The response of adipocyte glucose metabolism and fatty acid release to adenosine deaminase, insulin and perfusion

Investigation of intermediary metabolism by perfusion

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Adipocytes incubated with adenosine deaminase (ADA) showed: (1) increased amounts of fatty acids in the medium; (2) increased glucose incorporation into acylglycerol glycerol; (3) decreased glucose incorporation into acylglycerol fatty acids; (4) a co-ordinate decrease in the sensitivity of lipolysis and glucose incorporation into acylglycerol to insulin; (5) similar effects on glucose incorporations in perfused and normal incubations. The decrease in fatty acid synthesis by perfusion was found to be dependent on the presence of insulin or fatty acids, and independent of the effects of ADA. The significance of the effects of perfusion, ADA and insulin are discussed in relation to effects of fatty acids.

INTRODUCTION

In the presence of high concentrations of fatty acids or with mature rats, the major fate of glucose in adipocytes is incorporation into acylglycerol glycerol (Saggerson, 1972; Francendese & Digirolamo, 1981). The effects of the presence of adenosine and its interaction with insulin on acylglycerol glycerol synthesis have not been determined.

In adipocytes, fatty acids are known to have effects on acylglycerol glycerol and fatty acid synthesis from glucose with and without insulin (Saggerson, 1972). Fatty acids and their derivatives have effects on the enzymes involved in the regulation of glucose metabolism, such as 6-phosphofructo-1-kinase, PDH, acetyl-CoA carboxylase and phosphatidate phosphohydrolase. There is evidence that the effects of lipolytic hormones on glucose metabolism may in part be exerted through the effects of fatty acids on these enzymes (Halestrap & Denton, 1974; Smith & Saggerson, 1978; Sooranna & Saggerson, 1979; Lederer & Hers, 1984; Taylor & Saggerson, 1986).

Since the presence of ADA stimulates adipocyte lipolysis, as determined by glycerol production, incubations in flasks with this enzyme can be expected to accumulate fatty acids (Fain, 1973; Schwabe *et al.*, 1974; Fernandez & Saggerson, 1978; Green & Newsholme, 1979; Honnor *et al.*, 1985). However, investigations into the effects of adenosine on PDH activity, glucose uptake, glucose oxidation, lipid synthesis from glucose and the response of these processes to insulin in adipocytes have not included determinations of NEFA concentrations (Fain, 1973; Schwabe *et al.*, 1974; Green & Newsholme, 1979; Joost & Steinfelder, 1982; Green, 1983; Wong *et al.*, 1984, 1985; Kuroda *et al.*, 1987). It seems that some of the effects of adenosine may in part be exerted through changes in the concentrations of NEFA.

The release of fatty acids has been shown to be different in perfused incubations compared with those in flasks (Harper, 1984). This work also showed that the

method of incubation affected fatty acid synthesis from glucose.

The work presented here demonstrates the response of acylglycerol synthesis from glucose over a range of insulin concentrations, ADA being used to remove endogenously produced adenosine. The concentrations of NEFA were monitored and the effects of perfused incubations on the response to ADA and to insulin were determined.

MATERIALS AND METHODS

Chemicals and animals

Fatty-acid-free bovine serum albumin was from Sigma (London) Chemical Co., Poole, Dorset, U.K. ADA was from Boehringer Corp. (London), Lewes, East Sussex, U.K. Other chemicals, and the source and maintenance of the male Wistar rats, were as described by Harper (1984).

Experimental methods

Adipocytes were prepared by the method of Rodbell (1964) as described by Harper (1984), except that collagenase was used at 1 mg/ml and glucose (5 mM) was added to the medium.

Perfused and control incubations were carried out as described by Harper (1984) with 6 ml of Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932), with additions as indicated in the legends to the Tables and the Figure. The dilution rate in perfused incubations was 7.3 h⁻¹. All other incubations were carried out in 4 ml of the same medium, in 25 ml silicone-treated glass flasks.

For perfused incubations, the perfusate was collected during the incubation period. Incorporations, determinations and the analysis of statistical significance were as described by Harper (1984). Each determination was on a separate preparation of adipocytes.

Table 1. Effect of insulin and of palmitate on the response of acylglycerol synthesis to perfusion

Adipocytes were incubated as described in the Materials and methods section for 30 min in a medium containing 5 mM-[U-¹⁴C]glucose and albumin (10 mg/ml), with or without insulin (140 nM). (a) No further additions; (b) a separate series of determinations in which sodium palmitate (0.25 mM) was added. Glucose incorporations are the means $\pm$ S.E.M. for (a) five or (b) four determinations. Mean adipocyte DNA content was (a) 1.75 μ g/ml, or (b) 4.36 μ g/ml. * P < 0.05, ** P < 0.001 for comparison of incubations with and without insulin; † P < 0.05, †† P < 0.02 for comparison of perfused with normal incubations.

Additions		Incubation system	Glucose incorporation (μ g-atoms/h per 100 μ g of DNA) into:	
			Acylglycerol glycerol	Acylglycerol fatty acids
(a)	None	Normal	2.42 $\pm$ 0.26	3.76 $\pm$ 0.24
		Perfused	1.91 $\pm$ 0.07	4.04 $\pm$ 0.27
	Insulin	Normal	2.79 $\pm$ 0.43	5.81 $\pm$ 0.16**
		Perfused	2.16 $\pm$ 0.12	2.86 $\pm$ 0.38††
(b)	Palmitate	Normal	10.88 $\pm$ 1.06	7.43 $\pm$ 0.73
		Perfused	10.72 $\pm$ 1.19	5.21 $\pm$ 0.42†
	Palmitate + insulin	Normal	12.78 $\pm$ 1.61	14.99 $\pm$ 2.02*
		Perfused	10.68 $\pm$ 0.76	7.43 $\pm$ 0.79††

RESULTS

Effect of perfusion on acylglycerol synthesis

Perfusion in the presence of insulin (140 nM) decreased acylglycerol fatty acid synthesis by 51%, but had no effect in the absence of insulin (Table 1a). Hence the decrease in fatty acid synthesis reported by Harper (1984) was dependent on the presence of insulin in the incubations.

The net release of NEFA for the perfused incubations described in Table 1(a) was 3.07 and 2.51 μ mol/100 μ g of DNA for 0–15 min of incubation, and 1.48 and 1.81 μ mol/100 μ g of DNA for 15–30 min of incubation, in the presence and the absence of insulin respectively. This suggests that insulin potentiates the establishment of new steady-state values for fatty acid release (Harper, 1984).

Insulin stimulated acylglycerol fatty acid synthesis by 55% in normal incubations, but in perfused incubations decreased synthesis by 29% (Table 1a). The presence of added palmitate restored the ability of insulin to stimulate fatty acid synthesis in the perfused incubations. However, the stimulation at 43% was less than that in normal incubations, 102% (Table 1b).

Perfused incubations with and without palmitate showed a consistent, but not significant, small decrease in acylglycerol glycerol synthesis (Table 1).

Effect of ADA on lipolysis and acylglycerol synthesis

Lipolysis, as reflected by the concentration of NEFA in the medium, was markedly stimulated by the removal of endogenous adenosine (Fig. 1). This reflects the potent lipolytic action of ADA (Schwabe *et al.*, 1975), and is in agreement with previous observations of the effect of ADA on glycerol release cited in the Introduction.

ADA increased acylglycerol glycerol synthesis to 293% (P < 0.001) and decreased fatty acid synthesis to 21% (P < 0.001) of control values. Determination of glucose uptake, glucose oxidation or incorporations into

total lipid or fatty acid may mask these opposing effects on the disposition of glucose.

Effect of insulin on lipolysis and acylglycerol synthesis

In the absence of ADA, NEFA concentrations in the medium at high insulin concentrations (1.4 and 140 nM) were 21% lower (P < 0.02) than those at low insulin concentrations (0.014 and 0.14 nM). This demonstrates a small anti-lipolytic action of insulin on basal (unstimulated) lipolysis. In the presence of ADA, insulin at 140 nM decreased NEFA in the medium by 213% (P < 0.01) compared with insulin at 1.4 nM. Assuming complete esterification of the glycerol, the esterification of NEFA by increased acylglycerol glycerol synthesis only accounts for 17% of this decrease in NEFA. Hence the results demonstrate the well-characterized anti-lipolytic effect of insulin in the presence of ADA (Schwabe *et al.*, 1974; Green & Newsholme, 1979; Londos *et al.*, 1985).

Assuming that the values at 140 nM are maximal, the concentrations of insulin giving 50% of maximal effect for lipolysis, acylglycerol glycerol and fatty acid synthesis were 7.0 and 0.5 nM for incubations with and without ADA respectively (Fig. 1). A similar decrease in the sensitivity to insulin was observed by Schwabe *et al.* (1974), Wong *et al.* (1985) and Kuroda *et al.* (1987) for glucose oxidation, by Joost & Steinfelder (1982) for fatty acid synthesis, by Green (1983) for glucose uptake and by Wong *et al.* (1984) for PDH activity. A decrease by ADA in the sensitivity of lipolysis to insulin has been observed for ADA-stimulated compared with hormone-stimulated lipolysis (Schwabe *et al.*, 1974; Green & Newsholme, 1979; Londos *et al.*, 1985). A decrease in sensitivity compared with basal lipolysis does not appear to have been reported previously.

At 140 nM-insulin the decrease in fatty acid synthesis owing to addition of ADA is abolished, whereas for acylglycerol glycerol synthesis the 2.9-fold stimulation by ADA without insulin is increased to 5.8-fold at 140 nM-insulin.

Fig. 1. Effect of insulin and of ADA on fatty acid release and acylglycerol synthesis from glucose

Adipocytes were incubated in 25 ml flasks for 30 min as described in the Materials and methods section in a medium containing 5 mM-[U-¹⁴C]glucose, albumin (30 mg/ml) and insulin at the indicated concentrations, plus: ○, □, no additions; ●, ■, ADA (1 unit/ml). (a) Incorporation into acylglycerol fatty acid; (b) ○, ●, incorporation into acylglycerol glycerol; □, ■, concn. of NEFA. The results are the means of eight determinations. Incorporations are expressed as percentages of that without any additions; the latter were (a) 2.51 ± 0.63 and (b) 3.09 ± 0.62 μ g-atoms/h per 100 μ g of DNA (means $\pm$ S.E.M.). Mean adipocyte DNA content was 1.75 μ g/ml.

Table 2. Effect of perfusion on the response of acylglycerol synthesis from glucose and fatty acid release to ADA

Adipocytes were incubated as described in the Materials and methods section for 30 min in the same medium as for Fig. 1 with insulin (0.014 nM), in the presence and the absence of ADA (1 unit/ml). Glucose incorporations are the means $\pm$ S.E.M. for four determinations, and are expressed as percentage of that for normal incubation with no additions. Mean adipocyte DNA content was 1.5 μ g/ml. * $P < 0.05$, ** $P < 0.01$ for incubations with ADA compared with those without; † $P < 0.001$ for comparison of perfused with normal incubations.

Incubation system	Addition	Glucose incorporation (% of control) into:	
		Acylglycerol glycerol	Acylglycerol fatty acids
Normal	None	100	100
	ADA	$232 \pm 37^*$	$42 \pm 11^{**}$
Perfused	None	100 ± 14	$68 \pm 5^\dagger$
	ADA	$240 \pm 30^{**}$	$26 \pm 7^{**}$

Effect of ADA under perfused incubation conditions

The increased NEFA concentrations found with ADA in the experiment described in Fig. 1 reflect the accumulation of NEFA in the incubation vessels, so the effect of ADA was tested under perfused conditions such that NEFA would not accumulate (Table 2). Addition of ADA had the same effect in perfused and normal incubations: acylglycerol glycerol synthesis was increased to 235% and fatty acid synthesis was decreased to 40% of control values. The concentration of NEFA in the perfusate increased 230% ($P < 0.05$) from 39 ± 12 to 90 ± 17 μ M in the presence of ADA.

The presence of ADA had no effect on the 35% decrease in fatty acid synthesis by perfusion (Table 2).

DISCUSSION

Perfusion and fatty acid synthesis

The decrease in fatty acid synthesis by perfusion requires the presence of added fatty acids or of insulin (Table 1). It was shown previously that in the presence of insulin the decrease was little affected by added fatty acids (Harper, 1984). The requirement of insulin or of added fatty acids for the effect of perfusion suggests that insulin, fatty acids and perfusion have a common site of action on fatty acid synthesis.

The decrease in fatty acid synthesis by insulin under perfused conditions is opposite to the usual stimulatory effect of insulin (Table 1a; Rodbell, 1964). This may be related to the ability of insulin to potentiate the response of fatty acid release to perfusion. The ability of added fatty acids to restore the stimulatory response to insulin suggests that the decrease by insulin involves the common site of action discussed above. That the stimulation of fatty acid synthesis by insulin in the presence of added palmitate, and that by added palmitate (0.25 mM) in the presence of insulin, are halved under perfused conditions supports this view (Table 1b; Harper, 1984). That these stimulations by insulin and added palmitate are lower under perfused conditions suggests that their ability to promote the effects of perfusion opposes their normal stimulatory effect on fatty acid synthesis.

The results are consistent with the following proposal. Basal lipolysis is in part constrained by a cellular pool of fatty acids derived from lipolysis, possibly through effects on adenylate cyclase (Fain & Shepherd, 1975). This pool also constrains fatty acid synthesis through inhibition of acetyl-CoA carboxylase by a pool of acyl-CoA as proposed by Halestrap & Denton (1974), and through inhibition of PDH (Sooranna *et al.*, 1977; Smith & Saggerson, 1978). Exogenous fatty acids, with or without insulin, strongly promote esterification of both pools, hence relieving the constraints, but allow perfusion to increase lipolysis and in part re-impose the

constraint on fatty acid synthesis (Saggerson, 1972; Harper, 1984). The weaker stimulation of esterification by insulin relieves the constraint on lipolysis, but not on fatty acid synthesis. This allows perfusion-stimulated lipolysis to constrain further fatty acid synthesis.

ADA exerts its effect on lipolysis through cyclic AMP, as discussed below, and shows an additive decrease of fatty acid synthesis with that of perfusion (Table 2). This is not inconsistent with the above proposal, since intracellular fatty acids can be expected to exert their effect on adenylate cyclase independently of the R_1 receptor for adenosine. However, neither the mode of action of fatty acids on adenylate cyclase nor that of ADA on fatty acid synthesis is known.

The decrease in fatty acid synthesis by perfusion was observed with 0.014 nM-insulin (Table 2; R. D. Harper, unpublished work). This concentration is over 10-fold lower than that required to exhibit stimulatory effects on acylglycerol synthesis or anti-lipolytic effects (Fig. 1). However, this concentration may stimulate esterification of lower acylglycerols, thus relieving constraints on lipolysis as proposed above. That this concentration of insulin raised the NEFA concentration in the medium by 33% (Fig. 1; not statistically significant) supports this suggestion.

Effect of ADA and insulin on lipolysis

ADA increases cyclic AMP, and hence protein kinase activity and lipolysis (Fain, 1973; Schwabe *et al.*, 1974; Honnor *et al.*, 1985). The increase in NEFA reflects this lipolytic effect of ADA (Fig. 1).

The anti-lipolytic action of insulin is mediated by a decrease in cyclic AMP, with resulting decreases in protein kinase activity and also by cyclic-AMP-independent effects (Schwabe *et al.*, 1974; Londos *et al.*, 1985). In adipocytes this anti-lipolytic insulin action has been shown to be dependent on the presence of prostaglandins or adenosine (Lambert & Jacquemin, 1980, 1983). The anti-lipolytic effect of insulin with ADA presumably reflects the accumulation of prostaglandins in these incubations.

Effect of ADA and insulin on acylglycerol fatty acid synthesis

The increased NEFA concentrations present with ADA may decrease fatty acid synthesis through effects on acetyl-CoA carboxylase or PDH activities, as discussed above. However, an increase in cyclic AMP by ADA may contribute to any inhibition of acetyl-CoA carboxylase, as discussed by Brownsey (1983) for lipolytic hormones. Similarly, the abolition of adenosine stimulation of PDH may contribute to any decrease in PDH activity (Honeyman *et al.*, 1983). The relative contributions of fatty-acid-dependent and cyclic-AMP-dependent and -independent effects on fatty acid synthesis are not known.

Insulin (140 nM) abolishes the decrease in fatty acid synthesis produced by ADA and stimulates synthesis to values found in the absence of ADA. A similar pattern of effects was found for glucose oxidation and PDH activity (Schwabe *et al.*, 1974; Wong *et al.*, 1984). Smith & Saggerson (1978) showed that fatty acid synthesis is correlated with PDH activity and that PDH activity is inversely related to fatty acid release. The effects of ADA in the above reports and Fig. 1 are only observed at submaximal concentrations of insulin. This may be

related to the observation that adipocyte PDH activity is only decreased by fatty acids without insulin (Sooranna & Saggerson, 1979).

The results are consistent with the view that the ability of insulin to abolish the decrease in fatty acid synthesis by ADA is, at least in part, due to effects on fatty acid concentrations through its anti-lipolytic effect and the stimulation of esterification. The stimulation of fatty acid synthesis by insulin in the presence and absence of ADA may also reflect well-characterized stimulations of PDH and acetyl-CoA carboxylase activities, and of glucose uptake (Randle, 1981; Brownsey, 1983; Simpson & Cushman, 1986).

Effect of insulin and of ADA on acylglycerol glycerol synthesis

The activities of diacylglycerol acyltransferase, phosphatidate phosphohydrolase, ATP-dependent long-chain fatty acyl-CoA synthetase and glycerol phosphate acyltransferase may be implicated in the regulation of fatty acid esterification in adipose tissue (Nimmo & Houston, 1978; Sooranna & Saggerson, 1978; Cheng & Saggerson, 1978; Rider & Saggerson, 1983; Hall & Saggerson, 1985). However, the major enzyme or enzymes involved in the regulation of acylglycerol synthesis have not been unequivocally identified. It is unclear whether any of the enzymes listed above are covalently modified by phosphorylation. Fatty acids may be involved in the regulation of glycerol phosphate acyltransferase and phosphatidate phosphohydrolase, but this similarly remains unclear (Rider & Saggerson, 1983; Taylor & Saggerson, 1986).

The reports cited above showed that lipolytic agents decrease the activity of key enzymes involved in fatty acid esterification in adipocytes. That ADA does not decrease acylglycerol glycerol synthesis may be related to the finding in liver that fatty acids can overcome cyclic AMP inhibition of acylglycerol synthesis (Pelech *et al.*, 1983). However, the effects of adenosine on the activity of these enzymes are not known.

The stimulation of lipolysis by lipolytic hormones can exert opposite effects on acylglycerol fatty acid and glycerol synthesis similar to those reported here for addition of ADA (Rodbell, 1964; Saggerson, 1972). In the presence of glucose, acylglycerol glycerol synthesis is limited by the supply of fatty acids (Saggerson, 1972). Fatty acids may also exert effects through the activity of enzymes involved in the regulation of acylglycerol synthesis, as discussed above.

In the absence of insulin, the stimulation of acylglycerol synthesis by ADA appears to be secondary to the stimulation of lipolysis, and to be exerted through NEFA.

Acylglycerol glycerol synthesis stimulated by ADA is further stimulated by insulin (140 nM). There are few reports showing direct stimulation of potential regulatory enzymes by insulin (Sooranna & Saggerson, 1976). However, for all the potential regulatory enzymes cited above, insulin abolished the decrease in activity produced by lipolytic hormones. The stimulation by insulin in the presence of ADA may reflect a similar response. The mechanism of such an effect by insulin remains unclear, as discussed above. Glucose uptake is also stimulated by insulin, but acylglycerol glycerol synthesis does not appear to be limited by glucose under these conditions (Saggerson, 1972; Simpson & Cushman, 1986).

Effect of ADA on the sensitivity of lipolysis and acylglycerol to insulin

The results demonstrate co-ordinate regulation of lipolysis and acylglycerol synthesis by insulin in the presence and the absence of ADA. The requirement for such co-ordinate regulation was discussed by Saggerson *et al.* (1977). For lipolysis and glucose uptake, it has been suggested that this co-ordination is at the level of the R_s and R_i membrane receptors and the N_s and N_i regulatory components of adenylate cyclase for both cyclic-AMP-dependent and -independent effects (Londos *et al.*, 1985; Kuroda *et al.*, 1987). Whether a similar relationship holds for acylglycerol glycerol and fatty acid synthesis, and whether fatty acid metabolism contributes to this co-ordinate regulation, remain to be established.

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