Effects of hormones on the rate of the triacylglycerol/fatty acid substrate cycle in adipocytes and epididymal fat pads

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1. INTRODUCTION

It has been suggested by some workers that substrate ('futile') cycles are either of little of no significance in metabolism [1] whereas others suggest that they are of considerable importance in increasing sensitivity in metabolic control [2–4]. One prediction of the hypothesis that cycling increases sensitivity is that the rate will vary from one condition to another and that some hormones may specifically increase the cycling rate. Evidence for the existence of the triacylglycerol/fatty acid cycle in adipose tissue was presented by Steinberg [5] who compared the rates of fatty acid and glycerol production by isolated epididymal fat pads. This method has been used to investigate the effect of some hormones on the rate of the triacylglycerol/fatty acid cycle in isolated adipocytes of the rat. In addition, a radiochemical method that depends upon the incorporation of tritium from tritiated water into the glycerol and fatty acid moieties of triacylglycerol has been developed for the measurement of the rate of this cycle in epididymal fat pads of the rat. The effects of some hormones have been investigated using both techniques and the results are reported and discussed here.

2. MATERIALS AND METHODS

Male Wistar rats were obtained from OLAC (1976) Ltd. (Blackthorn, Bicester, Oxon OX6 OTP). Chemicals, biochemicals and enzymes were obtained from sources given in [6] except that crystalline glucagon, ACTH and TSH were obtained from Sigma Chemical Co. (Poole, Dorset) and radiochemicals from The Radiochemical Centre (Amersham HP7 9LL).

Rats were killed by cervical dislocation and the epididymal fat pads were removed. Only the thinner distal portion of the pad was used for incubation and up to 200 mg fresh tissue was incubated in 1.5 ml Krebs–Ringer bicarbonate buffer containing 1 mM glucose and 4% (w/v) defatted albumin [6]. Fat cells were prepared as in [7] and incubated in 1.3 ml Krebs–Ringer buffer as in [6]. The incubation was terminated by addition of 300 μl 2 M H_2SO_4 (which does not precipitate albumin); 200 μl was taken for assay of fatty acid and 1 ml for assay of glycerol. For the latter, the cells and albumin were precipitated by addition of trichloroacetic acid (200 μl, 40% (w/v)). The precipitate was removed by centrifugation and universal indicator (15 μl) was added to the supernatant and neutralised by addition of 7 M KOH from a microsyringe. The concentrations of glycerol and fatty acids were determined as in [8,9]. The rate of cycling was calculated as follows:

$$\text{cycling} = [(3 \times \text{rate of glycerol production}) - (\text{rate of fatty acid production})]$$

For the radiochemical measurement of the triacylglycerol/fatty acid cycle, fat pads rather than cells were used. The pads were incubated with ^3H_2O (1–2 mCi/ml incubation buffer). Incuba-

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tions were stopped by dropping the fat pads into 1.5 ml ice-cold $HClO_4$ (5%, w/v), petroleum ether (5 ml 40–60 fraction) was added and the tissue was homogenised using a Polytron tissue grinder. After centrifugation, the petroleum ether fraction was aspirated and kept in a ground glass stoppered test tube, and the homogenisation procedure was repeated with a further 5 ml petroleum ether. The organic phases were combined and washed (to remove ^3H_2O) by shaking 3 separate times with a mixture of 0.5 M NaCl and 0.5 M H_2SO_4 [10]. After each shaking the tubes were briefly centrifuged to sharpen the phase boundary and the aqueous layer was aspirated and discarded. The petroleum ether phase was evaporated to dryness and the triacylglycerol was hydrolysed in 1.5 ml 3:1 (v/v) ethanol : KOH (60%, w/v) for 3 h at 60–70°C. After cooling, the hydrolysate was acidified using 6 M H_2SO_4. Fatty acids were removed from the hydrolysate by 2 extractions using 5 ml 40–60 fraction petroleum ether. A volume of the aqueous hydrolysate (0.25 ml) was dissolved in 10 ml of scintillant (which contained 2.0 g 2,5-diphenyloxazole (PPO) and 0.05 g 1,4-bis(5-phenyloxazolyl)-2-benzene (POPOP) in 500 ml toluene + 250 ml Triton X-100 [11]) and the radioactivity (i.e., [^{3}H]-glycerol) was measured in a liquid scintillation counter (Beckman model LS 200). The petroleum ether washings were combined in a scintillation vial and evaporated to dryness, after which 10 ml scintillant (which contained 3.0 g PPO/l and 0.1 g POPOP/l toluene) was added and radioactivity (i.e., [^{3}H]fatty acid) was measured. The degree of quenching in each sample was obtained by use of internal standards; this enabled radioactivity to be calculated in dpm. It was assumed, for the calculation of triacylglycerol and fatty acid synthesis, that the specific activity of water in the adipocyte was identical to that in the incubation flask and that each glycerol incorporated into triacylglycerol con-

Table 1

Effects of some hormones, β-blockers, α-blockers and adenosine deaminase on rates of release of glycerol, fatty acids and triacylglycerol/fatty acid cycling in isolated fat cells

Conditions	Rates (μmol · h^{-1} · g dry cells^{-1})		
	Glycerol release	Fatty acid release	Cycling
Control	0.35 ± 0.17	1.13 ± 0.66	−0.07 ± 0.75
Noradrenaline	95.8 ± 3.8[a]	174 ± 7[a]	114 ± 6[a]
Propanol	0.62 ± 0.21	0.59 ± 0.98	1.27 ± 1.33
Noradrenaline + propanolol	13.1 ± 4.5[a]	11.2 ± 1.5[a]	27.8 ± 1.9[a]
Phentolamine	0.62 ± 0.21	2.88 ± 1.50	−1.02 ± 2.05
Noradrenaline + phentolamine	85.2 ± 3.1	156 ± 6[a]	99.7 ± 5.0[a]
Fenoterol	91.2 ± 2.9[a]	177 ± 9[a]	96.7 ± 2.3[a]
Adenosine deaminase	44.0 ± 2.5[a]	97.8 ± 5.4[a]	34.1 ± 4.3[a]
Control	−1.08 ± 1.04	2.38 ± 3.51	−5.61 ± 2.83
Glucagon	42.8 ± 1.6[a]	25.9 ± 3.2[a]	102 ± 4[a]
ACTH	122 ± 1[a]	191 ± 4[a]	174 ± 6[a]
TSH	122 ± 1[a]	170 ± 2[a]	165 ± 4[a]
Insulin	0.06 ± 0.61	−0.28 ± 1.12	0.45 ± 2.24

[a] $P < 0.001$; Statistical significance (Student's t-test) of the difference between control and treated cells

Cycling rates were measured by the non-isotopic method (section 2). Results are presented as means ± SEM for 6 incubations from 1 cell preparation

tained 3.3 atoms and each fatty acid 13.3 atoms of tritium [12,13]. Hence the rate of triacylglycerol synthesis from glycerol phosphate in vivo can be calculated; at steady-state, this rate will be equal to the rate hydrolysis of triacylglycerol (i.e., the rate of triacylglycerol/fatty acid cycle) except for the rate of de novo fatty acid synthesis within the adipose tissue cell. Hence, the rate of cycling was obtained from the following equation:

$$\text{Cycling rate} = [3 \times (\text{rate of triacylglycerol–glycerol synthesis}) - (\text{rate of fatty acid synthesis})]$$

3. RESULTS AND DISCUSSION

The rate of triacylglycerol/fatty acid cycling in isolated adipocytes was increased markedly by a number of hormones including noradrenaline, glucagon, ACTH and TSH but insulin had no effect (table 1). The effect of noradrenaline appeared to be via a β-receptor since the β-blocker propranolol markedly decreased the effect of the hormone whereas the α-blocker, phentolamine, had no effect. The β-adrenergic agonist, fenoterol, also markedly stimulated the rate of cycling. Increased cycling rates in response to noradrenaline, ACTH and glucagon were also observed when the rate of cycling was measured by the radiochemical assay (see section 2 and table 2) whereas triiodothyronine had no effect. There are several important findings of this work that should be emphasised:

(1) Except in one experiment, the rates of cycling measured by the nonisotopic method were very similar to those measured by the completely different radiochemical method (table 2). This provides additional evidence that the simpler non-isotopic method is measuring the rate of the triacylglycerol/fatty acid cycle.

(2) A number of hormones can markedly increase the rate of cycling. It is difficult to calculate the precise increase in cycling by the non-isotopic method in fat cells since the control-rate depends

Table 2

Effects of some hormones on the rate of the triacylglycerol/fatty acid cycle in incubated epididymal fat pads of the rat measured by non-isotopic and isotopic methods

Conditions	Rate of cycling (μmol . h^{-1} . g wet wt^{-1})	
	Non-isotopic method	Incorporation of tritium into triglyceride
Control (10 mM glucose and insulin)	3.99 ± 0.27 (7)	3.29 ± 0.20
Noradrenaline (10 mM glucose and insulin)	9.16 ± 1.51 (7)[a]	6.06 ± 0.82[a]
Control	0.49 ± 0.13 (9)	3.29 ± 0.51[c]
Glucagon (5 μg/ml)	10.3 ± 0.62 (9)[b]	9.51 ± 0.57[b]
ACTH (5 μg/ml)	7.96 ± 1.40 (9)[b]	7.51 ± 0.72[b]
Control	5.39 ± 1.19 (10)	5.18 ± 0.71
Triiodothyronine (1,25 μg/ml)	4.00 ± 0.44 (10)	5.09 ± 0.44

[a] $P < 0.05$; [b] $P < 0.001$; Statistical significance (Student's t-test) of the difference between control and treated fat pads

The methods for measurement of cycling rates are given in section 2. Control conditions contained 5 mM glucose unless otherwise indicated. Results are presented as means ± SEM with number of different animals used given in parentheses

upon the measurement of very small concentrations of fatty acid and glycerol so that any error in measurement is compounded by the need to multiply by 3 in the calculation of the cycling rate. Although the hormones that stimulate the rate of cycling in fat pads also stimulate the rate in adipocytes, the extent of the stimulation is considerably smaller in the fat pads. The reason for this difference is not known, but the stimulation observed in the fat pad is similar to that observed in vivo [14] suggesting that the magnitude of the effect in the pads is more physiological.

(3) Since noradrenaline and the β-adrenergic agonist, fenoterol, increase the rate of cycling, they must increase the rate of esterification. This increase occurs despite the fact that treatment of adipose tissue with catecholamines decreases the activities of all the enzymes involved in re-esterification of fatty acids [15]. Furthermore, there is evidence that catecholamines stimulate the phosphorylation of glycerol phosphate acyltransferase, probably a key regulatory enzyme in triacylglycerol synthesis and this causes inhibition rather than activation of the enzyme [16].

These findings suggests that factors other than the state of phosphorylation of the enzyme (e.g., concentration of fatty acids and fatty acyl-CoA) must play an important role in the regulation of the rate of esterification in adipose tissue.

Since the cycling rate can be specifically stimulated, at least several fold, by a number of hormones, the cycle probably plays an important physiological role in controlling the sensitivity of lipolysis of and/or esterification to other hormones and other regulators.

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