RAPID EFFECTS OF NORADRENALINE ON Mg^{2+}-DEPENDENT PHOSPHATIDATE PHOSPHOHYDROLASE ACTIVITY IN RAT ADIPOCYTES

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1. Introduction

The reaction catalysed by phosphatidate phosphohydrolase is a potential site of metabolic regulation since it occurs at an important branch-point in glycerolipid synthesis. There are several lines of evidence which support a role for this enzyme in the regulation of neutral lipid formation in liver and adipose tissue (reviewed [1]). Previous studies have been concerned with relatively long-term changes in this enzyme activity. In the present investigation we have attempted to detect rapid, hormone-induced changes in adipose tissue phosphatidate phosphohydrolase activity. This can be seen when rat adipocytes are briefly exposed to noradrenaline, freeze-stopped and the enzyme assayed immediately after preparation of tissue homogenates. We report here a noradrenaline dose-dependent decrease in phosphatidate phosphohydrolase activity that is both rapid and reproducible, and is essentially confined to the Mg^{2+}-dependent activity.

2. Materials and methods

Male Sprague-Dawley rats weighing 170–180 g were used throughout. Chemicals were obtained and treated as in [2]. In addition egg lecithin and phospholipase D (EC 3.1.4.4 from cabbage) were obtained from Sigma (London) Chemical Co. (Kingston-upon-Thames, Surrey). Phosphatidic acid was prepared by the action of phospholipase D on egg lecithin, followed by extraction and conversion of the product into the sodium salt [3]. On thin-layer chromatography on silica gel G plates in chloroform + methanol + water + formic acid solvent system (65: 26: 3.5: 7, by vol.) this product generally gave a single spot at a position corresponding to synthetic dipalmitoyl phosphatidic acid. Phosphatidate was dispersed in water by sonication before use as substrate in the phosphatidate phosphohydrolase assay. The amount of phosphatidate recovered was determined by hydroxamate formation [4]. Isolated adipocytes were prepared by the method in [5]. Cells from 6 epididymal fat pads were suspended in final vol. 10 ml in Krebs-Ringer bicarbonate containing fatty acid-poor albumin (40 mg/ml) and other additions indicated in figure legends. After the desired period of incubation, the contents of the flasks were transferred to siliconised homogeniser tubes, centrifuged for 10 s at approx. $130 \times g_{av}$, the resulting infranatant removed by aspiration and the tubes containing adipocytes plunged into liquid N_2. Cells were stored under liquid N_2 until extraction and enzyme assay. Samples of incubation media were saved for assay of glycerol and non-esterified fatty acids.

Extracts from frozen adipocytes were prepared by homogenisation in 1.2 ml ice-cold 0.25 M sucrose medium containing 1 mM EDTA, 1 mM dithiothreitol, 10 mM Tris–HCl buffer (pH 7.4) in an ultraturrax homogeniser for 3×10 s periods with 10 s intervals. The extracts were centrifuged for 20 s in an Eppendorf 5412 centrifuge and the resulting fat-free homogenates used directly for enzyme assays.

Lactate dehydrogenase (EC 1.1.1.27) was assayed as in [6].

Samples, 100 μl, of tissue extract were assayed for phosphatidate phosphohydrolase activity (EC 3.1.3.4) in final vol. 0.5 ml containing 100 mM Tris–maleate

buffer (pH 6.8) and 1.4 mM phosphatidate with or without the addition of 2.5 mM $MgCl_2$ [7]. The reaction was started by addition of tissue extract and the incubation carried out for 30 min at 37°C. The reaction was terminated by addition of 0.5 ml 10% (w/v) trichloroacetic acid, precipitated protein removed by centrifugation and inorganic phosphate in the supernatant estimated by the method in [8]. Appropiate blanks were conducted in parallel with all experiments. The enzyme activity is expressed as μmol inorganic phosphate released/30 min/unit lactate dehydrogenase activity. This form of expression corrects for any incomplete cell recovery after incubation and for any incompleteness of cell breakage during homogenisation.

Non-esterified fatty acids and glycerol in incubation media were assayed as in [9] and [10], respectively. Adipocyte DNA content was estimated by the method in [11].

Statistical analysis of data was performed on a paired basis and statistical significance determined by the Student's *t* test.

3. Results and discussion

Incubation of adipocytes with adrenaline for 1 h in the absence of substrates was found [2] to result in a dose-dependent decrease in glycerolphosphate acyltransferase and pyruvate dehydrogenase$_a$ activities. Using the same experimental procedure, Mg^{2+}-dependent phosphatidate phosphohydrolase was found to be considerably decreased by noradrenaline in a dose-dependent fashion (fig.1). The Mg^{2+}-independent phosphatidate phosphohydrolase was only marginally affected. The effect of noradrenaline on Mg^{2+}-dependent phosphatidate phosphohydrolase activity was clearly apparent even at 50 nM noradrenaline and the decrease in enzyme activity paralleled the stimulation of lipolysis seen either as glycerol or non-esterified fatty acid accumulation in the incubation media. Dose-dependent decreases in Mg^{2+}-dependent phosphatidate phosphohydrolase activity of a similar magnitude were also seen when adipocytes were exposed to noradrenaline for 1 h in the presence of 5 mM glucose (results not shown). In these experiments the adipocyte lactate dehydrogenase content was unchanged by noradrenaline. This suggested that

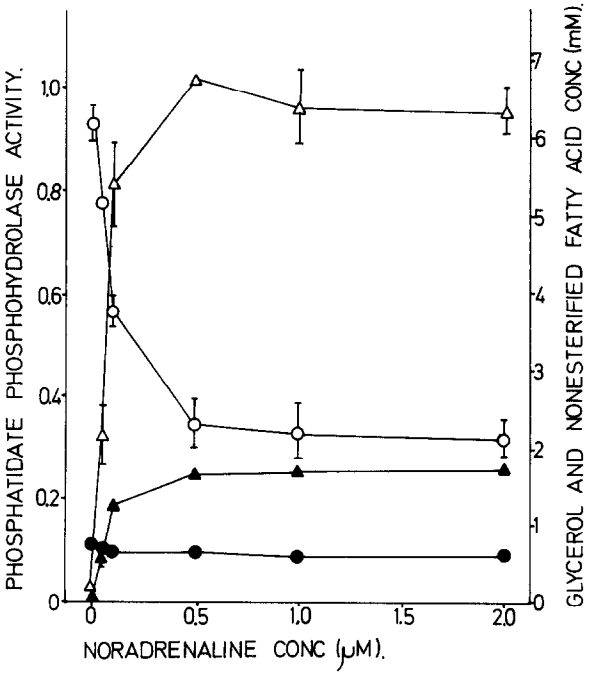

Fig.1. Effect of noradrenaline on adipocyte phosphatidate phosphohydrolase activity after 1 h. Adipocytes were incubated for 1 h without substrates as described in section 2 with the indicated concentrations of noradrenaline. (○) Mg^{2+}-Dependent phosphatidate phosphohydrolase activity. (●) Mg^{2+}-Independent phosphatidate phosphohydrolase activity. (△) Extracellular non-esterified fatty acid concentration. (▲) Extracellular glycerol concentration. The results are means of 4 separate experiments and the bars represent SEM. The mean adipocyte DNA was 11.7 μg/ml of incubation medium. Compared against the zero-noradrenaline controls, *P* values for percentage changes in Mg^{2+}-dependent phosphatidate phosphohydrolase activity at each noradrenaline concentration are: 50 nM, <0.05; 0.1 μM, <0.01; 0.5 μm, <0.01; 1 μM, <0.01; 2 μM, <0.001.

changes in enzyme leakage from the cells were unlikely to be responsible for the effects of the hormone.

Although these experiments clearly indicated an interesting effect of noradrenaline on phosphatidate phosphohydrolase activity, they may be unsatisfactory in one respect. After 1 h, non-esterified fatty acid concentrations in incubation media were considerably higher than levels encountered in vivo [12]; e.g., above 0.5 μM noradrenaline these values were approx. 6.4 mM and 5.6 mM in the absence and presence of glucose, respectively.

Further studies therefore were undertaken using

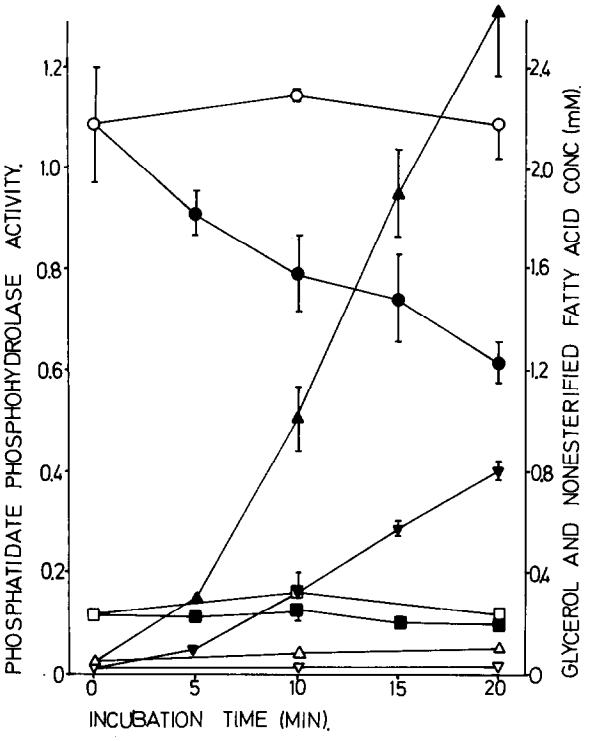

Fig.2. Time courses of noradrenaline effect on adipocyte phosphatidate phosphohydrolase activity. Adipocytes were incubated as described in section 2 with 5 mM glucose for the indicated times. The results are means of 4 separate experiments and the bars represent SEM. The mean adipocyte DNA was 8.3 μg per ml of incubation medium. open symbols, without noradrenaline; closed symbols, with 0.5 μM noradrenaline. (○, ●) Mg^{2+}-Dependent phosphatidate phosphohydrolase activity. (□, ■) Mg^{2+}-Independent phosphatidate phosphohydrolase activity. (△, ▲) Extracellular non-esterified fatty acid concentration. (▽, ▼) Extracellular glycerol concentration. In the presence of noradrenaline, *P* values for percentage changes in Mg^{2+}-dependent phosphatidate phosphohydrolase activity versus zero-time are: 5 min, <0.1; 10 min, <0.01; 15 min, <0.01; 20 min, <0.001.

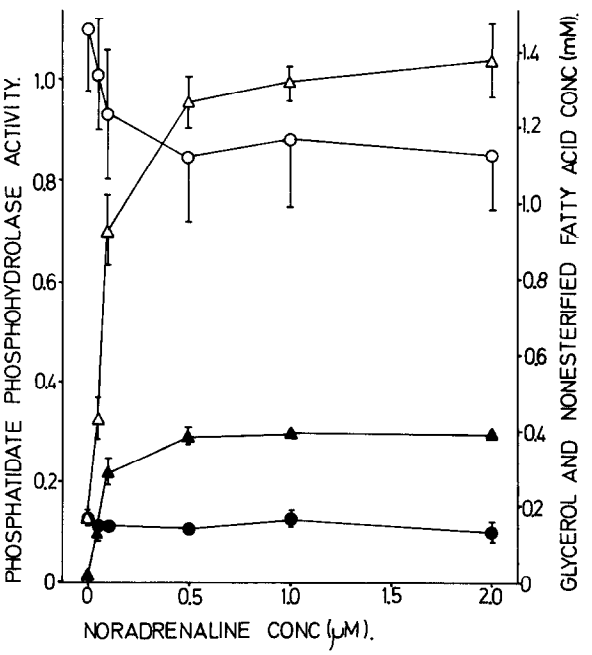

Fig.3. Effect of noradrenaline on adipocyte phosphatidate phosphohydrolase activity after 10 min. Adipocytes were incubated for 10 min as described in section 2 with 5 mM glucose. The results are means of 4 separate experiments and the bars represent SEM. The mean adipocyte DNA was 10.3 μg/ml incubation medium. (○) Mg^{2+}-Dependent phosphatidate phosphohydrolase activity. (●) Mg^{2+}-Independent phosphatidate phosphohydrolase activity. (△) Extracellular non-esterified fatty acid concentration. (▲) Extracellular glycerol concentration. Compared against the zero-noradrenaline controls, *P* values for percentages changes in Mg^{2+}-dependent phosphatidate phosphohydrolase activity at each noradrenaline concentration are: 50 nM, <0.01; 0.1 μM, <0.05; 0.5 μM, <0.01; 1 μM, <0.01; 2 μM, <0.01.

shorter incubation times with glucose present throughout. In fig.2 it may be seen that a decrease in Mg^{2+}-dependent phosphatidate phosphohydrolase activity was apparent with 0.5 μM noradrenaline as early as 5 min after the start of incubation. Enzyme activity continued to decrease over the 20 min period of these experiments. During this time lipolysis proceeded linearly and non-esterified fatty acid accumulation was not excessive (reaching 2.6 mM at 20 min with 0.5 μM noradrenaline). Figure 3 shows the dose dependence of the effect of noradrenaline on lipolysis and phosphatidate phosphohydrolase activity after only 10 min of exposure of the cells to the hormone in the presence of glucose. As at 1 h, maximum effects were obtained with 0.5–2.0 μM noradrenaline. Non-esterified fatty acid accumulation was within the physiological range encountered in rat plasma and the maximum decrease in Mg^{2+}-dependent phosphatidate phosphohydrolase activity was 24 ± 3%.

We conclude therefore that adipocyte Mg^{2+}-dependent phosphatidate phosphohydrolase activity can be rapidly decreased by quite low noradrenaline concentrations under incubation conditions that are physi-

ological with respect to glucose concentration and the range of extracellular fatty acid accumulation. It is as yet unknown whether the observed effects are secondary to stimulation of lipolysis, thereby implying inhibition of the enzyme by some lipolysis product, or involve a more direct effect of the hormone (through its second messenger(s)). This is the subject of further investigations. Whatever molecular mechanisms are involved, they are persistent enough to survive after freeze-stopping and homogenisation of the tissue.

It is suggested that the phenomenon reported here is relevant to the regulation of adipose tissue neutral lipid synthesis.

Acknowledgements

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