Changes in the sensitivity to glucagon of lipolysis in adipocytes from pregnant and lactating rats

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1. Rates of lipolysis were measured at different concentrations of glucagon in adipocytes prepared from parametrial adipose tissue of fed or starved rats in different reproductive states. All experiments were performed in the presence of a high concentration of adenosine deaminase (1 unit/ml). 2. Maximal rates of lipolysis (elicited by 25 nM-glucagon in each instance) were higher in adipocytes from peak-lactating rats than those from pregnant animals in both the fed and starved states. 3. Of adipocytes from fed animals, those from peak-lactating rats were the most sensitive to glucagon, whereas those from late-pregnant and early-lactating rats were 1–2 orders of magnitude less sensitive. 4. Adipocytes from 24 h-starved rats showed a much smaller stimulation of lipolysis by glucagon, making the assessment of sensitivity difficult. Therefore, rates of lipolysis were also measured in the presence of a maximally anti-lipolytic dose of insulin. The presence of insulin did not alter the relative sensitivities to glucagon of adipocytes from fed animals in different reproductive states, although all dose–response curves were shifted to the right. When lipolysis in adipocytes from starved animals was measured in the presence of insulin, it became evident that starvation for 24 h markedly increased the sensitivity of adipocytes from late-pregnant rats to glucagon, but did not affect that of cells from animals in the other reproductive states. 5. It is concluded that the large changes in sensitivity to glucagon that occurred during the reproductive cycle may enable the modulation of adipose-tissue lipolysis *in vivo* to satisfy the different metabolic requirements of the animal in the transition from pregnancy to peak lactation.

INTRODUCTION

Adipose tissue plays an important role in the partitioning of carbohydrate and lipid substrates during pregnancy and lactation. The reciprocal changes in the lipogenic activity of adipose and mammary tissues in the transition between these two physiological conditions are achieved partly through long-term changes in their respective enzyme contents and partly by differences in the ability of the two tissues to respond acutely to hormonal changes that occur in response to specific stimuli, e.g. feeding (see Zammit, 1985). In this respect, it was previously suggested that the sensitivity of adipose, but not of mammary, tissue to glucagon (Robson *et al.*, 1984) may be involved in such short-term tissue-specific direction of lipogenic substrates during lactation.

Whereas mammary tissue influences whole-body metabolism primarily through the presence or absence of its anabolic activity, adipose tissue has a central role to play both when it is the primary site of peripheral lipogenesis and when it is in a lipolytic state. Furthermore, even under conditions when adipose tissue is capable of high rates of lipogenesis, it needs to be acutely responsive to physiological stimuli (e.g. food deprivation) by mobilizing stored triacylglycerols. Glucagon is a potential modulator of adipose-tissue metabolism with respect to dietary status of the animal. The glucagon/insulin molar ratio may be a determinant of the balance between lipolysis and lipogenesis in the tissue (see Zammit & Corstorphine, 1982b; Robson *et al.*, 1984). This is especially true during lactation, when the glucagon/insulin molar ratio in the circulation is elevated (Robinson *et al.*, 1978) and the

major tissue of substrate utilization (i.e. mammary tissue) is insulin-sensitive but glucagon-insensitive (Robson *et al.*, 1984).

In the present study the changes in sensitivity of lipolysis to glucagon, under defined conditions, have been studied for adipocytes from fed or starved animals at different stages in the reproductive cycle. The results indicate that the tissue undergoes marked changes in the sensitivity of lipolytic rate to glucagon. The data are discussed in relation to the metabolic requirements of the pregnant and lactating rat.

MATERIALS AND METHODS

Animals

The source and maintenance of the female Wistar rats used were as described previously (Robson *et al.*, 1984). Virgin rats were age-matched with pregnant and lactating animals. Starved animals had their food withdrawn 24 h before being killed, but had constant access to water. Pregnant animals were used 19 days after mating, early-lactating animals 1 day after the day of parturition and peak-lactating animals 10–12 days after parturition. All experiments were performed between mid-November and mid-February.

Preparation and incubation of adipocytes

Animals were anaesthetized with pentobarbitone (60 mg/kg body wt.) and, after 10 min, samples of parametrial adipose tissue were removed for the preparation of adipocytes by collagenase digestion as described by

Abbreviations used: EC₅₀, concn. giving 50% of maximal effect; PIA, N⁶-[R-(–)-1-methyl-2-phenylisopropyl]adenosine.

Honnor *et al.* (1985a). All procedures were performed at 37 °C. The digestion medium contained 3 mg of collagenase and 1 mg of soya-bean trypsin inhibitor/ml, and final concentrations (in 10 ml) of 8 mM-glucose, 4% (w/v) fatty-acid-free albumin and 200 nM-adenosine in Krebs–Henseleit (1932) medium (1.3 mM-Ca²⁺) supplemented with Hepes buffer (12.5 mM, pH 7.4). Digestion was continued for 60 min, and the cells were washed twice in Krebs–Henseleit medium containing 200 nM-adenosine and finally resuspended in the same medium (25 ml). They were then gently mixed for 7 min before addition of portions of cell suspension to the experimental flasks. This period of equilibration after the final wash was found to give much more highly reproducible rates of lipolysis in duplicate flasks, for which times of addition of cells were necessarily separated by several minutes, since 20 flasks were incubated simultaneously in each experiment. Such an effect was also observed by Vega & Kono (1979) for glucose transport in adipocytes. Samples (1 ml) of adipocyte suspension (equivalent to $\frac{1}{25}$ th the parametrial-adipose-tissue mass of one rat) were then added to the experimental flasks with 4 ml of Krebs–Henseleit medium (see above) that contained 8 mM-glucose, 4% albumin, 1 unit of adenosine deaminase/ml and the indicated concentrations of glucagon and/or insulin. The final incubation volume was 5 ml, and the flasks were shaken (160 oscillations/min) at 37 °C and continuously gassed with O₂/CO₂ (19:1), throughout the 60 min incubation period. It was ascertained in preliminary experiments that rates of glycerol release were linear over this period. Incubations were terminated by addition of 0.5 ml of cold 60% (w/v) HClO₄, and the glycerol released was assayed after neutralization of the 500 g supernatants (Eggstein & Kuhlmann, 1974). Samples of adipocyte suspension were retained and frozen at –20 °C for determination of DNA content by the method of Labarca & Paigen (1978).

Chemicals

The sources of these were as given by Robson *et al.* (1984). In addition, collagenase was obtained from BCL Chemicals (Lewes, Sussex, U.K.). Pig glucagon, trypsin inhibitor, adenosine, crystalline insulin, glycerol kinase and glycerol-3-phosphate dehydrogenase were from Sigma Chemical Co. (Poole, Dorset, U.K.). Adenosine deaminase was dialysed overnight against Krebs–Henseleit medium before use. The activity of the enzyme was assayed at 25 °C as described previously (Zammit & Corstorphine, 1982b).

Expression of results

Rates of glycerol release in Figs. 1–4 are expressed as a percentage of that observed at 25 nM-glucagon, in the absence of insulin for the same preparation of adipocytes. Values of glycerol release in the presence of insulin (Figs. 3 and 4) were all obtained with the same adipocyte preparations used to generate the data in Figs. 1 and 2, such that paired *t* tests could be performed (see the legend to Fig. 3). Absolute values of the rates of lipolysis are given in Table 1.

RESULTS AND DISCUSSION

Removal of adenosine from the incubation medium resulted in a several-fold increase in the rate of lipolysis

of adipocytes from animals in all physiological conditions tested (results not shown; cf. data of Honnor & Saggerson, 1980; Honnor *et al.*, 1985a,b). However, the extent to which further stimulation of lipolysis by addition of an optimal glucagon concentration (i.e. 'maximal' lipolysis) occurred depended on physiological state, and varied greatly between individual animals (see Fig. 1; cf. Honnor *et al.*, 1985a). In adipocytes from fed peak-lactating animals, the lipolytic rate achieved by removal of adenosine with 1 unit of adenosine deaminase/ml ranged from 15.8% to 112% of that obtained after further addition of 25 nM-glucagon (which was the optimal concentration of hormone for all cell preparations used). A similar degree of variability was observed for cells from fed early-lactating (3.3–91.5%) and fed non-mated (5.5–98.9%) animals. However, the parameter was much more reproducible in adipocytes from fed late-pregnant rats (range 27.7–47.9%). The reason for this variability in the stimulation of lipolysis by removal of adenosine for cells from different individual animals in the fed state is not known (see Honnor *et al.*, 1985a). The present observation that, in at least one physiological condition, the variability is diminished may suggest that this parameter may respond to subtle physiological differences between individual animals, which may be decreased in certain physiological states. Certainly, the adenosine deaminase-induced increase in lipolytic rate was less variable (as a percentage of maximal lipolysis) for cells prepared from starved rats (Fig. 2), and the mean ratios were considerably higher than those for cells prepared from fed rats in the same reproductive state (e.g. $45.6 \pm 19.4\%$ for fed non-mated rats, compared with $80.3 \pm 9.8\%$ for cells from starved non-mated animals). These observations were in agreement with those of Honnor *et al.* (1985a) on adipocytes from epididymal fat-pads of starved male rats. However, the fact that lipolysis in adipocytes from starved early-lactating animals was capable of a greater degree of further stimulation by glucagon than for cells from animals in the other physiological conditions (see Fig. 2) again suggests that the degree of lipolytic stimulation achievable through removal of adenosine (relative to maximal) may be a function not only of season (see Honnor *et al.*, 1985a) but also of physiological state. In this respect, it may be relevant that cells from starved early-lactating animals were also the least sensitive to glucagon (see below).

Changes in sensitivity and responsiveness to glucagon

The dose–response curves in Fig. 1 indicate that cells from fed animals in peak lactation were the most sensitive (EC₅₀ approx. 0.2 nM), but cells from fed late-cytes from fed non-mated rats were marginally less sensitive (EC₅₀ approx. 0.2 nM), but cells from fed-late pregnant and early-lactating rats showed EC₅₀ values that were approx. 1.0 and 1.5 orders of magnitude higher respectively.

The sensitivity of lipolysis to glucagon was difficult to assess in cells from starved animals. As indicated above, glucagon did not produce much further stimulation of lipolysis over and above that already achieved by removal of adenosine in these cells. Only in cells from starved early-lactating rats was there a substantial further increase in response to glucagon (Fig. 2). Because of these difficulties, it was necessary to antagonize the effects of the removal of endogenously produced

Fig. 1. Glucagon dose-response curves for the rate of lipolysis in adipocytes isolated from fed female rats in different reproductive states

Adipocytes isolated from fed non-mated (●), late-pregnant (▲), early-lactating (○) and peak-lactating (□) rats were incubated with the concentrations of glucagon shown, in the presence of 1 unit of adenosine deaminase/ml. Rates of lipolysis are normalized with respect to that observed at 25 nM-glucagon. Lipolytic rates at 100 nM-glucagon were 10–15% lower than maximal rates in each instance (results not shown). Values are means ($\pm$ S.E.M.) for four or five separate preparations. Absolute values of maximal rates of lipolysis are given in Table 1.

Fig. 3. Glucagon dose-response curves for lipolysis in adipocytes from fed rats in the presence of insulin

Adipocytes from the same preparations used in the experiments depicted in Fig. 1 were used. Rates of lipolysis are expressed as percentages (means $\pm$ S.E.M.) of the maximal rate (obtained at 25 nM-glucagon in the absence of insulin; see Fig. 1) observed for the relevant adipocyte preparation. The insulin concentration was 1.6 units/ml. *Value at 25 nM-glucagon is significantly different ($P < 0.02$) from that obtained (100%) in the absence of insulin (paired t test). Notation is as in Fig. 1.

Fig. 2. Glucagon dose-response curves for the rate of lipolysis in adipocytes isolated from 24 h-starved female rats in different reproductive states

See the legend to Fig. 1 for details.

Fig. 4. Glucagon dose-response curves for lipolysis in adipocytes from 24 h-starved rats in the presence of insulin

See the legend to Fig. 3 for details.

adenosine and the expression of a wider range of glucagon-stimulated lipolysis, thus enabling a more accurate estimation of the changes in the dose-response curves that were induced by starvation. This was done by measuring rates of lipolysis in the presence of a maximally effective (anti-lipolytic) dose of insulin. Insulin was preferred to a non-degradable adenosine analogue [e.g. N^6 -[R-(—)-1-methyl-2-phenylisopropyl]adenosine, PIA], as it is known that glucagon cannot overcome a maximally effective dose of PIA, (Honnor *et al.*, 1985b) and that PIA decreases the maximal lipolytic response of glucagon (Saggerson, 1986). Moreover, the sensitivity of lipolysis to PIA may vary with nutritional and reproductive state (Chohan *et al.*, 1984; Vernon *et al.*, 1983). The insulin experiments were run concurrently with those reported above, on the same adipocyte preparations, such that paired observations were obtained. For cells from all physiological states, 1.6 munits of insulin/ml gave a maximal anti-lipolytic response.

The maximal response to insulin in the absence of glucagon was markedly lower in cells from fed peak-lactating rats than in those from fed animals in any of the other reproductive states (50% versus 85–96% inhibition). This observation is consistent with those by others (Burnol *et al.*, 1983, 1986; Jones *et al.*, 1984; Kilgour & Vernon, 1987, 1988), who showed that aspects of adipose-tissue metabolism may be refractory to insulin in peak-lactating animals. Interestingly, this blunted anti-lipolytic effect of insulin was not yet established on day 2 of lactation (see Fig. 3) and was not apparent at all in adipocytes from 24 h-starved peak-lactating rats (cf. Figs. 3 and 4).

Inclusion of insulin in the incubation medium resulted in a shift of the glucagon dose-response curves to the right for adipocyte preparations from fed animals (Figs. 1 and 3). However, the relative sensitivities to glucagon, among different physiological states, were maintained. Cells from fed peak-lactating animals remained the most sensitive (EC_{50} approx. 0.35 nM) and those from fed early-lactating animals were least sensitive (EC_{50} approx. 4.2 nM-glucagon). In addition, only in cells from fed early-lactating animals was a maximally effective dose of glucagon not able fully to overcome the anti-lipolytic effect of insulin (Fig. 3). In these adipocytes insulin still inhibited the rate of glycerol release by 40% ($P < 0.01$ for paired observations) at maximally effective glucagon concentrations. Lipolysis in cells from fed animals in all the other reproductive states was not inhibited significantly by insulin in the presence of 25 nM-glucagon (Fig. 3).

These experiments performed in the presence of insulin showed that starvation had altered the relative sensitivities to glucagon of adipocytes in one important respect, namely, there was a marked increase in sensitivity in adipocytes from pregnant rats, such that they were now equally sensitive to those from peak-lactating and non-mated animals. By contrast, the very low sensitivity of cells from early-lactating rats was unaffected by 24 h starvation. Maximal rates of lipolysis in the presence of insulin were not significantly lower than those in its absence for cells from starved animals (Fig. 4).

Physiological considerations

Extrapolation of the above observations to the situation *in vivo* must be attempted with caution, particularly because concomitant changes in the sensi-

Table 1. Maximal rates of glycerol release by adipocytes from parametrial adipose tissue of fed or starved female rats in different reproductive states

Adipocytes were incubated at 37 °C (see the Materials and methods section) for 60 min in the presence of 1 unit of adenosine deaminase/ml and 25 nM-glucagon. These conditions gave maximal lipolytic rates for all preparations used (see Figs. 1–4). Values are means ($\pm$ S.E.M.) for the numbers of separate determinations shown in parentheses. Statistical significance of differences between means was measured by Student's *t* test; * $P < 0.1$ versus late-pregnant.

Animals	Glycerol release (μ mol/h per 100 μ g of DNA)	
	Fed	Starved
Non-mated	0.68 $\pm$ 0.13 (4)	0.73 $\pm$ 0.13 (4)
Late-pregnant	0.59 $\pm$ 0.06 (4)	0.52 $\pm$ 0.12 (5)
Early-lactating	0.72 $\pm$ 0.22 (4)	1.01 $\pm$ 0.25 (3)*
Peak-lactating	0.79 $\pm$ 0.08 (5)*	1.01 $\pm$ 0.20 (4)*

tivity of adipose-tissue lipolysis to adenosine (see Vernon *et al.*, 1983) may counteract those to glucagon. Present methodology does not allow determination of the likely concentrations of adenosine (and hormones) at the cell receptor sites *in vivo*. Consequently, it is only possible to discuss data obtained under well-defined conditions *in vitro*.

The maximal rates of lipolysis (Table 1) were not significantly different between adipocytes obtained from either fed or starved lactating and non-mated rats (cf. Aitchison *et al.*, 1982). However, maximal glycerol release by cells from pregnant rats tended to be lower in both the fed and starved states. This decrease attained moderate statistical significance when late-pregnant values were compared with those for peak lactation (Table 1) for both the fed and the starved states. Thus in the peak-lactating animal there is likely to be an increased mobilization of triacylglycerol compared with the late-pregnant rat, particularly in the fed state, because adipocytes from peak-lactating rats also had the highest sensitivity to glucagon (Figs. 1 and 4). This increased sensitivity to glucagon, coupled with the prevalence of an increased glucagon/insulin molar ratio during lactation (Robinson *et al.*, 1978), should favour adequate rates of triacylglycerol mobilization during peak lactation and the consequent provision of preformed fatty acids to the mammary gland for secretion into milk.

By contrast, in early-lactating animals, although the maximal rates of lipolysis were very similar to those in peak-lactating rats, the sensitivity to glucagon was 1–2 orders of magnitude lower in both the fed and starved states. It is suggested that this insensitivity of lipolysis to glucagon in adipose tissue of early-lactating animals may represent a general adaptation that spares triacylglycerol in the face of the large decrease in insulin concentrations that occurs around parturition (Saudek *et al.*, 1975; Robinson *et al.*, 1978; Flint *et al.*, 1979). Decreased effectiveness of the action of glucagon would counteract the diminution of the antilipolytic effect of insulin and thus prevent triacylglycerol mobilization at a time when lactation is not yet established.

Adipocytes from fed late-pregnant rats too were relatively insensitive to glucagon (compared with peak-lactating and not-mated states). This may be an adaptation to the hyperglucagonaemia of late pregnancy (Girard *et al.*, 1973), and may ensure that rates of triacylglycerol mobilization are not elevated before parturition and lactation. This adaptation should be reinforced *in vivo* by the prevalence of high circulating concentrations of insulin during late pregnancy in the fed state (see above). After 24 h starvation, sensitivity of lipolysis in adipocytes from pregnant rats was markedly increased (Figs. 2 and 4), although the maximal rate of lipolysis remained unchanged (Table 1). This suggests that triacylglycerol mobilization would be acutely facilitated during starvation, and it may contribute towards the increased susceptibility of late-pregnant animals to ketonaemia during starvation (see Zammit, 1985), often referred to as 'accelerated starvation'. Concomitant changes in the metabolic profile of the liver would facilitate the partitioning of the metabolism of circulating fatty acids towards ketone-body formation (Zammit, 1980, 1981; Zammit & Corstorphine, 1982a).

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