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Effect of noradrenaline on triacylglycerol synthesis in rat brown adipocytes

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Incubation of rat brown adipocytes with noradrenaline in the presence of insulin and palmitate caused a decrease in the rate of triacylglycerol synthesis as measured by [U-^{14}C]glucose incorporation into acylglycerol glycerol. Concomitantly, the ratio of [1-^{14}C]palmitate oxidized to CO_2 to that esterified was increased. This alteration in the rate of triacylglycerol synthesis by noradrenaline was not observed when fatty acid oxidation was inhibited by etomoxir. Noradrenaline did not cause any acute inactivation of enzymes of the triacylglycerol-synthesis pathway. It is suggested that the decrease in triacylglycerol synthesis seen with noradrenaline is secondary to activation of fatty acid oxidation.

INTRODUCTION

It is well established that, when stimulated by noradrenaline, brown adipose tissue substantially increases its rate of respiration in a manner that generates heat (see reviews by Nedergaard & Lindberg, 1982; Nicholls & Locke, 1984; Cannon & Nedergaard, 1985). This and subsequent work has led to the concept that β-oxidation of fatty acids provides the major source of reducing equivalents for this enhanced respiration (Isler *et al.*, 1987; Saggerson *et al.*, 1988). It is also quite obvious that brown adipose tissue is capable of synthesizing triacylglycerols from fatty acids, since these are stored in multilocular droplets within brown adipocytes. In addition, the enzymic activities of the triacylglycerol-synthesis pathway have been described, together with rates of metabolic flux into triacylglycerols in these cells (Baht & Saggerson, 1988).

In brown adipocytes, maximum flux rates for fatty acid oxidation and esterification are quite similar, and this is matched by comparable cell activities of the initiating enzymes for these two competing pathways, namely overt carnitine palmitoyltransferase and GPAT (Saggerson & Carpenter, 1982; Baht & Saggerson, 1988). This therefore allows the possibility that regulation of either pathway could influence the other.

It is worth making comparisons with two other tissues. In the liver the rate of fatty acid oxidation appears to be a major determinant of the rate of esterification. This is evidenced by the finding that tetradecylglycidate, although inhibiting fatty acid oxidation, restored triacylglycerol formation by livers from fasted rats back to rates observed in the fed state (Ide & Ontko, 1981). By contrast, in white adipose tissue it is highly unlikely that control of fatty acid oxidation could ever influence esterification, since generally at least 99% of the fatty acid metabolic flux is towards esterification (Harper & Saggerson, 1976). In addition, the ratio of GPAT activity to overt carnitine acyltransferase activity in white adipocytes is approx. 100:1 (Baht & Saggerson, 1988). Both long- and short-term regulation of the white-adipose-tissue triacylglycerol synthesis pathway has been documented. The activities of several enzymes were decreased in starvation or diabetes (Taylor & Saggerson, 1986; Saggerson & Carpenter, 1987), and this was paralleled by decreased metabolic flux rates (Harper & Saggerson, 1976; Baht & Saggerson, 1988). In addition, catecholamine hormones decrease the activities of acyl-CoA synthetase, GPAT and Mg^{2+}-dependent PPH in white adipocytes in a rapid and reversible manner (Cheng & Saggerson, 1978; Rider & Saggerson, 1983; Hall & Saggerson, 1985; Saggerson, 1988), and under appropriate experimental conditions also decrease metabolic flux in the esterification pathway (Grahn & Davies, 1980; Saggerson, 1985).

The regulation of triacylglycerol synthesis in brown adipose tissue has been less extensively investigated. In the longer term hypothyroidism and cold-acclimation increase the activities of enzymes in this pathway (Baht & Saggerson, 1988; Darnley *et al.*, 1988). It appears that no studies of acute hormonal regulation have been made. In the present study we have posed two questions. First, when respiration and fatty acid oxidation in brown adipocytes are stimuated by noradrenaline, is there a concomitant decrease in the capability of the esterification pathway? Second, if so, is this change secondary to diversion of fatty acid metabolic flux into oxidative metabolism, or is control by noradrenaline exerted directly upon the esterification process? We have sought answers by measuring metabolic fluxes in the presence and absence of etomoxir, an inhibitor of fatty acid oxidation, and by investigating acute effects of noradrenaline on the activities of triacylglycerol-synthesizing enzymes.

MATERIALS AND METHODS

Chemicals

These were obtained and treated as described by Baht & Saggerson (1988) and Saggerson *et al.* (1988). In addition, [1-^{14}C]palmitate was obtained from Amersham International (Little Chalfont, Bucks., U.K.), and 2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylate

Abbreviations used: GPAT, glycerolphosphate acyltransferase (EC 2.3.1.15); MGPAT, monoacylglycerolphosphate acyltransferase (EC 2.3.1.51); PPH, phosphatidate phosphohydrolase (EC 3.1.3.4).

(etomoxir) was a gift from Byk Gulden Pharmazeutika (Konstanz, German Federal Republic).

Animals

Male Sprague–Dawley rats (6 weeks old; 160–180 g) were bred at University College London. Animals had constant access to water and Rat & Mouse No. 3 Breeding Diet (Special Diet Services, Witham, Essex, U.K.), which contained (w/w) 21% digestible crude protein, 4% digestible crude oil and 39% starches and sugars. They were maintained at approx. 21 °C on a 13 h-light/11 h-dark cycle, with light from 06:00 to 19:00 h.

Isolation of adipocytes

Brown adipocytes were isolated from the interscapular depot (Woodward & Saggerson, 1986) by the procedure originally described by Fain *et al.* (1967) and elaborated by Nedergaard & Lindberg (1982).

Measurements of metabolic flux rates in incubated adipocytes

Freshly isolated cells (approx. 8×10^5 cells/ml) were dispensed into 25 ml silicone-treated Erlenmeyer flasks containing 4 ml of Krebs–Ringer bicarbonate medium (Krebs & Henseleit, 1932), fatty acid-poor albumin (40 mg/ml), 5 mM-glucose and 2 mM-sodium palmitate. [U-^{14}C]Glucose (0.1 μCi/μmol), [1-^{14}C]palmitate (0.19 μCi/μmol), insulin (4 nM) or noradrenaline (1 μM) was added as appropriate. The flasks were incubated for 30 min at 37 °C with shaking under O_2/CO_2 (19:1). [^{14}C]Glucose incorporation into fatty acids, acylglycerol glycerol and CO_2 was measured as described by Saggerson & Greenbaum (1970). [1-^{14}C]Palmitate incorporation into acylglycerol fatty acids and CO_2 was measured as described by Harper & Saggerson (1976). For measurement of glycerol release by cells, the procedure of Woodward & Saggerson (1986) was followed and glycerol was assayed enzymically (Garland & Randle, 1962).

Measurement of oxygen consumption

This was as described by Woodward & Saggerson (1986), by using 3 ml final volumes of Krebs–Ringer bicarbonate medium containing fatty acid-poor albumin (40 mg/ml) and approx. 9×10^5 brown adipocytes/ml. Additions of glucose, palmitate and hormones were made as appropriate (see Fig. 1 and Table 1) at the same concentrations as were used for measurements of metabolic fluxes (see above). Etomoxir was added as an aqueous solution.

Investigation of effects of hormones on enzyme activities

Brown adipocytes (approx. 8×10^5 cells/ml) were incubated with shaking for 30 min at 37 °C in 4 ml of Krebs–Ringer bicarbonate medium containing fatty acid-poor albumin (40 mg/ml) and 5 mM-glucose. Insulin (4 nM) and noradrenaline (1 μM) were added as appropriate (Table 3). After incubations, adipocytes were recovered by centrifugation at 200 $g_{av.}$ for 20 s, and the underlying medium was removed by aspiration. The cells were freeze-stopped and stored in liquid N_2 for approx. 1 h. Homogenates were prepared in 1 ml volumes of ice-cold 0.25 M-sucrose medium containing 10 mM-Tris/HCl buffer, pH 7.4, 1 mM-EDTA and 1 mM-dithiothreitol. This procedure is identical with that used for white adipocytes by Sooranna & Saggerson (1976) and Rider & Saggerson (1983). Portions of homogenates were assayed for activities of mitochondrial GPAT, microsomal GPAT, MGPAT, Mg^{2+}-dependent PPH and lactate dehydrogenase as described by Baht & Saggerson (1988). To compensate for any incompleteness of recovery of cells from incubations, GPAT, MGPAT and PPH activities were expressed relative to lactate dehydrogenase activity (Sooranna & Saggerson, 1976), where 1 unit of lactate dehydrogenase reduces 1 μmol of pyruvate/min at 25 °C.

Measurement of DNA

Adipocyte DNA was measured by the method of Switzer & Summer (1971), with calf thymus DNA as standard.

Statistical methods

Statistical significance was assessed by Student's *t* test for paired samples.

RESULTS AND DISCUSSION

To study rates of fatty acid esterification, brown adipocytes were incubated with 2 mM-palmitate (fatty acid/albumin molar ratio of 3.3:1). This fatty acid concentration is well in excess of that needed to saturate the esterification process in brown adipocytes (Saggerson *et al.*, 1988). Addition of long-chain fatty acids has previously been shown to increase respiration by brown adipocytes (Bukowiecki *et al.*, 1981), and accordingly (in the presence of 5 mM-glucose) palmitate doubled O_2 uptake (Table 1; $P < 0.005$). A further significant enhancement of respiration occurred when noradrenaline was added in addition to palmitate (Table 1) giving a rate indistinguishable from that with noradrenaline alone. Both conversion of [U-^{14}C]glucose into acylglycerol glycerol and [1-^{14}C]palmitate esterification were used as estimates of triacylglycerol synthesis. The former approach has been justified by Baht & Saggerson (1988). The latter approach must be an underestimate in the presence of noradrenaline, because lipolysis will dilute the [^{14}C]palmitate precursor pool. However, if it is assumed that this dilution equally affects both oxidation and esterification, changes in the oxidation/esterification ratio (B/C in Table 1) should be informative in different hormonal states. Evidence of precursor dilution can be gleaned from Table 1. First, the ratio $^{14}CO_2$ production from palmitate/O_2 uptake was decreased by 38% in the presence of noradrenaline. Second, the ratio acylglycerol glycerol formation/palmitate esterification (A/C in Table 1) was significantly increased by noradrenaline (with or without insulin). Third, in a separate experiment it was calculated that fatty acid release by lipolysis could dilute the labelled fatty acid pool by at least 20%. It is noteworthy, and convenient, that insulin did not show an anti-lipolytic effect under these conditions, i.e. that insulin did not modify the dilution effect of noradrenaline.

We attempted to minimize the dilution effect by decreasing the cell concentration as far as was practicable and by using the relatively high 2 mM concentration of palmitate. As discussed above, these attempts were only partially successful, so that the meaningful data in Table 1 are the rates of acylglycerol glycerol formation and the ratio of palmitate oxidation/palmitate esterification (B/C). Table 1 shows that, in the presence of insulin,

Table 1. Effects of hormones on metabolic flux rates

Incubations were performed as described in the Materials and methods section. The values are means ± S.E.M. for the numbers of separate measurements shown in parentheses. Significant effects of noradrenaline are indicated by [a]$P < 0.025$, [b]$P < 0.01$, [c]$P < 0.005$. Significant effects of insulin are indicated by [d]$P < 0.05$, [e]$P < 0.025$, [f]$P < 0.005$, [g]$P < 0.0005$ respectively. The absolute values for palmitate flux were not analysed statistically.

Additions to cell incubation	O_2 uptake (μmol/30 min per 100 μg of DNA)	[^{14}C]Glucose conversion into acylglycerol glycerol (μg-atoms of C/30 min per 100 μg of DNA) (A)	[1-^{14}C]Palmitate conversion (μg-atoms of C/30 min per 100 μg of DNA)		Glycerol release (μmol/30 min per 100 μg of DNA)	Ratio A/C	Ratio B/C
			Into CO_2 (B)	Into acylglycerol fatty acid (C)			
None	14.1 ± 3.1 (4)	–	–	–	–	–	–
Noradrenaline	42.0 ± 8.5[a] (3)	–	–	–	–	–	–
Palmitate	27.7 ± 2.7 (4)	0.30 ± 0.03 (8)	0.87 ± 0.26 (7)	0.44 ± 0.04 (8)	0.03 ± 0.01 (3)	0.69 ± 0.08 (8)	2.07 ± 0.53 (7)
Palmitate + noradrenaline	38.9 ± 3.3[a] (4)	0.36 ± 0.05 (8)	0.76 ± 0.22 (8)	0.31 ± 0.04 (8)	2.25 ± 1.15 (3)	1.26 ± 0.17[c] (8)	2.67 ± 0.71 (8)
Palmitate + insulin	–	1.22 ± 0.18[g] (8)	1.08 ± 0.35 (7)	1.22 ± 0.15 (8)	0.04 ± 0.01 (3)	0.99 ± 0.06[f]	0.92 ± 0.29[f] (7)
Palmitatate + noradrenaline + insulin	–	0.75 ± 0.12[cf] (7)	0.81 ± 0.22 (7)	0.52 ± 0.08 (7)	2.20 ± 1.16 (3)	1.48 ± 0.14[bd] (7)	1.64 ± 0.45[ce] (7)

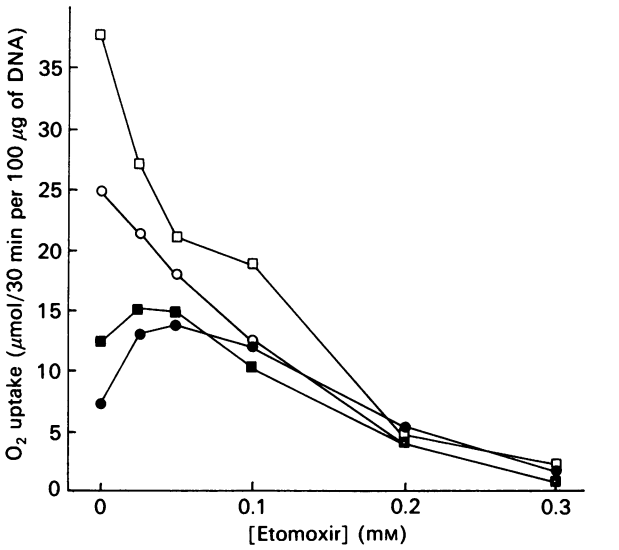

Fig. 1. Effect of etomoxir on O_2 consumption by brown adipocytes

The values are from a single representative experiment. Incubations contained fatty acid-poor albumin (40 mg/ml). ●, No further additions; ■, with glucose (5 mM); ○, with glucose + palmitate (2 mM); □, with glucose + palmitate + noradrenaline (1 μM).

noradrenaline decreased triacylglycerol synthesis, as evidenced by a 39% decrease in acylglycerol glycerol formation. Partitioning of fatty acid metabolism into oxidation rather than esterification (ratio B/C) was also significantly increased by noradrenaline in the presence of insulin. No significant effects of noradrenaline on these parameters were noted in the absence of insulin. It is also noteworthy that insulin increased acylglycerol glycerol formation in both the presence and the absence of noradrenaline and significantly decreased the partitioning of palmitate metabolism towards oxidation. Thus these two hormones appear to have opposing effects on both the partitioning of fatty acid metabolism and on the absolute rate of triacylglycerol synthesis in brown adipocytes.

The drug etomoxir is one of a series of oxirane carboxylic acid derivatives which include tetradecylglycidate and 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate (POCA). These all have the property of suppressing fatty acid oxidation as a result of their inhibition of overt carnitine palmitoyltransferase (Kiorpes *et al.*, 1984; Turnbull *et al.*, 1984). At 0.3 mM etomoxir caused a substantial decrease in O_2 uptake (Fig. 1) and in [1-^{14}C]palmitate oxidation (Table 2). It is noteworthy that lower concentrations of the drug actually increased respiration in the absence of palmitate. This effect was not investigated further, but might possibly be due to an interaction with the brown-adipocyte mitochondrial uncoupling protein.

Table 2 confirms the previously noted effect of noradrenaline to decrease acylglycerol glycerol formation (in the presence of insulin). Etomoxir totally abolished this effect, suggesting that the suppression of triacylglycerol synthesis by noradrenaline is likely to be secondary to the concomitant increase in fatty acid oxidation by this hormone. It is clear from Table 2 that

Table 2. Effects of etomoxir on metabolic flux rates

Incubations were performed as described in the Materials and methods section. The values are means ± S.E.M. for the numbers of separate measurements shown in parentheses. Significant effects of insulin are indicated by [a]$P < 0.005$, and of noradrenaline by [b]$P < 0.005$. The values for palmitate flux were not analysed statistically.

Additions to cell incubation	Etomoxir (0.3 mM)	[1-^{14}C]Palmitate conversion into CO_2 (μg-atoms of C/30 min per 100 μg of DNA)	[U-^{14}C]Glucose conversion (μg-atoms of C/30 min per 100 μg of DNA)	
			Into acylglycerol glycerol	Into fatty acid
Palmitate	−	0.60 ± 0.13 (4)	0.31 ± 0.04 (5)	0.03 ± 0.008 (5)
	+	0.01 ± 0.01 (3)	0.23 ± 0.04 (5)	0.02 ± 0.008 (5)
Palmitate + insulin	−	0.63 ± 0.14 (4)	1.26 ± 0.27[a] (5)	0.30 ± 0.05[a] (5)
	+	0.04 ± 0.02 (4)	0.36 ± 0.08 (5)	0.12 ± 0.02[a] (5)
Palmitate + insulin + noradrenaline	−	0.53 ± 0.09 (4)	0.77 ± 0.19[b] (4)	–
	+	0.01 ± 0.007 (3)	0.36 ± 0.08 (4)	–

Table 3. Effect of hormones on activities of enzymes of triacylglycerol synthesis

Incubations, freeze-stopping and assays were performed as described in the Materials and methods section. The values are means ± S.E.M. for the numbers of separate measurements shown in parentheses.

Additions to cell incubation	Enzyme activity (nmol/min per unit of lactate dehydrogenase activity)			
	Mitochondrial GPAT [5]	Microsomal GPAT [5]	MGPAT [3]	Mg^{2+}-dependent PPH [3]
None	0.61 ± 0.08	2.80 ± 0.33	92 ± 19	23.5 ± 5.4
Noradrenaline	0.63 ± 0.06	3.27 ± 0.24	82 ± 12	20.3 ± 2.7
Insulin	0.63 ± 0.04	2.90 ± 0.23	103 ± 14	22.8 ± 3.9
Noradrenaline + insulin	0.71 ± 0.10	3.43 ± 0.28	90 ± 15	20.7 ± 1.2

etomoxir does have metabolic side effects in this experimental system. Flux of [^{14}C]glucose both into acylglycerol glycerol and into fatty acids was decreased by the drug (Table 2), and $^{14}CO_2$ formation was also decreased (results not shown). This suggested a generalized effect of etomoxir to decrease glucose metabolism. We considered the possibility that etomoxir might simply mimic the effect of noradrenaline by limiting glycerol phosphate provision from glucose, and thereby might negate the effect of the hormone. We believe this to be unlikely, since noradrenaline actually increases glucose metabolism, as evidenced by increased lactate + pyruvate output (Saggerson *et al.*, 1988) and increased glycolytic flux (F. Sobrino & E. D. Saggerson, unpublished work). We also considered the possibility that etomoxir might have profound toxic effects. However, the cells still appeared to be viable, since fatty acid synthesis could be stimulated 6-fold by insulin to give rates in excess of those seen in the absence of hormone and the drug (the rates of fatty acid synthesis are low because palmitate inhibits this process in brown adipocytes; see Saggerson *et al.*, 1988).

Table 3 describes an experiment in which brown adipocytes were exposed to noradrenaline for 30 min, recovered, freeze-stopped and assayed for enzymic activities. In identical experiments noradrenaline has previously been demonstrated to decrease the activities of mitochondrial and microsomal GPAT and of Mg^{2+}-dependent PPH in white adipocytes (Sooranna & Saggerson, 1976; Cheng & Saggerson, 1978; Rider & Saggerson, 1983). By contrast, no changes in these or in the activity of MGPAT were observed in brown adipocytes, suggesting that noradrenaline does not acutely regulate these activities in the brown adipocyte.

In conclusion, acute stimulation of brown adipocytes by noradrenaline decreases triacylglycerol synthesis. This change does not appear to be due to direct regulation of the triacylglycerol-synthesis pathway, but does appear to be secondary to the concomitant activation of fatty acid oxidation. In the longer term, the brown adipocyte may be able to adapt and restore its rate of triacylglycerol synthesis even while being stimulated by noradrenaline, since cold-acclimation for more than 1 day leads to substantial increases in the activities of some triacylglycerol-synthesizing enzymes (Darnley *et al.*, 1988).

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