Role of growth hormone in the adaptations of lipolysis in rat adipocytes during recovery from lactation

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Removal of the litter from lactating rats results in a fall in the rate of noradrenaline-stimulated lipolysis of adipocytes. This adaptation can be prevented by administration of growth hormone (somatotropin) to such rats and mimicked by injecting lactating rats with an antiserum to growth hormone, whereas lowering serum prolactin by injecting bromocriptine had no effect. The anti-lipolytic effect of adenosine is increased during lactation and is still increased by 2 days after litter removal. Injection of growth hormone into lactating rats decreased slightly the response to adenosine, whereas injection of growth hormone into rats after removal of their litters resulted in a much greater decrease in the response to adenosine, to that found in virgin and pregnant rats.

INTRODUCTION

Lactation usually results in the use of a substantial proportion of the lipid reserves of adipose tissue to help meet the demands for milk production (see Vernon & Flint, 1984). Lipid lost during lactation is replaced on removal of the young, so reproductively active female animals undergo cycles of lipid loss and accumulation (Johnson, 1973).

The switch from lipid mobilization to lipid accumulation in adipose tissue at the end of lactation is accompanied by an increase in the activity of lipoprotein lipase (Hamosh *et al.*, 1970; Scow *et al.*, 1977; Flint *et al.*, 1981) and the rates of fatty acid synthesis (see Vernon & Flint, 1983) and esterification (Flint *et al.*, 1981). The factors responsible for these adaptations have not been fully resolved, but an increase in serum insulin (Agius *et al.*, 1979; Burnol *et al.*, 1983) and a decrease in serum prolactin (Amenomori *et al.*, 1970; Agius *et al.*, 1979; Flint *et al.*, 1981), which accompany litter removal, are probably involved.

In addition to an increased capacity for lipid synthesis, litter removal also results in a substantial fall in the rate of noradrenaline-stimulated lipolysis (Vernon & Finley, 1986), but, in contrast with lipid synthesis, this adaptation does not appear to result from the changes in serum prolactin and insulin noted above. We have therefore investigated the role of GH in these adaptations. This was prompted by the observations that hypophysectomy decreased the response to lipolytic stimuli, and that this could be rectified by treatment with GH (see Goodman & Schwartz, 1974), and that an antiserum to rat GH acted synergistically with bromocriptine (which decreases serum prolactin) to diminish milk yield in rats (Madon *et al.*, 1986).

EXPERIMENTAL

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 eight 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, and were killed by cervical dislocation at about 10:00 h. Rats were accustomed to handling, and to minimize effects of stress further they were killed in a room immediately adjacent to the room where they were housed. Rats received the following, either alone or in combinations as given in Table 1: bromocriptine (a gift from Sandoz), 500 µg/injection; γ-globulin fraction of anti-rGH serum, 220 mg/injection, equivalent to 4.5 ml of anti-rGH serum (see below); sheep prolactin (NIADDK-oPRL-16), 1.5 mg/injection; sheep growth hormone (NIADDK-oGH-13), 1 mg/injection; or carrier solutions (Madon *et al.*, 1986). Sheep prolactin and growth hormone were gifts from NIADDK, Bethesda, MD, U.S.A. Details of the preparation and characterization of the anti-rGH serum and its γ-globulin fraction have been described previously (Madon *et al.*, 1986). Rats and their litters were weighed daily between 09:00 and 10:00 h. All experiments were performed from September through to December.

Immediately after killing, samples of blood and parametrial adipose tissue were removed. Serum was prepared and stored at –20 °C for assay of GH (Madon *et al.*, 1986). Parametrial adipocytes were prepared and their size and number determined as described previously (Aitchison *et al.*, 1982). The rate of lipolysis (glycerol release) of isolated adipocytes was determined as described previously in Medium 199 containing Earle's salts, L-glutamine, 25 mM-Hepes, pH 7.3, and 4% (w/v) essentially fatty-acid-free bovine serum albumin as incubation medium (Aitchison *et al.*, 1982). Albumin and adenosine deaminase were dialysed before use (Vernon *et al.*, 1983).

Results are expressed as means ± S.E.M. and were analysed by Student's *t* test for unpaired or paired observations as appropriate.

Table 1. Effects of GH and prolactin on the rates of lipolysis in parametrial adipocytes from lactating rats and from such rats after removal of their litters

Parametrial adipocytes were prepared from rats at 14–17 days of lactation and from such rats after their litters had been removed 2 days previously. Some rats were injected with bromocriptine, anti-rGH serum, prolactin or GH, either alone or in various combinations twice daily, for 2 days before being killed (see below). The amount of glycerol released by the adipocytes over 1 h in the presence or absence of various agents (below) was determined as described in the text. Incubations contained about 10^6 adipocytes/ml and, when added, $100 \mu\text{M}$ -noradrenaline, $0.8 \mu\text{g}$ of adenosine deaminase/ml, 2 mM -theophylline or 100 nM -PIA. Results are means $\pm$ S.E.M. for five or six observations.

State	Injections	Additions to medium ...	Glycerol released (nmol/h per 10^6 cells)			
			None	Noradrenaline	Noradrenaline, adenosine deaminase, theophylline	Noradrenaline, adenosine deaminase, PIA
Lactating	None (carrier solutions)		27 $\pm$ 5	176 $\pm$ 19	785 $\pm$ 72	238 $\pm$ 32
	Bromocriptine		25 $\pm$ 8	208 $\pm$ 37	911 $\pm$ 106	276 $\pm$ 48
	Anti-rGH serum		28 $\pm$ 7	84 $\pm$ 14	440 $\pm$ 51	79 $\pm$ 10
	Bromocriptine and anti-rGH serum		26 $\pm$ 6	76 $\pm$ 8	275 $\pm$ 44	75 $\pm$ 10
	Bromocriptine, anti-rGH serum, prolactin and GH		23 $\pm$ 7	241 $\pm$ 46	798 $\pm$ 90	259 $\pm$ 52
Litter removed	None (carrier solutions)		23 $\pm$ 6	322 $\pm$ 54	809 $\pm$ 58	340 $\pm$ 60
	Prolactin		15 $\pm$ 6	117 $\pm$ 13	458 $\pm$ 45	161 $\pm$ 41
	GH		16 $\pm$ 4	167 $\pm$ 33	628 $\pm$ 48	201 $\pm$ 33
	GH and prolactin		7 $\pm$ 3	511 $\pm$ 142	886 $\pm$ 146	591 $\pm$ 161
			24 $\pm$ 6	484 $\pm$ 98	855 $\pm$ 94	565 $\pm$ 113

RESULTS

Maximum rates of lipolysis were determined by measuring the rate of glycerol release in the presence of 100 μ M-noradrenaline plus 0.8 μ g of adenosine deaminase/ml (to prevent the accumulation of adenosine in the medium) and 2 mM-theophylline (and adenosine antagonist and inhibitor of cyclic AMP phosphodiesterase).

Removal of the litter had no effect on the basal rate of lipolysis, but significantly decreased the rate in the presence of noradrenaline alone ($P < 0.05$) or in combination with adenosine deaminase ($P < 0.01$) or adenosine deaminase plus theophylline ($P < 0.01$) (Table 1).

Administration of bromocriptine to lactating rats (to lower the serum prolactin concentrations) had no effect on the rate of lipolysis in the presence of any of the agents used (Table 1). In contrast, injections of anti-rGH serum (to decrease the serum GH concentration) decreased the rate of lipolysis in the presence of noradrenaline alone ($P < 0.01$) or in combination with adenosine deaminase ($P < 0.01$) or with adenosine deaminase plus theophylline ($P < 0.05$). Injection of anti-rGH serum into lactating rats had no effect on the sensitivity to noradrenaline (concentration of noradrenaline required to achieve half-maximal effect) (results not shown) whereas previous studies showed that the sensitivity to noradrenaline was not altered by lactation or litter removal (Vernon & Finley, 1986). When bromocriptine was administered with anti-rGH serum, it had no further effect on the rate of lipolysis in the presence of noradrenaline alone, but produced a greater decrease in the rate in the presence of noradrenaline plus adenosine deaminase ($P < 0.05$) and adenosine deaminase plus theophylline ($P < 0.001$) than did anti-rGH serum alone. These effects of bromocriptine plus anti-rGH serum on lipolysis were prevented by the concurrent administration of prolactin plus GH (Table 1). Injections of GH alone to untreated lactating rats increased the rate of lipolysis in the presence of noradrenaline alone ($P < 0.05$), but had no effect when theophylline and/or adenosine deaminase was included.

Injections of prolactin partly prevented the effect of weaning on the rates of stimulated lipolysis (Table 1). Injections of GH, however, completely prevented the effect of weaning on the rate of lipolysis under all conditions tested (Table 1) and in addition increased ($P < 0.001$) the effects of noradrenaline on lipolysis to those found in virgin and pregnant rats (Vernon & Finley, 1986).

The difference in the rate of noradrenaline-stimulated lipolysis in the presence and absence of adenosine deaminase should reflect the effect of adenosine; this can be confirmed by addition of the analogue PIA, which is not metabolized by adenosine deaminase. Addition of 100 nM-PIA decreased the rate of lipolysis in the presence of noradrenaline plus adenosine deaminase to values similar to those obtained in the presence of noradrenaline alone in treated and untreated rats alike (Table 1). A previous study showed that 100 nM-PIA was sufficient to elicit the maximum effect of PIA under the conditions used with adipocytes from both lactating and pregnant rats (Vernon *et al.*, 1983).

The serum GH concentration in blood taken at the time of killing was 23.0 ± 7.4 ng/ml in weaned rats and

7.3 ± 1.1 ng/ml in lactating rats (results are means $\pm$ S.E.M. for six and five observations respectively and do not differ significantly); the various treatments used (except for injection of anti-rGH serum) had no effect on the concentration of endogenous (i.e. rat) serum GH (results not shown).

DISCUSSION

The present study confirms our previous observation that the maximum rate of noradrenaline-stimulated lipolysis (i.e. in the absence of adenosine) is decreased on weaning and that the effect of weaning cannot be mimicked by lowering serum prolactin concentration with bromocriptine (Vernon & Finley, 1986). Rather, GH appears to have a dominant role in the changes in maximum lipolytic response on weaning, for lowering serum GH concentrations during lactation decreased the maximum lipolytic response, whereas giving GH to rats on litter removal prevented the decline. However, the decrease in lipolytic response on litter removal is clearly not due to a decrease in serum GH itself, at least as assessed by radioimmunoassay. This suggests that endogenous rat GH may become less effective on litter removal, possibly owing to a change in GH-binding proteins in blood (Silverlight *et al.*, 1985) or a change in phosphorylation state of GH (Liberti & Joshi, 1986). Alternatively, adipocytes may become less sensitive to rat GH on litter removal, although they clearly respond to sheep GH.

Although lowering the serum prolactin concentration had no effect on lipolysis in untreated lactating rats, its presence did limit the fall in maximum lipolytic rate induced by giving anti-rGH serum, whereas in weaned rats, in which endogenous rat GH appears to have become ineffective, administration of prolactin partly mimics the effect of GH, showing that prolactin can modulate lipolysis, but only in the effective absence of GH.

The response of adipocytes to the anti-lipolytic effect of adenosine is paradoxically increased, despite increased mobilization of fat reserves during lactation (Vernon *et al.*, 1983). The sensitivity to adenosine, however, appears to be unchanged by lactation (Vernon *et al.*, 1983). The physiological advantage of this increased response to adenosine is still unclear, and the factor(s) responsible have yet to be ascertained. Decreasing serum prolactin in lactating rats has no effect on the response to adenosine (Table 1; Vernon & Finley, 1986), so prolactin would not appear to be responsible. The present study shows that GH can decrease the response to adenosine (i.e. increase the response to noradrenaline in the presence of adenosine) in both lactating and weaned rats. However, some other factor must be involved to account for the increased response to adenosine during lactation, for serum GH, as assessed by radioimmunoassay, does not decrease in this state (R. J. Madon & D. J. Flint, unpublished work). Endogenous GH appears to be biologically effective during lactation, as the response to noradrenaline, in the absence of adenosine, does not decrease. Also, the effect of exogenous sheep GH on the response to adenosine is much less in lactating rats than in weaned rats. Lactation in sheep results in a rise in serum GH (Vernon *et al.*, 1981) and an increase in the lipolytic effect of noradrenaline (Vernon & Finley, 1985), but also an increased response to adenosine (Vernon &

Finley, 1985). Thus some further factor must be involved to explain the effects of lactation on the response to adenosine. Nevertheless, the study clearly shows that, once the litter is removed, GH can markedly affect the response of adipocytes to the anti-lipolytic effects of adenosine as well as noradrenaline.

The present results thus suggest that, after removal of the litter from lactating rats, serum GH becomes effectively inactive, resulting in a fall in the maximum lipolytic response to noradrenaline, which should facilitate the replenishment of the reserves of lipid in adipose tissue. GH can also modulate the response of adipose tissue to adenosine, but some factor other than growth hormone (and prolactin) is responsible for the increased response of adipocytes to adenosine during lactation.

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