Antagonistic effects of insulin on the corticosterone-induced increase of phosphatidate phosphohydrolase activity in isolated rat hepatocytes

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Corticosterone *Insulin* *L-α-Phosphatidate phosphohydrolase* *Phospholipids* *Triglycerides*

1. INTRODUCTION

Insulin and glucocorticoids probably both control hepatic triacylglycerol synthesis (but in different ways). Insulin is known to stimulate the synthesis of fatty acids de novo and their subsequent conversion to tiracylglycerols in the liver. This increase in esterification would also be favoured by the increase in the supply of glycerol 3-phosphate and dihydroxyacetone phosphate, and by an increase in the relative concentration of malonyl-CoA, an inhibitor of pamitoyl carnitine acyltransferase (EC 2.3.1.21). The latter event would redirect acyl-CoA esters to the various acyltransferases of glycerolipid synthesis, and hence promote esterification [1]. Furthermore, insulin may enhance esterification by increasing the mitochondrial glycerol 3-phosphate acyltransferase (EC 2.3.1.15) activity. However, the glycerol phosphate acyltransferase of the endoplasmic reticulum, which is situated at the major site of hepatic triacylglycerol synthesis, is unaltered [2,3].

High concentrations of circulating glucocorticoids also stimulate hepatic triacylglycerol synthesis in some situations in which fatty acids are being released by adipose tissue [4]. The major effect [5] of glucocorticoids on the enzymes of hepatic triacylglycerol synthesis is the increase in the activity of phosphatidate phosphohydrolase (EC 3.1.3.4). This stimulation can be directly demonstrated with isolated perfused livers [6], or with hepatocytes [7]. The increased capacity of phosphatidate phosphohydrolase facilitates the production of triacylglycerol, especially when the supply of substrates is high and the production of phosphatidate is not rate-limiting [4].

Since both insulin and corticosterone stimulate hepatic triacylglycerol synthesis independently we investigated whether these hormones interact in controlling the activity of phosphatidate phosphohydrolase in isolated hepatocytes. Alone, insulin produced a very small decrease (8%) in phosphatidate phosphohydrolase activity. However, in the presence of corticosterone, the stimulation of phosphatidate phosphohydrolase activity was progressively inhibited by increasing concentrations of insulin. The inhibition was observed only if insulin was included at the beginning of the incubation; no effect was observed if the insulin was added 330 min after exposure to corticosterone.

2. MATERIALS AND METHODS

2.1. *Materials*

Most of the materials employed have been described in [7]. The bovine insulin was a gift specially prepared and purified by the Boots Co. (Nottingham). The potency of the insulin was 25–26 U/mg. Glucagon, vasoactive intestinal peptide and somatostatin were not detected in the sample whilst the pro-insulin content was < 0.5%.

2.2. *Preparation and incubation of hepatocytes and determination of enzyme activities*

The methods for preparing and incubating hepatocytes, and for the determination of phosphatidate phosphohydrolase activity have been described

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[7]. The latter activity was expressed relative to that of lactate dehydrogenase which was measured as in [8]. This was done to compensate for cell breakage.

There was no consistent effect of insulin, or corticosterone on the recovery of lactate dehydrogenase in the hepatocytes after incubation, and cell breakage was normally < 5%. The metabolic viability of the cells was established by their high ATP contents and by the ability of glucocorticoids to stimulate the synthesis of phosphatidate phosphohydrolase [7].

3. RESULTS AND DISCUSSION

As expected from [7], the incubation of hepatocytes with corticosterone for 6 h increased the activity of phosphatidate phosphohydrolase and maximum stimulations were obtained over 10^{-6}–10^{-5} M (fig.1). Corticosterone levels that are encountered in vivo in these rats are 10^{-7}–3×10^{-6} M [5,9,10]. The addition of 20 mU insulin /ml to the incubations decreases the stimulating effects of corticosterone at 10^{-7}–10^{-4} M (fig.1; table 1). The effect of 10^{-5} M corticosterone was abolished when insulin was increased to 10–100 mU/ml (fig.2). If insulin was added to the incubations at 330 min, rather than at the beginning, it did not significantly modify the ability of corticosterone to increase the phosphohydrolase activity (table 1). Thus insulin probably inhibits the known effects of glucocorticoids in stimulating the synthesis of phosphatidate phosphohydrolase [6,7]. When insulin alone was added to the incubations there was a very small decrease in the activity of phosphatidate phosphohydrolase which was statistically significant (fig.1, table 1). This may result from the inhibition of phsophohydrolase synthesis which is stimulated by endogenous corticosterone in the hepatocytes.

Initially the insulin antagonism of the corticosterone-induced stimulation of phosphatidate phosphohydrolase activity seems incongruous because both hormones are involved in stimulating triacylglycerol synthesis in the liver. However, several examples of this particular antagonism have been found in vivo.

(1) The raised concentrations of circulating corticosterone associated with the acute feeding of

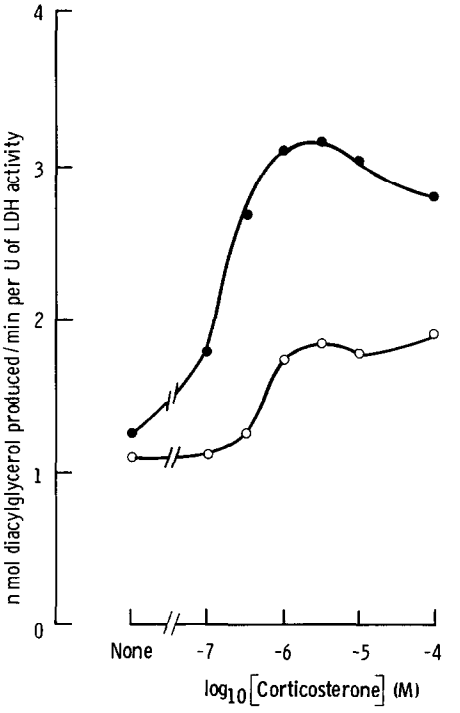

Fig.1. Effect of corticosterone on the activity of phosphatidate phosphohydrolase in hepatocytes incubated for 6 h in the presence of 20 mU insulin/ml (○), or in its absence (●).

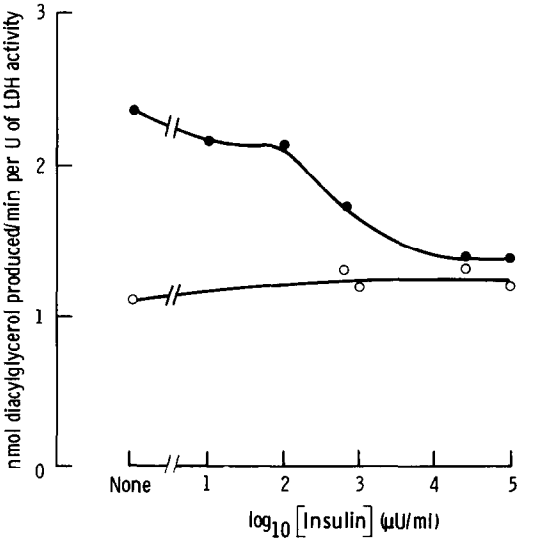

Fig.2. Effect of insulin on the activity of phosphatidate phosphohydrolase in hepatocytes incubated for 6 h in the presence of 10^{-5} M corticosterone (●), or in its absence (○).

Table 1

Effects of insulin and corticosterone on the activity of phosphatidate phosphohydrolase in isolated rat hepatocytes

Additions	Incubation time (min)	Relative activity (%) of phosphatidate phosphohydrolase	Sig. diff.[a]
I None	360	100 (13)	
II Corticosterone (10^{-5} M)	360	184 ± 26 (13)	I *vs* II, $P < 0.001$
III Insulin (20 mU/ml)	360	92 ± 9 (11)	I *vs* III, $P < 0.05$
IV Corticosterone (10^{-5} M) + insulin (20 mU/ml)	360	121 ± 23 (11)	I *vs* IV, $P < 0.02$ II *vs* IV, $P < 0.001$
V Corticosterone (10^{-5} M) + insulin (20 mU/ml after 330 min)	360	198 ± 64 (3)	II *vs* V, n.s.

[a] Calculated by using a paired *t*-test

Results are shown as means ± SD (no. independent expt) and are expressed relative to the activity when no hormone was added. The value of this control was 1.39 ± 0.48 nmol diacylglycerol produced . min^{-1} . U lactate dehydrogenase $^{-1}$ for the 13 independent expt.

fructose, sorbitol, glycerol or ethanol [9,10] produced an increase in hepatic phosphatidate phosphohydrolase activity [10,11]. However, the acute feeding of glucose to rats maintained on a standard laboratory diet raised insulin concentrations in concert with the increased corticosterone concentrations [9], and it did not affect the activity of phosphatidate phosphohydrolase in the liver [11]. Rats that were fed on semi-purified diets which were rich in starch or lard did show an increase in the phosphohydrolase activity after glucose feeding [10]. Their corticosterone responses were much more pronounced, but their insulin responses were similar to the rats fed on the standard diet [10].

(2) The circulating concentrations of corticosterone in rats normally reach a peak 2 h before the beginning of the dark period of the daylight cycle [12]. At this time the insulin concentrations are relatively low. These conditions favour the synthesis of the phosphohydrolase which reaches a peak activity 2 h after dark [12]. This is the time when the rats are feeding so that insulin concentrations rise, whereas those of corticosterone fall. There should be no immediate effect on the phosphohydrolase activity (table 1), but its rate of synthesis should decrease and the activity should subsequently decline [12].

(3) High phosphatidate phosphohydrolase activities in the liver are characteristic of a variety of conditions in which the importance of glucocorticoids relative to insulin in controlling metabolism is increased [4]. The high phosphohydrolase activities that are observed in the livers of rats with ketotic diabetes are decreased by insulin therapy [13,14].

It is still not entirely clear why insulin should antagonise the glucocorticoid-induced increase in the phosphohydrolase activity. However, it should be remembered that insulin in vivo would also inhibit the release of fatty acids from adipose tissue and their supply to the liver. The glucocorticoid-induced increase of the phosphohydrolase activity probably enables the liver to partially maintain its production of diacylglycerol when the synthesis of phosphatidate declines because of low insulin concentrations and when substrate availability is restricted. This would help to preserve the synthesis of phosphatidylcholine and phosphatidylethanolamine which are required for membrane turnover and for bile production. If the substrate supply is high then the increased phosphohydrolase activity facilitates the synthesis of triacylglycerols. These triacylglycerols may accumulate to produce a fatty liver, or they may be secreted as very low density lipoprotein. In the latter case the fatty acids

are subsequently taken up by heart and skeletal muscle in which the lipoprotein lipase activity is maintained by glucocorticoids rather than by insulin [4].

Insulin also antagonises the effects of glucocorticoids in stimulating:

(i) The synthesis of phsophatidylcholine in cells cultured from foetal lung [15];
(ii) The activity of argininosuccinate synthetase and argininosuccinate lyase [16];
(iii) The activity of phosphoenolpyruvate carboxykinase in Reuber H35 cells [17,18];
(iv) The activity of tryptophan oxygenase in rat hepatocytes [19];
(v) The activity of tyrosine aminotransferase in foetal hepatocytes [20].

By contrast, insulin and glucocorticoids can produce additive stimulations in the activity of tyrosine aminotransferase in Reuber H35 cells [17,18].

In a number of the examples given above the antagonistic interactions of insulin and glucocorticoids seem to be related to the function of the liver in exporting energy in periods of metabolic stress. For instance, the brain is receiving ketones and glucose, and heart and skeletal muscle are being supplied with ketones and triacylglycerols. One of the functions of the glucocorticoid-induced stimulations of phosphatidate phosphohydrolase may be to redirect fatty acids towards muscle tissue in these conditions.

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