period	Okadaic acid (1 μ M)	Glycerol release (μ mol/25 min per 10^6 cells)	
		Lactating	Litter removed
Preincubation (25 min)	—	0.10 $\pm$ 0.02	0.13 $\pm$ 0.02
	+	0.30 $\pm$ 0.07	0.22 $\pm$ 0.02
Incubation	—	1.86 $\pm$ 0.19	0.51 $\pm$ 0.12
+ noradrenaline	+	1.71 $\pm$ 0.15	0.49 $\pm$ 0.15

(Barber et al., 1992). In addition, litter removal results in an increased proportion of adipocyte acetyl-CoA carboxylase in the active state (Barber et al., 1992), which suggests that AMP-stimulated kinase activity is not enhanced (Hardie, 1989).

Hormone-sensitive lipase activity was assayed by measurement of the release of fatty acids from endogenous triacylglycerols. In order to obtain an estimate of total hormone-sensitive lipase activity, tissue was preincubated with noradrenaline and adenosine deaminase for 25 min before homogenization. Inclusion of noradrenaline increased enzyme activity slightly in tissue from lactating rats and rats treated with GH after litter removal (Table 4). In addition, in some experiments 50 units of the catalytic subunit of A-kinase was included during the assay. This did not increase the hormone-sensitive lipase activity further if tissue was preincubated with noradrenaline plus adenosine deaminase in any of the three states tested in Table 4, indicating that maximum activity was probably achieved by preincubation. To ensure that the A-kinase subunit was operative in the assay, in two experiments with tissue from lactating rats the tissue was preincubated with 100 nM *N*⁶-phenylisopropyladenosine (PIA) to decrease the phosphorylation state of the lipase. Addition of PIA gave a lipase activity with tissue from lactating rats of 993 nmol/h per 10^6 cells (compared with 2515 nmol/h per 10^6 cells after incubation with noradrenaline plus adenosine deaminase), and this was increased to 2346 nmol/h per 10^6 cells by addition of the A-kinase subunit, showing that the A-kinase was effective (mean value from two experiments). Three com-

parable studies with tissue from litter-removed rats revealed a much smaller effect of preincubation with PIA (lipase activity 2190 ± 232 nmol/h per 10^6 cells), and a smaller increase on addition of A-kinase catalytic subunit (2480 ± 270 nmol/h per 10^6 cells) was found. This is consistent with previous studies (Vernon and Finley, 1986) which showed that response to PIA is enhanced during lactation. Previous studies also found that A-kinase increased the activity of hormone-sensitive lipase in homogenates by 50–100% (Wise and Jungas, 1978; Fredrikson et al., 1981).

Litter removal did not change maximum hormone-sensitive lipase activity of whole homogenates (i.e. of tissue after preincubation with noradrenaline) (Table 4); however, treatment with GH increased lipase activity significantly ($P < 0.01$) above that of lactating and untreated litter-removed rats (Table 4). Culture of 3T3-F442A adipocytes with GH also increased hormone-sensitive lipase activity (Dietz and Schwartz, 1991). As a difference in total lipase activity did not explain the diminished response to catecholamines on litter removal, we measured the lipase activity associated with the fat-cake obtained after centrifugation of adipose-tissue homogenates. Hirsch and Rosen (1984) reported that exposure of 3T3-L1 adipocytes to catecholamines resulted in a movement of hormone-sensitive lipase from the cytosol to a sedimentable particulate fraction (they did not check the fat-cake). After preincubation with adenosine deaminase alone, lipase activity of the fat-cake was similar for tissue from lactating, litter-removed and GH-treated litter-removed rats (Table 4), and represented about 25% of the activity of the whole homogenate. After preincubation with noradrenaline, however, a significantly higher ($P < 0.01$) lipase activity was found in the fat-cake from lactating and GH-treated litter-removed rats than in that from litter-removed rats (Table 4). This suggests that an impaired ability of lipase to associate with the fat-cake, and hence the fat droplet in the intact cell, is largely responsible for the diminished ability of catecholamines to stimulate lipolysis in adipocytes from litter-removed rats.

General discussion

Adaptation during the lactation cycle

Modifications of the β -adrenergic signal-transduction system during the lactation cycle in the rat are more complicated than expected, with changes in β -receptor number, adenylate cyclase and cyclic AMP phosphodiesterase activity and presumably some component involved in the association of hormone-sensitive lipase with the fat droplet. The increased β -receptor number and decreased cyclic AMP phosphodiesterase activity during lac-

Table 4 Effect of litter removal, with and without treatment with GH, on hormone-sensitive lipase activity of rat adipose tissue

Pieces of adipose tissue from lactating rats and rats after litter removal for 48 h treated with and without GH were incubated for 25 min with adenosine deaminase (0.8 μ g/ml) with or without 0.1 mM noradrenaline, after which hormone-sensitive lipase activity was assayed in whole tissue homogenates and in the fat-cake obtained by centrifugation of the homogenate. Results are means $\pm$ S.E.M. (derived from analysis of variance), with the numbers of observations in parentheses. Values in a row without the same superscript (a, b) differ significantly ($P < 0.05$).

Tissue preparation	Noradrenaline in preincubation	State...	Hormone-sensitive lipase activity (nmol of fatty acid released/h per 10^6 cells)		
			Lactating	Litter removed	Litter removed, GH treated
Whole homogenate	—		2691 $\pm$ 301 (9)	3337 $\pm$ 319 (8)	3492 $\pm$ 451 (4)
	+		3208 ^a $\pm$ 211 (11)	3472 ^a $\pm$ 211 (11)	4814 ^b $\pm$ 350 (4)
Fat-cake	—		699 $\pm$ 81 (6)	978 $\pm$ 99 (4)	794 $\pm$ 114 (3)
	+		1424 ^a $\pm$ 88 (8)	861 ^b $\pm$ 95 (7)	1395 ^b $\pm$ 125 (4)

tation might be expected to result in some enhancement of response to catecholamines in this state, but activation of A-kinase was unaltered. The decrease in maximum (forskolin-stimulated) adenylate cyclase activity is probably at least partly responsible for the failure of changes in β -receptor number and cyclic AMP phosphodiesterase activity to enhance the response, although there may be some additional changes, for the ability of catecholamines to activate adenylate cyclase was not clearly enhanced by lactation, despite the increased number of receptors. Thus lactation, paradoxically, induces changes in some individual components of the β -adrenergic signal-transduction system which apparently cancel each other out, so that there is no overall change in response or sensitivity to the signal.

A decrease in β -receptor number and also the amount of the GTP-binding protein G_s on litter removal from lactating rats was reported by Ros et al. (1992), who suggested that these adaptations might account for the decreased lipolytic response to catecholamines of adipocytes on litter removal. The present study shows that, although a fall in receptor number occurs on litter removal, this is in fact a restoration of β -receptor number from elevated levels in lactation to levels found in virgin rats. Also, contrary to the suggestion of Ros et al. (1992), the key impairment responsible for the diminished response to catecholamine lies downstream of A-kinase and indeed of hormone-sensitive lipase, for the maximum assayable activity of this latter enzyme appears to be unchanged by litter removal and changes in protein phosphatase and AMP-stimulated kinase activity, which modulate hormone-sensitive lipase activity, appear to be excluded. Rather, the defect seems to be at the level of association of hormone-sensitive lipase with the lipid droplet. At present this mechanism is very poorly understood; the lipid droplet is known to be surrounded by a protein complex (Franke et al., 1987) and one protein at least is known to be phosphorylated by A-kinase (Egan et al., 1990; Greenberg et al., 1991; Mooney and Bordwell, 1991). A number of studies (Wise and Jungas, 1978; Oschry and Shapiro, 1980; Okuda et al., 1983; Ninomiya et al., 1990) suggest that stimulation of lipolysis by catecholamines involves another mechanism apart from phosphorylation and activation of hormone-sensitive lipase, intracellular translocation of hormone-sensitive lipase being an obvious possibility (Hirsch and Rosen, 1984). The present study supports this view and shows that the translocation mechanism is under some form of chronic control.

Role of growth hormone

The role of growth hormone in the regulation of lipolysis has been a matter of considerable controversy, with claims that the hormone has and does not have direct lipolytic effects (see Vernon and Flint, 1989). Previous studies, mostly with domestic ruminants, show that growth hormone acts chronically to increase lipolytic response to catecholamines (Peters, 1986; Sechen et al., 1990; Watt et al., 1991), and we previously showed that treatment with GH prevented the decrease in response to catecholamines found on litter removal from lactating rats (Vernon et al., 1987). The mechanism whereby GH modulates response of adipocytes to catecholamines has not been fully elucidated, although we found that chronic exposure of sheep adipose tissue to GH *in vitro* increased ligand binding to the β -receptor (Watt et al., 1991). The present study shows that GH can increase the number of β -receptors of rat adipocytes and decrease the cyclic AMP phosphodiesterase activity, although these adaptations are not the cause of the altered response to catecholamine. In addition, GH treatment increases the maximum hormone-sensitive lipase activity and the proportion of the

enzyme associated with the lipid droplet. Dietz and Schwartz (1991) found that GH increased hormone-sensitive lipase activity of 3T3-F442A adipocytes *in vitro*, and Smith and McNamara (1990) found that hormone-sensitive lipase activity is increased in bovine adipose tissue during lactation; serum GH concentrations are elevated in high-milk-yielding lactating cattle (Peel and Bauman, 1987). These various observations show that GH modulates several components of the β -adrenergic signal-transduction cascade, although it would appear that changes after A-kinase are most important for altered lipolytic response, at least during the lactation cycle.

Finally, although GH treatment prevents the fall in response to catecholamines on litter removal, it is not clear if GH is involved *in vivo*, for we previously found no evidence for a fall in serum GH concentration on litter removal (Vernon et al., 1987). As serum GH secretion is highly pulsatile (Jansson et al., 1985), it is not easy to obtain a representative estimate of the serum GH concentration; hence we assayed serum IGF-I concentration, which should provide a measure of the biologically effective concentration of GH in serum. Serum IGF-I increases on litter removal from the low level found in lactation, which is not consistent with a fall in serum GH concentration, and would argue against changes in the amount of biologically active GH or amount of GH-binding protein during this period. This suggests that the response or sensitivity of adipocytes to GH is decreased on litter removal; alternatively, some other factor may be responsible for the fall in response to catecholamines, and GH can overcome the effect of this putative factor.

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