RAPID ANTAGONISTIC ACTIONS OF NORADRENALINE AND INSULIN ON RAT ADIPOCYTE PHOSPHATIDATE PHOSPHOHYDROLASE ACTIVITY

Christopher H. K. CHENG and E. David SAGGERSON
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, England

Received 30 June 1978

1. Introduction

Mg^{2+}-dependent phosphatidate phosphohydrolase activity in rat adipocytes is rapidly decreased by exposure of the cells to noradrenaline in vitro [1]. It is unknown whether this effect relates to longer-term changes in this activity which have suggested a regulatory role for this enzyme [2,3]. In this study both insulin and propranolol (a β-adrenergic blocking agent) have been found to inhibit the action of noradrenaline upon adipocyte phosphatidate phosphohydrolase activity. Furthermore, insulin was observed to rapidly restore phosphatidate phosphohydrolase activity after it had been decreased by noradrenaline.

2. Materials and methods

Male Sprague-Dawley rats, 170–180 g, were used throughout. Chemicals were obtained and treated as in [1]. In addition, bovine insulin (6 × recrystallized) was a gift from Boots Pure Drug Co. (Nottingham). Noradrenaline and propranolol were obtained from Sigma (London) Chemical Co. (Kingston-upon-Thames, Surrey). Sodium palmitate was obtained from Nu-Chek Prep. (Elysian, MI). Palmitate was bound to fatty acid-poor albumin as in [4].

Isolated adipocytes were prepared by the Rodbell method in [5]. Cells from 6 epididymal fat pads were suspended in final vol. 10 ml in Krebs-Ringer bicarbonate containing 10 mg albumin/ml. From this stock cell suspension 1 ml portions were then dispensed into siliconised 25 ml flasks and incubated with shaking at 37°C in total vol. 4 ml Krebs-Ringer bicarbonate containing albumin (40 mg/ml), unless otherwise stated, under $O_2 + CO_2$ (95 : 5%).

Other additions are indicated in the table and figure legends. After the desired incubation period, the cells were separated from the incubation medium and frozen in liquid N_2 [1]. Cells were stored under liquid N_2 until extraction. Samples of incubation media were saved for measurement of glycerol and nonesterified fatty acids.

Extracts from frozen adipocytes were prepared by homogenization in 0.8 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 at 10 s intervals. The extracts were centrifuged for 1 min in an Eppendorf 5412 centrifuge and the resulting fat-free homogenate used for enzyme assay.

Samples of tissue extract, 50 μl, were assayed for phosphatidate phosphohydrolase (EC 3.1.3.4) activity in final vol. 0.5 ml containing 100 mM Tris–maleate (pH 6.8), 2.5 mM $MgSO_4$, 1.4 mM phosphatidate, 1 mM dithiothreitol, 0.1 mM EGTA. The reaction was started by addition of tissue extract and incubated for 10 min at 37°C. The reaction was stopped by adding 0.5 ml 10% (w/v) trichloroacetic acid, precipitated protein removed by centrifugation and inorganic phosphate in the supernatant determined as in [6]. The blank in each assay was prepared by addition of trichloroacetic acid before adding the tissue extract. The enzyme activity is expressed as nmol inorganic phosphate released/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.

Measurements of lactate dehydrogenase activity,

 Elsevier/North-Holland Biomedical Press

nonesterified fatty acids, glycerol and DNA were as in [7], [8], [9] and [10], respectively.

3. Results and discussion

Incubation of rat adipocytes with noradrenaline has been found to cause a rapid, dose-dependent and time-dependent decrease in Mg^{2+}-dependent phosphatidate phosphohydrolase activity [1]. The experiment shown in table 1 demonstrates the ability of insulin and propranolol to abolish the decrease in phosphatidate phosphohydrolase activity produced by noradrenaline. In this experiment the antagonists insulin and propranolol were added at the start of the 20 min incubation. Both antagonists at the tested concentrations had no effect upon basal enzyme activity. The blocking action of propranolol suggested the involvement of a β-type adrenergic receptor in the action of noradrenaline upon the enzyme activity.

Figure 1 shows a time course of the restoration of phosphatidate phosphohydrolase activity by insulin after 10 min pre-incubation of cells with 0.5 μM noradrenaline in the presence of 5 mM glucose. After addition of insulin, not only was the noradrenaline-dependent decrease in enzyme activity arrested, but a progressive increase in activity was seen leading to the re-establishment of the zero-time activity within 20 min. In the 10–30 min time interval insulin increased the enzyme activity by 8.1 ± 0.9 arbitrary units. It may also be seen that insulin promptly reduced lipolysis, as measured by glycerol release, and eventually promoted some removal of non-esterified fatty acids from the incubation medium (presumably by re-esterification).

Figure 2 shows a similar experiment except for the absence of glucose from the incubations. Insulin again significantly restored the activity, although the rate of restoration was lower than seen in the presence of glucose. In this case insulin increased the enzyme activity by 5.3 ± 1.3 arbitrary units in the 10–30 min

Table 1
Inhibition of noradrenaline action upon phosphatidate phosphohydrolase activity by insulin and propranolol

Additions to incubation medium	Phosphatidate phosphohydrolase activity	Glycerol accumulation in incubation media (mM)
None	45.3 ± 2.4	0.02 ± 0.00
Insulin (200 μunits/ml)	46.4 ± 2.1	0.02 ± 0.00
Noradrenaline (0.5 μM)	26.8 ± 3.1[a]	0.76 ± 0.02[a]
Noradrenaline (0.5 μM) + insulin (200 μunits/ml)	46.8 ± 1.9[b]	0.07 ± 0.00[a,b]
Propranolol (2.5 μM)	45.8 ± 2.7	0.02 ± 0.00
Propranolol (2.5 μM) + noradrenaline (0.5 μM)	41.8 ± 1.9	0.18 ± 0.01[a]

[a] Indicates $P < 0.001$ for comparison of noradrenaline-treated samples versus the appropriate controls
[b] Indicates $P < 0.001$ for effects of insulin versus noradrenaline-treated controls

Adipocytes were incubated for 20 min as described in section 2 with 5 mM glucose and the indicated additions. The results are the mean ± SEM of 4 separate experiments. The mean adipocyte DNA was 7.47 μg/ml flask contents

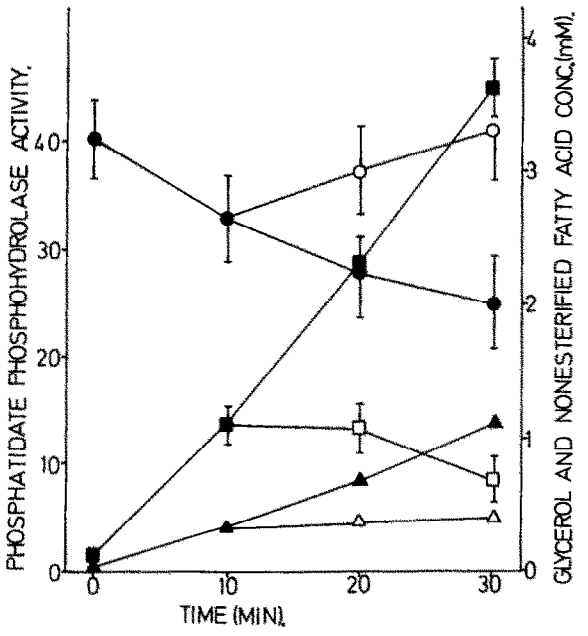

Fig.1. Effect of insulin on phosphatidate phosphohydrolase activity previously decreased by noradrenaline in the presence of glucose. Adipocytes were incubated as described in section 2 with 5 mM glucose and 0.5 μM noradrenaline (closed symbols). After 10 min insulin (200 μunits/ml) was added (open symbols). The results are means of 4 separate experiments. The bars indicate SEM. The mean fat cell DNA was 8.16 μg/ml flask contents. (●, ○) Phosphatidate phosphohydrolase activity. Versus zero-time value: $P < 0.05$ for 20 min value + insulin; $P > 0.5$ for 30 min value + insulin. Versus 10 min value: $P < 0.02$ for 20 min value + insulin; $P < 0.01$ for 30 min value + insulin. (■, □) Nonesterified fatty acids in incubation media. (▲, △) Glycerol in incubation media.

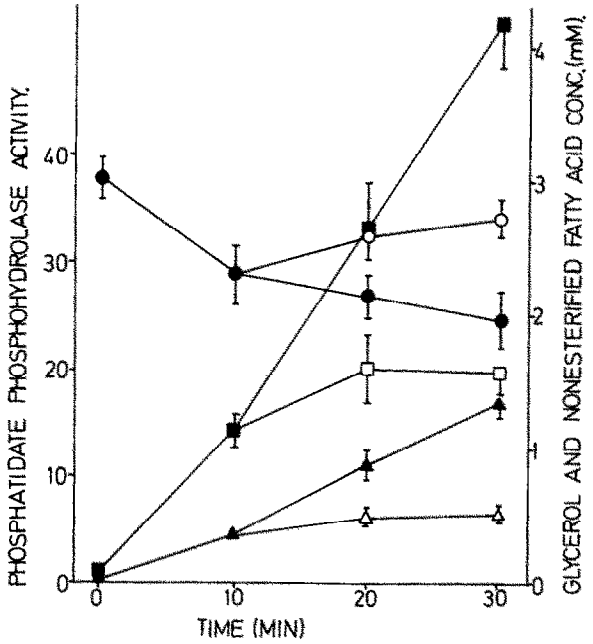

Fig.2. Effect of insulin on phosphatidate phosphohydrolase activity previously decreased by noradrenaline in the absence of glucose. Procedure and symbols as for fig.1 except that glucose was omitted from incubations. The results are means ± SEM of 4 separate experiments. The mean fat cell DNA was 7.88 μg/ml flask contents. For phosphatidate phosphohydrolase activity. Versus zero-time value: $P < 0.02$ for 20 min and 30 min values + insulin. Versus 10 min value: $P < 0.05$ for 20 min and 30 min values + insulin.

time interval. Also, although addition of insulin again diminished lipolysis, the profile of nonesterified fatty acid accumulation differed from the experiment shown in fig.1. The possibility was considered that glucose might facilitate the action of insulin on the enzyme by promoting fatty acid removal. Accordingly, the effect of fatty acid accumulation upon phosphatidate phosphohydrolase activity was investigated.

Table 2 shows that phosphatidate phosphohydrolase activity was decreased slightly after incubation of adipocytes with palmitate for 30 min. This was chosen as a representative long chain fatty acid to mimic the accumulation of these metabolites after noradrenaline treatment. As for the action of noradrenaline upon the enzyme activity, the mechanism responsible for this decrease is unknown. Incubation for 30 min with 4 mM fatty acid only decreased phosphatidate phosphohydrolase activity by ~ 20% whereas incubation with 0.5 μM noradrenaline for the same period resulted in ~ 40% decrease in activity and a final accumulation of ~ 4 mM nonesterified fatty acid (fig.1). Incubation for 10 min with noradrenaline resulted in ~ 20% decrease in enzyme activity and an accumulation of ~ 1 mM fatty acids (fig.1). Palmitate, 1 mM, on the other hand was ineffective in this respect (table 2). It is concluded that fatty acid accumulation might contribute in part to the inactivation of phosphatidate phosphohydrolase seen with noradrenaline. However, much of the hormone action cannot be considered as being secondary to fatty acid accumulation.

Table 2
Effect of incubation with palmitate on adipocyte phosphatidate phosphohydrolase activity

Initial concentration of sodium palmitate in incubation media (mM)	Phosphatidase phosphohydrolase activity	% Decrease in phosphatidate phosphohydrolase activity
0	40.8 ± 2.0	–
0.5	40.7 ± 1.2	0.1 ± 2.5
1.0	39.5 ± 1.8	3.2 ± 2.7
2.0	35.8 ± 1.6[a]	12.2 ± 0.7[b]
3.0	34.7 ± 1.3[a]	14.9 ± 1.2[a]
4.0	32.7 ± 1.4[a]	19.8 ± 1.9[a]

[a,b] Indicate $P < 0.01$, < 0.001, respectively, versus the control without palmitate

Adipocytes were incubated for 30 min as described in section 2 with 5 mM glucose, albumin (45.4 mg/ml) and the indicated concentrations of sodium palmitate. The results are mean ± SEM of 4 separate experiments. The mean adipocyte DNA was 6.45 μg/ml flask contents

Figure 3 shows that addition of propranolol also rapidly arrested the decrease in phosphatidate phosphohydrolase activity due to noradrenaline. The inhibition of lipolysis by propranolol was as great as that seen with insulin (fig.1). Although there was some restoration of phosphatidate phosphohydrolase activity after addition of propranolol (fig.3), this was smaller than that seen after insulin addition. The enzyme activity was only increased by 2.5 ± 0.6 arbitrary units in the 10–30 min time interval. It is likely therefore that at least part of the action of insulin on the enzyme activity is separate from its antilipolytic action.

In conclusion, the rapid effect of noradrenaline on phosphatidate phosphohydrolase activity [1] is reversible by insulin treatment. In the main, the actions of noradrenaline or insulin do not appear to be attributable to their ability to increase or decrease respectively the accumulation of lipolysis products.

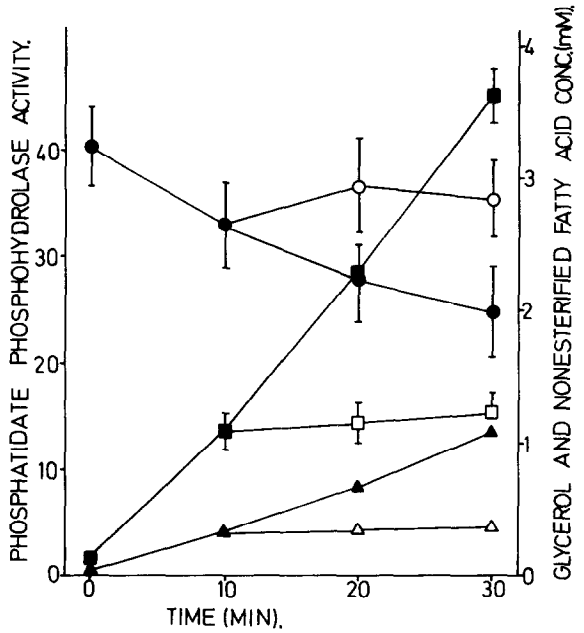

Fig.3. Effect of propranolol on phosphatidate phosphohydrolase activity previously decreased by noradrenaline in the presence of glucose. Adipocytes were incubated as described in section 2 with 5 mM glucose and 0.5 μM noradrenaline (closed symbols). After 10 min propranolol (2.5 μM) was added (open symbols). The results are means ± SEM of 4 separate experiments. The mean fat cell DNA was 8.16 μg/ml flask contents. (●, ○) Phosphatidate phosphohydrolase activity. Versus zero-time value: $P < 0.05$ for 20 min value + propranolol; $P < 0.01$ for 30 min value + propranolol. Versus 10 min value: $P < 0.01$ for 20 min value + propranolol; $P < 0.05$ for 30 min value + propranolol. (■, □) Nonesterified fatty acids in incubation media. (▲, △) Glycerol in incubation media.

Acknowledgements

We wish to thank Miss C. Carpenter for skilled technical assistance. This work was supported by a Medical Research Council project grant to E.D.S. C.H.K.C. wishes to thank the Shell Company of Hong Kong Ltd., and the Chinese University of Hong Kong for sponsorship and financial support.

References

[1] Cheng, C. H. K. and Saggerson, E. D. (1978) FEBS Lett. 87, 65–68.
[2] Fallon, H. J., Lamb, R. G. and Jamdar, S. C. (1977) Biochem. Soc. Trans. 5, 37–40.
[3] Brindley, D. N. (1978) in: Proc. 11th FEBS Meet., Copenhagen, vol. 46, symp. A5 pp. 31–40, Pergamon Press, Oxford, New York.
[4] Evans, H. H. and Mueller, P. S. (1963) J. Lipid Res. 4, 34–45.
[5] Rodbell, M. (1963) J. Biol. Chem. 239, 375–380.
[6] Ames, B. N. and Dubin, D. T. (1960) J. Biol. Chem. 235, 769–775.
[7] Saggerson, E. D. (1974) Biochem. J. 140, 211–224.
[8] Itaya, K. and Ui, M. (1965) J. Lipid Res. 6, 16–20.
[9] Garland, P. B. and Randle, P. J. (1962) Nature 196, 987–988.
[10] Burton, K. (1956) Biochem. J. 62, 315–323.