STIMULATION OF THE ACTIVITIES OF PHOSPHATIDATE PHOSPHOHYDROLASE AND TYROSINE AMINOTRANSFERASE IN RAT HEPATOCYTES BY GLUCOCORTICOIDS

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Received 18 August 1981

1. Introduction

Glucocorticoids stimulate the synthesis of triacylglycerols and the secretion of very low density lipoproteins by the liver [1–3]. The effect involves an increased activity of phosphatidate phosphohydrolase (EC 3.1.3.4) and this enzyme appears to facilitate an increased rate of triacylglycerol synthesis in a variety of physiological conditions [3–6]. Direct evidence for the involvement of cortisol in stimulating the phosphohydrolase activity has been demonstrated with isolated perfused liver [4], but not with isolated hepatocytes. This work involves the latter system which can be more readily employed to investigate how a variety of hormones interact to control hepatic glycerolipid synthesis. The soluble phosphatidate phosphohydrolase activity was stimulated by 10^{-9}–10^{-6} M dexamethasone and by 10^{-6}–10^{-5} M corticosterone, and increases of ~2-fold were observed after 4–7 h incubation. The dexamethasone stimulation was abolished by cycloheximide, or actinomycin D. The increases were of a similar magnitude to those observed with tyrosine aminotransferase (EC 2.6.1.5), whose rate of synthesis is known to depend on the availability of glucocorticoids.

2. Materials and methods

2.1. *Materials*

Collagenase (class I) was obtained from Millipore Corp., minimum essential medium with Earle's salts and foetal calf serum from Gibco: Bio-Cult Ltd, Sagital (sodium pentabarbitone) from May and Baker Ltd, and actinomycin D (Lysovac) from Merck, Sharp and Dohme Intl. Radiochemicals were obtained from The Radiochemical Centre, Amersham and general biochemicals and antibiotics were from Sigma Chemical Co. (London) Ltd.

2.2. *Preparation and incubation of hepatocytes*

Hepatocytes were prepared between 10:00–11:00 h from male Wistar rats fed ad libitum and kept in a room lit from 08:00–20:00 h. They were anaethetized with an intraperitoneal injection of 60 mg sodium pentabarbitone/kg body wt and hepatocytes were prepared essentially as in [7]. An initial non-cyclic perfusion was performed with calcium-free Krebs-Henseleit buffer [8] containing 5 mM glucose, 20 mM Hepes (pH 7.4) and equilibrated with O_2/CO_2 (95:5, v/v). This was followed by a cyclic perfusion with the same buffer, except that 1.4 mM $CaCl_2$ and 70 mg collagenase/100 ml was added. The liver was dispersed after 15–20 min by gentle shearing with scissors in the collagenase buffer and the cells were agitated in a Gallenkamp Orbital Shaker (70 rev./min) for 8 min.

The mixture was filtered through a course plastic strainer and then through 142 μm and 62 μm stainless steel meshes. The resulting cells were washed 3 times by centrifuging at 50 × *g* for 2 min and resuspended in Eagle's minimum essential medium containing Earle's salts and 20 mM Hepes. Samples (500 μl) were used for each incubation. All of the steps above were performed with buffers equilibrated with O_2/CO_2 (95:5, v/v) at 37°C.

Incubations were performed at 37°C in siliconized 25 ml conical flasks in which an atmosphere of O_2/CO_2 (95:5, v/v) was maintained by a rubber Subaseal. The gas was replaced at 2 h intervals. Each flask contained 4 ml Eagle's minimum essential medium including Earle's salts, 20 mM Hepes buffer (pH 7.4), 480 μg streptomycin, 800 U penicillin, 10% charcoal-

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.75

treated [9] foetal calf serum and hepatocytes equivalent to ~11 mg dry wt. Steroid hormones were dissolved in methanol and the final concentrations were achieved by adding 5 μl of this solution/flask. Control incubations received 5 μl methanol and this addition did not alter the observed enzyme activities.

2.3. *Determination of enzyme activities and of ATP concentrations*

Each incubation was centrifuged at 50 × *g* for 4 min. The resulting cell pellet was sonicated (22 kHz, 5 s) in 1 ml 0.25 M sucrose (pH 7.4) at 4°C containing 0.5 mM dithiothreitol; then frozen in liquid N_2 and stored at −20°C. Aliquots used for the determination of tyrosine aminotransferase activity contained 0.2 mM pyridoxal phosphate.

The methods used to determine the activities of tyrosine aminotransferase [10] and phosphatidate phosphohydrolase [11] were as described except that for phosphatidate phosphohydrolase determination, the specific radioactivity of [^{3}H]phosphatidate was increased to 0.9 Ci/mol. The ATP concentrations of hepatocytes was measured as in [12], and this was used as an index of the metabolic viability of the cells [13].

3. Results and discussion

Dexamethasone (10^{-9}–10^{-6} M) and corticosterone (10^{-6}–10^{-5} M) stimulated the activity of phosphatidate phosphohydrolase by ~2-fold (fig.1, table 1) and these stimulations were seen after ~4 h incubation (fig.2). The greater effect of dexamethasone at lower concentrations agrees with other work concerning the potency of glucocorticoids [9,14]. The magnitude of the increases for the phosphohydrolase were of the

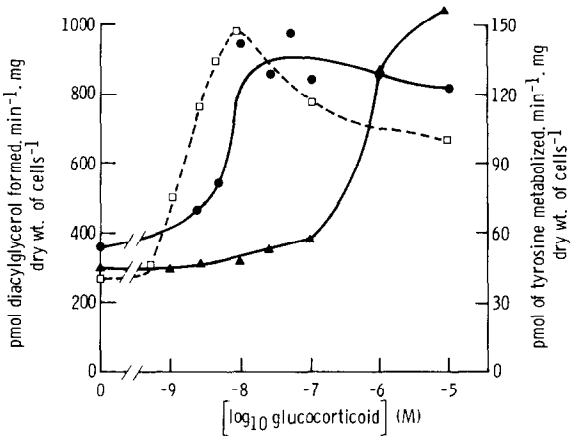

Fig.1. Effect of glucocorticoid concentration on the activities of phosphatidate phosphohydrolase and tyrosine aminotransferase in hepatocytes. The results from a representative experiment show the effects of varying the concentration of dexamethasone on the activities of phosphatidate phosphohydrolase (●) and tyrosine aminotransferase (□) and the effects of corticosterone on phosphatidate phosphohydrolase activity (▲).

Table 1
Effect of glucocorticoids on the activities of phosphatidate phosphohydrolase and tyrosine aminotransferase in rat liver hepatocytes (pmol substrate converted . min^{-1} . mg dry wt cells^{-1})

	Additions	Incubation time	Phosphatidate phosphohydrolase	Tyrosine aminotransferase
I	None	0 h	386 ± 119 (8) I *vs* III[a]	60 ± 23 (5)
II	None	6 h	364 ± 101 (10) II *vs* III[a]	39 ± 10 (5) II *vs* III[a]
III	Dexamethasone (10^{-7} M)	6 h	779 ± 171 (10) III *vs* IV[a]	120 ± 32 (5) I *vs* III[b]
IV	Dexamethasone (10^{-7} M) + actinomycin D (1 μg/ml)	6 h	302 ± 102 (3) III *vs* IV[a]	n.m.
V	Actinomycin D (1 μg/ml)	6 h	308 ± 136 (3)	n.m.
VI	Dexamethasone (10^{-7} M) + cycloheximide (5 μg/ml)	6 h	156 ± 9 (3) III *vs* VI[a]	n.m.
VII	Cycloheximide (5 μg/ml)	6 h	155 ± 6 (3)	n.m.
VIII	Corticosterone (10^{-5} M)	6 h	628 ± 213 (10) II *vs* VIII[c]	n.m.

Results are means ± SD (*n*); n.m., not measured. The significance of differences between groups was determined with an unpaired *t*-test and is indicated as follows: [a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.005$

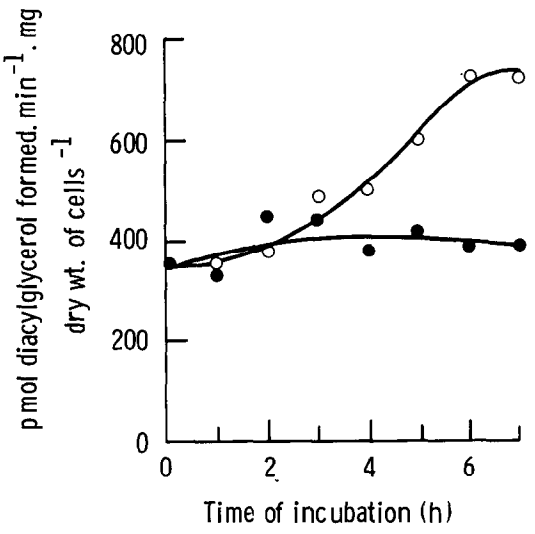

Fig.2. Time course of the stimulation of phosphatidate phosphohydrolase activity by dexamethasone. The activities in control hepatocytes (○) and those treated with 10^{-7} M dexamethasone (●) are shown from a representative experiment.

same order as for tyrosine aminotransferase (fig.1, table 1), whose synthesis is known to be induced by glucocorticoids [9,15]. Here, there was no significant difference in the concentrations of dexamethasone needed to stimulate these two activities. The increases in phosphatidate phosphohydrolase compare reasonably well with the 2.74-fold stimulation obtained in [4] by perfusing isolated livers with cortisol. In vivo, the phosphohydrolase activity has been observed to increase by 2–7-fold when the circulating levels of corticosterone increase from ~0.1 μM to 1–3 μM [16–19].

In other experiments we replaced the foetal calf serum in the medium used for hepatocyte incubations by 20 mg fatty acid-poor bovine serum albumin/ml. This caused an ~50% decrease in the phosphohydrolase activity during 6 h incubation, and dexamethasone failed to increase this low activity. In the presence of foetal calf serum the phosphohydrolase activity was maintained during the incubation, and glucocorticoid-induced stimulations were reproducibly seen (table 1). These differences may be related to the metabolic viability of the cells, since the initial ATP level of ~8 nmol/mg dry wt of cells fell by ~70% during the 6 h incubation with albumin. By contrast, only ~15% of the ATP was lost when foetal calf serum was used.

The glucocorticoids probably increased the phosphohydrolase activity by stimulating its synthesis, since both cycloheximide and actinomycin D block the increase (table 1). Actinomycin D also prevented the increases in activity that were observed in vitro after perfusing livers with cortisol [4], and those seen in vivo after subtotal hepatectomy [20].

This work characterises the conditions under which dexamethasone and corticosterone can increase the activity of phosphatidate phosphohydrolase in isolated hepatocytes. This system is suitable for determining how other hormones interact with glucocorticoids to control hepatic glycerolipid synthesis. The results also demonstrate that the glucocorticoid-induced increases in phosphohydrolase activity are similar to those for tyrosine aminotransferase. These findings indicate that the increased potential to synthesise triacylglycerols is probably co-ordinated with an increased capacity for gluconeogenesis. This may be particularly important in stress conditions in which the liver converts excess fatty acids to ketones and triacylglycerols which can then be exported to skeletal and cardiac muscles as a source of energy [6]. In a similar manner, the brain is being supplied with glucose and ketones by the liver.

Acknowledgements

The work was supported by a project grant from MRC; R. J. J. received a CASE award from SRC and Beecham Pharmaceuticals Ltd.

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