STUDIES OF THE EFFECT OF ADRENALINE ON GLYCEROL PHOSPHATE ACYLTRANSFERASE ACTIVITY IN RAT ADIPOCYTES

Suren R. SOORANNA and E. David SAGGERSON
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, England

Received 12 April 1978

1. Introduction

It was found [1] that glycerol phosphate acyltransferase (GPAT) activity was decreased in rat adipocytes incubated for 1 h with adrenaline. This dose-dependent effect of adrenaline on GPAT activity was accompanied by increased lipolysis [1], but could not be mimicked by incubation of adipocytes with fatty acids [2]. In this study we have investigated the time dependence of the adrenaline effect on GPAT activity and have shown that this effect is inhibited by the β-blocker propranolol and by insulin.

2. Materials and methods

Sources and treatments of animals and chemicals were as described in [1]. Adipocytes were prepared as in [3] and incubated and extracts prepared from freeze-stopped cells as described in [2]. Enzymes were assayed as described in [1]. GPAT (EC 2.3.1.15) was assayed with 0.5 mM [U-^{14}C]glycerol-3-phosphate and the activity expressed as nmol glycerol-3-phosphate incorporated into butanol-soluble products/min/unit adipocyte lactate dehydrogenase. This means of expression corrects for any incompleteness in homogenisation or recovery of cells from incubations. Non-esterified fatty acids in incubation media were measured as described in [1]. Where appropriate, statistical differences between experimental values were *t*-tested on the basis of paired differences.

3. Results and discussion

Effects of adrenaline on adipocyte GPAT activity were investigated [1] with cells incubated in the absence of a carbohydrate substrate. Under the same conditions (fig.1a), 0.63 μM adrenaline did not result in any appreciable decrease in GPAT activity until 45 min. The effect of adrenaline was significant at 60 min and 75 min ($P < 0.01$ in each case). When cells were incubated in the presence of 5 mM glucose (fig.1b) the effect of 0.63 μM adrenaline on GPAT activity was again delayed, in this case until 30 min of incubation with the hormone had elapsed. The effect of adrenaline was significant at 30 min, 45 min, 60 min and 75 min ($P < 0.05$ in every case). The molecular mechanism underlying this adrenaline-dependent decrease in GPAT is unknown. However, it is persistent in that it survives through freeze-stopping and preparation of dilute, defatted homogenates. It would seem unlikely that the decrease in GPAT activity is directly related to non-esterified fatty acid accumulation since these are not related on a temporal basis (fig.1a,b) and addition to incubations of palmitate at concentrations as high as 3.5 mM causes no decrease in GPAT activity [2]. Furthermore, lower concentrations of adrenaline than used here produce significant decreases in GPAT after 1 h whilst only producing modest accumulation of non-esterified fatty acids [1].

Table 1 show 2 experiments which demonstrate that the decrease in GPAT activity resulting from exposure of adipocytes to adrenaline for 75 min is

Table 1
Stability of GPAT activity in extracts prepared from adipocytes exposed to adrenaline

Additions to cell incubation	Extracellular unesterified fatty acid concn at the end of cell incubation (mM)	Incubation time of tissue extracts at 0–4°C (min)	GPAT activity	Additions to cell incubation	Extracellular unesterified fatty acid concn at the end of cell incubation (mM)	Incubation time of tissue extracts at 30°C (min)	GPAT activity
		0	4.93			0	3.85
		5	4.69			5	4.08
		15	5.09			15	4.03
None	0.28	30	5.17	None	0.21	30	4.19
		45	5.12			45	4.25
		70	5.28			70	4.29
		0	2.37			0	1.72
		5	2.22			5	1.59
		15	2.18			15	1.63
Adrenaline (0.63 μM)	4.15	30	2.06	Adrenaline (0.63 μM)	4.12	30	1.72
		45	2.08			45	1.60
		70	2.34			70	1.65

Adipocytes (8.8 μg DNA/ml flask contents) were incubated in a paired fashion with and without adrenaline for 75 min in 4 ml Krebs-Ringer bicarbonate containing fatty acid-poor albumin (32.5 mg/ml) and glucose (5 mM). After freeze-stopping [1] the cells were homogenized in 1 ml 0.25 M sucrose medium containing 1 mM EDTA, 1 mM dithiothreitol and 10 mM Tris–chloride buffer pH 7.4 as in [2]. These extracts were then incubated as shown below and aliquots taken for assay of GPAT activity at the indicated times. The results in each case are from 1 experiment

Fig.1a

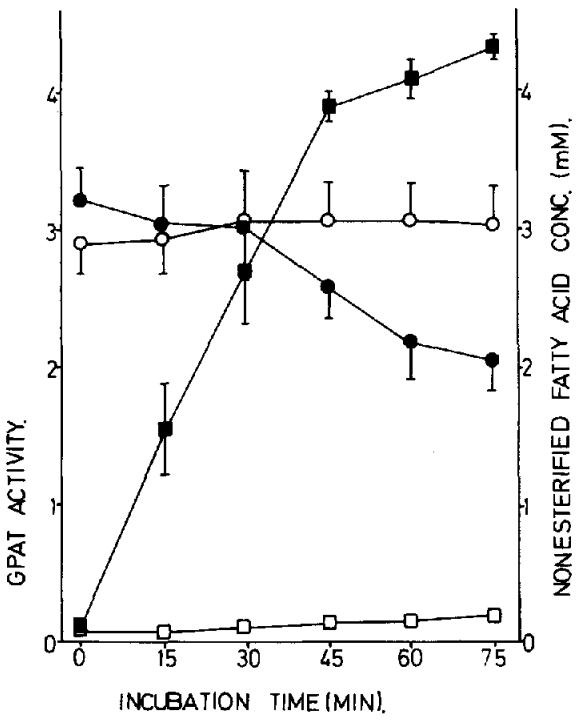

Fig.1b

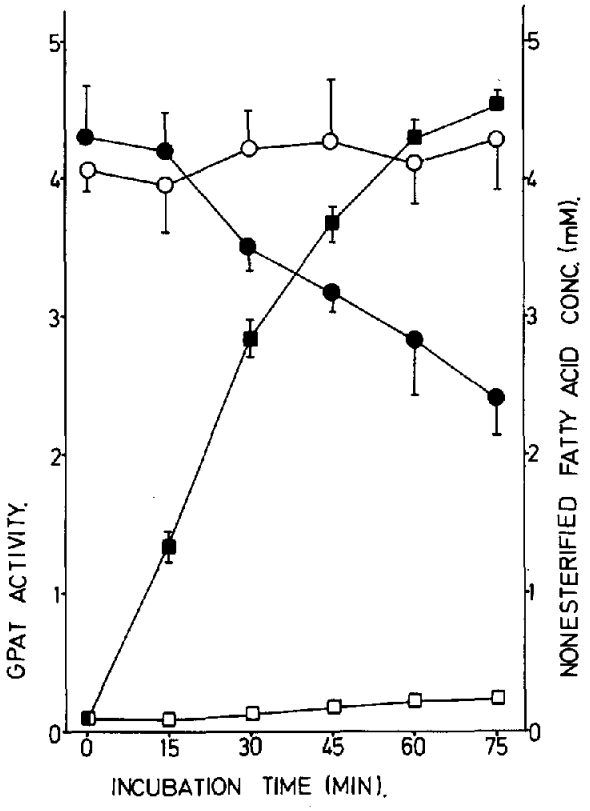

Fig.1. Time courses of adrenaline effect on adipocyte GPAT activity. Adipocytes were incubated for the indicated times in 4 ml Krebs-Ringer bicarbonate containing fatty acid-poor albumin (32.5 mg/ml). The bars represent SEM. GPAT activity: (○) without adrenaline; (●) with 0.63 μM adrenaline; Extracellular non-esterified fatty acid accumulation: (□) without adrenaline; (■) with 0.63 μM adrenaline. (a) Incubation without carbohydrate substrates. The values are means of 5 experiments. The mean adipocyte DNA was 8.4 μg/ml flask contents. (b) Incubation with 5 mM glucose. The values are means of 4 experiments. The mean adipocyte DNA was 8.2 μg/ml flask contents.

quite stable in tissue extracts kept at 4°C or incubated at 30°C for as long as 70 min. This finding should facilitate further investigation of the molecular mechanisms responsible for the adrenaline effect on GPAT.

It was found [1] that, in incubations of cells with no carbohydrate substrates, insulin alone had little effect on GPAT activity unless the enzyme was assayed with 1 mM glycerol phosphate, in which case a small stimulation by insulin was observed. Table 2 shows that insulin abolished the decrease in GPAT resulting from exposure of the cells to adrenaline. Surprisingly, adrenaline significantly increased GPAT activity in the presence of insulin although lipolysis was still significantly increased above the basal rate. The effects of insulin and adrenaline on GPAT therefore appear to interact with each other and insulin is more effective in opposing the action of adrenaline on GPAT than in altering the basal activity of the enzyme. Propranolol, 10 μM, which completely blocked the lipolytic action of adrenaline, also abolished the effect of adrenaline on GPAT implying the involvement of a β-adrenergic receptor in this action of the hormone.

Acknowledgements

We wish to thank Miss C. Carpenter for skilled technical assistance. This work was supported by grants from the Medical Research Council and the British Diabetic Association.

Table 2
Effect of adrenaline, insulin and propranolol an adipocyte GPAT activity

Additions	Extracellular unesterified fatty acid concn	GPAT activity
None	0.093 ± 0.012	3.13 ± 0.36
Insulin (20 munits/ml)	0.086 ± 0.011	2.87 ± 0.29
Adrenaline (0.63 μM)	3.994 ± 0.089[b]	2.36 ± 0.23[a]
Adrenaline (0.63 μM) + insulin (20 munits/ml)	0.430 ± 0.101[a,d]	3.49 ± 0.34[a,c]
Propranolol (10 μM)	0.102 ± 0.005	3.18 ± 0.28
Propranolol (10 μM) + adrenaline (0.63 μM)	0.098 ± 0.008	3.28 ± 0.23

[a,b] Indicate $P < 0.05$, 0.001 respectively, for adrenaline-treated cells versus appropriate controls
[c,d] Indicate $P < 0.01$, 0.001, respectively, for insulin-treated cells versus appropriate controls

Adipocytes were incubated without added carbohydrate substrates for 1 h in 4 ml of Krebs-Ringer bicarbonate containing fatty acid-poor albumin (32.5 mg/ml) and other additions as indicated. The results are the means and SEM of 5 separate experiments. The mean fat cell DNA was 7.5 μg/ml flask contents

References

[1] Sooranna, S. R. and Saggerson, E. D. (1976) FEBS Lett. 64, 36–39.
[2] Sooranna, S. R. and Saggerson, E. D. (1976) FEBS Lett. 69, 144–148.
[3] Rodbell, M. (1964) J. Biol. Chem. 239, 375–380.