# A DECREASE IN DIACYLGLYCEROL ACYLTRANSFERASE AFTER TREATMENT OF RAT ADIPOCYTES WITH ADRENALINE

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## 1. Introduction

The activities of several adipocyte enzymes involved in glyceride synthesis are decreased by relatively brief exposure of these cells to catecholamines [1–4]. The reaction catalysed by diacylglycerol acyltransferase (DGAT) is the only reaction that is exclusively concerned with the synthesis of triacylglycerol. On this basis, it might be considered as a possible site of regulation. We report here an effect of adrenaline on the activity of DGAT which appears to be both rapid and dose-dependent.

## 2. Materials and methods

Sources and treatments of animals and chemicals were as in [1]. In addition, [1-$^{14}$C]palmitoyl CoA was from NEN Chemicals GmbH (Frankfurt/Main) and 1,2-dioctadecenoyl-*sn*-glycerol (diolein) was from International Enzymes Ltd, (Windsor, Berks). Adipocytes, prepared by the method in [5], were incubated and tissue extracts prepared from freeze-stopped cells in a medium of 0.25 M sucrose containing 10 mM Tris–Cl buffer, (pH 7.4), 1 mM EDTA and 1 mM dithiothreitol as in [6]. Tissue extracts were diluted 1 in 5 in ice-cold 0.25 M sucrose medium containing 10 mM Tris–Cl buffer (pH 7.4) and 1 mM EDTA before use in enzyme assays.

DGAT (EC 2.3.1.20) was assayed at 30°C in final vol. 0.2 ml containing 50 mM Tris–Cl buffer (pH 8.0), 8 mM $MgCl_2$, 0.1 $\mu$Ci [1-$^{14}$C]palmitoyl CoA, 20 $\mu$g fatty acid-poor albumin, 2.5 mM diolein, 0.01 ml ethanol and 30 $\mu$M palmitoyl CoA [7]. The reaction was initiated with 0.1 ml diluted tissue extract. Assays were performed for 6 min but were linear for up to 15 min. The reaction was terminated by the addition of 2.0 ml propan-2-ol:*n*-hexane:0.5 M $H_2SO_4$ (40:10:1, by vol.). After the addition of 1.2 ml water and 1.2 ml *n*-hexane, the resulting hexane layer was washed with 4 vol. freshly-prepared 50 mM $NaHCO_3$ in ethanol: water (1:1, by vol.) pre-cooled to 4°C. From 2,5-bis (5-*t*-butylbenozoxazol-2-yl) thiophen scintillator (4 g/l in toluene) 10 ml was added to 0.9 ml hexane layer and this was counted with efficiency of 88%. Blanks without diolein were performed for each tissue extract. Appropriate zero-time blanks were also conducted in parallel with all experiments. DGAT activities were compared to lactate dehydrogenase activity in order to correct for any incompleteness in recovery of cells after incubation or for incomplete cell breakage during homogenisation. Lactate dehydrogenase (EC 1.1.1.27) was assayed as in [8] and 1 unit represents the reaction of 1 $\mu$mol substrate/min at 25°C.

Nonesterified fatty acids and glycerol in incubation media were measured by the methods in [9] and [10], respectively. Adipocyte DNA content was determined by the method in [11].

Statistical differences between experimental values were *t*-tested on the basis of paired differences.

## 3. Results and discussion

Table 1 shows that incubation of adipocytes for 1 h with adrenaline caused a dose-dependent decrease in the activity of DGAT which was significant with

Table 1
Effect of adrenaline on adipocyte DGAT activity

| Adrenaline (M) | [1-$^{14}$C]Palmitoyl incorporation into hexane-soluble products (nmol/min/unit lactate dehydrogenase) | | | Extracellular nonesterified fatty acid (mM) | Extracellular glycerol (mM) |
|---|---|---|---|---|---|
| | Total activity | Background activity | Diolein-dependent activity | | |
| 0 | 6.97 ± 0.64 | 2.82 ± 0.06 | 4.15 ± 0.67 | 0.09 ± 0.01 | 0.04 ± 0.00 |
| $10^{-8}$ | 6.84 ± 0.94 | 2.50 ± 0.17 | 4.35 ± 0.77 | 0.09 ± 0.10 | 0.07 ± 0.01 |
| | (−3.1 ± 5.2) | | (+5.0 ± 7.5) | | |
| $10^{-7}$ | 6.54 ± 1.08 | 2.58 ± 0.22 | 3.97 ± 0.88 | 1.70 ± 0.21$^{c}$ | 0.77 ± 0.11$^{c}$ |
| | (−8.0 ± 7.7) | | (−6.2 ± 8.9) | | |
| $5 \times 10^{-7}$ | 5.94 ± 0.72$^{a}$ | 2.73 ± 0.17 | 3.19 ± 0.64$^{a}$ | 5.88 ± 0.90$^{c}$ | 2.28 ± 0.22$^{c}$ |
| | (−15.6 ± 3.9)$^{a}$ | | (−24.1 ± 5.2)$^{b}$ | | |
| $10^{-6}$ | 5.13 ± 0.77$^{b}$ | 2.81 ± 0.16 | 2.31 ± 0.69$^{c}$ | 6.47 ± 1.14$^{c}$ | 2.53 ± 0.26$^{c}$ |
| | (−27.5 ± 5.5)$^{b}$ | | (−49.0 ± 10.4)$^{b}$ | | |

a,b,c Indicate $P < 0.05$, $< 0.02$, $< 0.01$ for effects of adrenaline

Adipocytes were incubated with 5 mM glucose for 1 h in 4 ml Krebs-Ringer bicarbonate containing fatty acid-poor albumin (40 mg/ml) and the indicated concentrations of adrenaline. The results are the means and SEM of 4 expts. The mean adipocyte DNA was 5.9 μg/ml flask contents. Values in parentheses indicate percentage effects of adrenaline

0.5 μM and 1 μM adrenaline. All cell extracts had a background incorporation of the [$^{14}$C]palmitoyl group into hexane-soluble products. This is presumably due to the presence of endogenous substances which can act as acyl acceptors. For this reason [$^{14}$C]palmitoyl incorporation was always measured in the presence and absence of diolein. Tables 1 and 2 both show that it was only the diolein-dependent [$^{14}$C]palmitoyl incorporation and not this background activity that was affected by adrenaline.

Incubation of cells for 1 h with 0.5 μM or 1 μM adrenaline resulted in large accumulations of lipolysis

Table 2
Effect of adrenaline and insulin on adipocyte DGAT activity

| Expt. no. | Additions to incubation medium | [1-$^{14}$C]Palmitoyl incorporation into hexane-soluble products (nmol/min per unit of lactate dehydrogenase) | | | Extracellular nonesterified fatty acid (mM) | Extracellular glycerol (mM) |
|---|---|---|---|---|---|---|
| | | Total activity | Background activity | Diolein-dependent activity | | |
| 1 | None | 5.50 ± 0.52 | 1.92 ± 0.25 | 3.58 ± 0.53 | 0.04 ± 0.01 | 0.03 ± 0.00 |
| | Adrenaline (0.63 μM) | 4.77 ± 0.46$^{a}$ | 2.09 ± 0.28 | 2.68 ± 0.41$^{a}$ | 1.66 ± 0.19$^{a}$ | 0.57 ± 0.03$^{a}$ |
| 2 | None | 5.37 ± 0.61 | 1.92 ± 0.30 | 3.44 ± 0.63 | 0.04 ± 0.01 | 0.03 ± 0.00 |
| | Insulin (2 munits/ml) (84 ng/ml) | 5.19 ± 0.68 | 1.81 ± 0.30 | 3.38 ± 0.76 | 0.02 ± 0.01$^{b}$ | 0.02 ± 0.00$^{b}$ |

a Indicates $P < 0.01$ for effects of adrenaline
b,c Indicate $P < 0.05$, $P < 0.02$ for effects of insulin

Adipocytes were incubated with 5 mM glucose for 20 min in 4 ml of Krebs-Ringer bicarbonate containing fatty acid-poor albumin (32.5 mg/ml) and the indicated additions. The results are the means and SEM of 6 and 5 experiments and the mean fat cell DNA was 4.9 and 5.0 μg/ml of flask contents for experiments 1 and 2 respectively.

products (table 1). It may be seen in table 2 however that DGAT activity was significantly decreased by 25% after incubation with 0.63 μM adrenaline for only 20 min. In this time interval the accumulation of nonesterified fatty acids was within the physiological range. It would seem unlikely therefore that the decreases in DGAT activity shown in table 1 are a consequence of the accumulation of nonesterified fatty acids.

Incubation with insulin alone for 20 min did not change DGAT compared with the basal activity (table 2). However, insulin was able to oppose the effect of adrenaline. This was seen in an experiment (4 independent measurements) in which incubation with 0.63 μM adrenaline for 20 min decreased the diolein-dependent activity by 29% from 2.48 ± 0.52 to 1.77 ± 0.36. In a paired incubation with 0.63 μM adrenaline and insulin (2 munits/ml) this activity was 2.15 ± 0.37 ($P < 0.01$ versus the activity found in cells incubated with adrenaline and $P > 0.3$ versus the basal activity).

The mechanism underlying these effects of hormones on DGAT activity is at present unclear. However, the observed changes in activity are persistent enough to survive freeze-stopping and homogenisation of cells.

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