Persistent activity modification of phosphatidate phosphohydrolase and fatty acyl-CoA synthetase on incubation of adipocytes with the tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate

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Incubation of rat adipocytes with the phorbol ester TPA caused a dose-dependent, persistent increase in phosphatidate phosphohydrolase activity and decrease in fatty acyl-CoA synthetase activity. Half-maximal effects were obtained with 1–2 × 10^{-10} M TPA. TPA did not alter basal or noradrenaline-stimulated lipolysis. It is suggested that these two enzymes might be modified by protein kinase C.

Adipocyte *Phorbol ester* *Phosphatidate phosphohydrolase* *Fatty acyl-CoA synthetase* *Lipolysis*

1. INTRODUCTION

12-*O*-Tetradecanoylphorbol 13-acetate (TPA) is the most potent of a series of phorbol diesters which are well known tumour promoters. Recent studies have provided evidence that a Ca^{2+}- and phospholipid-dependent protein kinase, protein kinase C, is a target for phorbol esters as the tumour promoters activate this enzyme both in vitro and in vivo [1]. TPA and other phorbol esters appear to stimulate protein kinase C by substituting for diacylglycerol which is required to activate the enzyme [2]. Structure-activity studies show good correlation between the ability of individual phorbol esters to promote tumours and to activate protein kinase C. The observed effects of TPA in a variety of cell types are extremely diverse but these include rapid effects which may relate to lipid and carbohydrate metabolism. For example, TPA causes inactivation of glycogen synthase in hepatocytes [3] and protein kinase C phosphorylates and inactivates liver and muscle glycogen synthase [4]. Furthermore, TPA increases the phosphorylation of hepatocyte cytosolic proteins whose phosphorylation is also increased by angiotensin II or vasopressin [5]. Other effects include stimulation of phospholipid synthesis [6], stimulation of 2-deoxyglucose oxidation [7], diminished elevation of cyclic AMP levels in response to glucagon [8] or β-adrenergic stimulation [9], diminution of the affinity of epidermal growth factor or insulin binding to receptors [10–12] and increased phosphorylation of these receptors [13,14].

Since protein kinase C is reported to be present in rat adipose tissue [15–17] we have investigated the effect of brief exposure of adipocytes to TPA on the activities of two enzymes in the triacylglycerol synthesis pathway. Both enzymes, Mg^{2+}-dependent phosphatidate phosphohydrolase (PPH, EC 3.1.3.4) and ATP-dependent fatty acyl-CoA synthetase (FAS, EC 6.2.1.3), may play a role in the regulation of triacylglycerol synthesis in adipose tissue and appear to exist in different activity states that are subject to rapid interconversion in response to hormones. For example, PPH in adipocytes is regulated by noradrenaline and insulin [18–20] and the hepatocyte enzyme is activated by vasopressin [21]. Likewise, FAS in

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adipose tissue is regulated by insulin, catecholamine hormones and corticotropin [21–25]. The mechanisms underlying these activity changes are unknown at present.

2. MATERIALS AND METHODS

Chemicals were obtained and treated as in [18,19,23,25]. In addition, TPA and 12-*O*-tetradecanoylphorbol 13-acetate 4-*O*-methyl ether were obtained from Sigma (London). Epididymal fat pads from fed male rats (170–190 g) were used as the source of adipocytes [26]. Adipocytes equivalent to 2/3 of a fat pad were incubated for 30 min in siliconised 25-ml flasks with shaking at 37°C under O_2 + CO_2 (95:5%). All flasks contained 4 ml Krebs-Ringer bicarbonate, albumin (40 mg/ml), 5 mM glucose, adenosine deaminase (4 munits/ml), 0.35 mM dimethyl sulphoxide (DMSO) and various additions of phorbol esters or noradrenaline. After 30 min the cells were separated from the incubation medium by centrifugation and frozen in liquid N_2 [18]. Samples of incubation media were saved for measurement of glycerol [27].

Extracts from frozen adipocytes were prepared by homogenization in 1.0 ml ice-cold 0.25 M sucrose medium containing 1 mM EDTA, 1 mM dithiothreitol, 10 mM Tris–HCl (pH 7.4) in an Ultra-Turrax homogeniser for 3 × 10-s periods with 10-s intervals. The extracts were centrifuged for 1 min in an Eppendorf 5412 centrifuge and the resulting fat-free homogenate used for enzyme assays.

Lactate dehydrogenase (EC 1.1.1.27) was assayed as in [28]. PPH was assayed as P_i release from phosphatidate as in [18]. FAS activity was assayed by following the incorporation of [^{3}H]palmitate into [^{3}H]palmitoyl-CoA [22,25]. Both PPH and FAS activities are expressed as nmol product produced/min per unit lactate dehydrogenase activity. Lactate dehydrogenase is unaffected by treatment of cells with noradrenaline or phorbol esters and therefore this form of expression corrects for any incompleteness of cell breakage and recovery during homogenization.

Statistical significance was determined by Student's *t*-test for paired samples. Throughout values are presented as means ± SE.

3. RESULTS AND DISCUSSION

Adipocytes were incubated for 30 min with a range of TPA concentrations which were added with 0.35 mM DMSO. Although this addition changed the basal activity of FAS from 32 ± 4 to 40 ± 4 (n = 6) and that of PPH from 35 ± 5 to 33 ± 5 (n = 5), these effects of the solvent were not statistically significant. Fig.1 shows that TPA brought about a dose-dependent increase in PPH activity and a decrease in FAS activity. In both cases the maximum change (+35% for PPH and −28% for FAS) was seen with 10^{-7} M TPA and half-maximal effects were observed at approx. 1–2 × 10^{-10} M TPA which is similar to, or lower than, the concentrations of TPA exerting half-maximal effects in other systems [1,3,4,8,11–13]. As a control experiment, the 4-*O*-methyl ether of TPA, which is not a tumour promoter, was tested at a concentration of 10^{-8} M in 4 separate

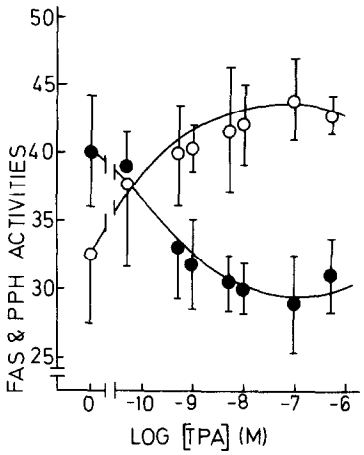

Fig.1. Effect of TPA concentration on PPH and FAS activities. Adipocytes were incubated as described in section 2 for 30 min with the indicated concentrations of TPA. The activities are means ± SE of 4–5 separate experiments in every case and are expressed as nmol/min per unit lactate dehydrogenase. For FAS all changes in activity are significant to the level of $P < 0.05$ over the TPA concentration range 5 × 10^{-10} M – 5 × 10^{-7} M. For PPH all changes in activity are significant to the level of $P < 0.025$ over the TPA concentration range 5 × 10^{-11} – 10^{-8} M. (○) PPH, (●) FAS.

adipocyte preparations and was found to be ineffective (a 2 ± 2% increase in PPH and a 1 ± 2% decrease in FAS activity were observed). Parallel incubations of cells with 10^{-6} M noradrenaline were performed. This treatment resulted in a 56 ± 8% decrease in FAS activity (n = 6) and 20 ± 8% decrease in PPH activity (n = 4) in accord with previous findings [18–20,23,25]. The decrease in FAS activity caused by noradrenaline was therefore approximately double that caused by the maximally effective dose of TPA. Incubation of cells with 10^{-6} M noradrenaline + 5×10^{-7} M TPA resulted in a 52 ± 2% decrease in FAS activity, i.e., the effects of the two agents on this activity were non-additive. Addition of 10^{-6} M noradrenaline to cells incubated with 10^{-7} M TPA resulted in a 38 ± 5% decrease in PPH activity compared with incubations with TPA alone, i.e., noradrenaline appeared to override the stimulatory effects of TPA on this activity and TPA slightly enhanced the inactivating effect of noradrenaline.

Glycerol accumulation in incubation media was measured as an index of lipolysis. Basal lipolysis resulted in 0.05 ± 0.00 mM glycerol in incubation media after 30 min. TPA ($5 \times 10^{-11} - 5 \times 10^{-7}$ M) did not increase lipolysis above the basal rate. 10^{-6} M noradrenaline raised the glycerol release to 0.84 ± 0.06 mM. Lipolysis in the presence of 10^{-6} M noradrenaline + 5×10^{-7} M TPA was not significantly different from that in the presence of noradrenaline alone. The phorbol ester therefore does not appear to modify the activity state of the hormone-sensitive lipase.

It is concluded that FAS and PPH, or uncharacterized systems regulating their activity, may be modified by the action of protein kinase C present in adipocytes. In the physiological setting protein kinase C could be stimulated by diacylglycerol arising from phosphoinositide breakdown. In the rat adipocyte this is probably increased by α_1-adrenergic stimulation [29,30] and possibly by insulin [30–32]. At present no coherent picture emerges since both FAS and PPH are decreased by noradrenaline and these effects are opposed by insulin [19,25]. However, it is noteworthy that the increase in PPH activity seen in this study with TPA is matched by a comparable increase in PPH activity when hepatocytes are incubated with vasopressin [21] – a hormone likely to activate protein kinase C in hepatocytes [5,33].

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