Insulin antagonises the growth hormone-mediated increase in the activity of phosphatidate phosphohydrolase in isolated rat hepatocytes

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Rat hepatocytes were incubated in monolayer culture, under serum-free conditions for 8 h. Rat growth hormone (up to 100 nM) increased the activity of phosphatidate phosphohydrolase by up to 47%. Insulin (500 pM or 35 nM), cycloheximide or actinomycin D reversed this effect. The ability of growth hormone to modify the effects of insulin is discussed in relation to the control of the phosphohydrolase activity and glycerolipid synthesis.

Glycerolipid *Growth hormone* *Insulin* *L-α-Phosphatidate phosphohydrolase*

1. INTRODUCTION

The role of growth hormone in the control of hepatic lipid metabolism has received relatively little attention. Growth hormone can inhibit acetyl-CoA carboxylase through an increase in its phosphorylation state [1] and can stimulate the β-oxidation of saturated fatty acids [2]. Injection of growth hormone into rats led to an increase in hepatic phosphatidate phosphohydrolase activity after a period of 4 h [3], but it was not clear whether this was a direct effect of the hormone itself.

The activity of phosphatidate phosphohydrolase has been shown to be increased in the long term by glucagon and dexamethasone, these increases being reversed by insulin [4,5] and to some extent by spermine (unpublished). The metabolic expression of the phosphohydrolase activity occurs when the cytosolic form of the enzyme translocates to the endoplasmic reticulum. This occurs when the supply of fatty acids to the liver increases [6,7]. It is thought that changes in the activity and subcellular distribution of this enzyme are important in the control of hepatic triacylglycerol synthesis [7].

It has been extensively reported that growth hormone has the ability to modify insulin sensitivity in a variety of tissues [8–11]. Generally, growth hormone excess leads to a state of insulin resistance, whereas growth hormone deficiency leads to a state of increased insulin sensitivity. Although the exact mechanism of growth hormone action is not fully understood, modifications of the insulin receptor [11,12] or of cyclic AMP metabolism [13–15] have been implicated. Paradoxically however, growth hormone has also been shown to possess insulin-like activity [16,17]. An increase in plasma growth hormone has been reported to lead to a transient insulin-like effect on glucose clearance (after 1–2 h) followed by insulin antagonistic effects (after 6 h) [16]. It has also been reported that a growth hormone fragment (amino acids 6–13) stimulated glycogen synthesis whereas another fragment (amino acids 177–191) led to a stimulation of glycogenolysis [17]. This may be ex-

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plained by variants of growth hormone having metabolic effects [18].

The following experiments were performed to determine whether growth hormone can modify the activity of phosphatidate phosphohydrolase directly or by modifying the effects of insulin, or other hormones. To do this the enzyme was measured in a rat hepatocyte culture system under serum-free conditions. We have shown that growth hormone can directly increase phosphatidate phosphohydrolase activity and that this effect is antagonised by insulin.

2. MATERIALS AND METHODS

The sources of the rats and most of the materials have been described [4]. Rat growth hormone was kindly donated by the National Institute of Arthritis, Metabolism and Digestive Diseases, USA. The activity was 1.7 IU/mg, containing 0.07 IU/mg of TSH activity.

Hepatocytes were prepared and attached to Primaria tissue culture dishes in Leibowitz-L15 medium containing 10% (v/v) newborn calf serum. They were maintained for the next 12–18 h in Leibowitz-L15 medium containing 0.2% (w/v) fatty acid-poor bovine serum albumin before beginning the experiments [4]. Phosphatidate phosphohydrolase [19] and lactate dehydrogenase activities [20] were measured in cell homogenates [4] as described. The total phosphohydrolase activity was expressed relative to that of lactate dehydrogenase to compensate for slight variations in cell number between different culture dishes.

3. RESULTS AND DISCUSSION

Incubation of hepatocytes for 8 h with rat growth hormone increased the activity of phosphatidate phosphohydrolase by up to 47% in 10 independent experiments. A significant increase was seen with a hormone concentration of 0.1 nM (table 1, column A). This increase was not apparent until after 4–6 h and seemed to reach a maximum after approx. 12 h (fig.1). The presence of the protein synthesis inhibitors, actinomycin D

Table 1

Effects of growth hormone, insulin, dexamethasone and inhibitors of protein synthesis on the activity of phosphatidate phosphohydrolase in cultured hepatocytes

Growth hormone concentration (nM)		Additions					
		(A) None	(B) Insulin (500 pM or 35 nM)	(C) Dexamethasone (100 nM)	(D) Dexamethasone (100 nM) + insulin (35 nM or 500 pM)	(E) Actinomycin (1 μg/ml)	(F) Cycloheximide (5 μg/ml)
I	0	100 (10)	106 ± 32 (8)	385 ± 123 (9)	134 ± 38 (6)	92 ± 22 (3)	102 ± 30 (3)
II	0.1	119 ± 11 (7) I vs II[c]	95 ± 17 (3)	363 ± 110 (4)	217 ± 87 (5)		
II	1.0	121 ± 14 (7) I vs III[c]	102 ± 7 (4)	431 ± 99 (5)	193 ± 51 (5)		
IV	10	147 ± 42 (10) I vs IV[c]	103 ± 20 (6) AIV vs BIV[a]	472 ± 113 (6)	182 ± 24 (5)	119 ± 39 (3)	82 ± 4 (3)
V	100	144 ± 35 (6) I vs V[a]	111 ± 38 (4) AV vs BV[b]	427 ± 97 (4)	155 ± 10 (2)		

Cultured rat hepatocytes were incubated for 8 h with the indicated concentrations of hormones and inhibitors. The total phosphohydrolase activity is expressed relative to incubations in which no extra additions were made which was 0.332 ± 0.235 nmol diacylglycerol produced/min per unit lactate dehydrogenase activity. Results are given as means ± SD (numbers of independent experiments). The significance of the effects of growth hormone and insulin were calculated by using a paired *t*-test and shown by [a] $P < 0.05$; [b] $P < 0.02$ and [c] $P < 0.01$

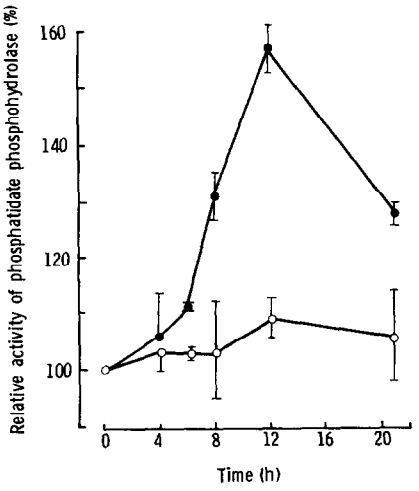

Fig.1. Effect of growth hormone on the activity of phosphatidate phosphohydrolase in cultured rat hepatocytes. The cells were incubated in the absence (○) or presence of 100 pM growth hormone (●) for the times indicated. The results show the mean ± ranges of the relative phosphohydrolase activity for duplicate plates in one experiment. The activity at the beginning of the incubation period was taken as 100% and was 0.35 mmol diacylglycerol produced/min per unit lactate dehydrogenase activity

(1 μg/ml) or cycloheximide (5 μg/ml) abolished this increase without significantly affecting the phosphohydrolase activity in control incubations (table 1, columns E,F). This suggests that growth hormone, like dexamethasone and glucagon [5], may be acting by increasing the synthesis of the enzyme. Insulin at either 500 pM or 35 nM was equally effective in abolishing the effects of growth hormone without affecting the phosphohydrolase activity when added alone.

The incubation of hepatocytes with growth hormone and dexamethasone in combination produced additive increases in the phosphohydrolase activity in 4 out of 6 independent experiments (table 1, column C). However, these effects were mostly masked by the variability of the dexamethasone-mediated increase in the phosphohydrolase activity when compared with the relatively small effects of growth hormone in these experiments. If cyclic AMP did accumulate within the cell on incubation with growth hormone, then additive or synergistic increases in the phosphohydrolase would be expected, as was seen with incubations containing dexamethasone and cyclic AMP [4] or glucagon [5].

Growth hormone appeared to increase the activity of phosphatidate phosphohydrolase in incubations that contained dexamethasone and insulin (table 1, column D). This effect was however quite varied in magnitude and not statistically significant. The increases seen were larger than the effects of growth hormone alone in some experiments and may occur because the growth hormone antagonises the effects of insulin.

Growth hormone and glucocorticoids [11,12] have both been reported to lead to a state of insulin resistance through a decrease in the affinity of insulin binding to its receptor or in receptor number [11,12]. If this were the case then a preincubation of hepatocytes with growth hormone could enhance the possible anti-insulin-like effects thus decreasing the effects of insulin in antagonising the dexamethasone- or glucagon-mediated increases in the phosphohydrolase activity.

The long-term regulation of phosphatidate phosphohydrolase activity is under complex hormonal control. Dexamethasone, cyclic AMP analogues, glucagon [4,5] and growth hormone can increase its activity, probably by increasing its rate of synthesis. Insulin has the ability to antagonise these increases, although insulin sensitivity may be decreased in the long term by growth hormone and glucocorticoids [11,12]. It is not yet known whether growth hormone can acutely alter the activation of the phosphohydrolase through its translocation from the cytosolic to membrane-associated compartments in response to an increased fatty acid supply. Growth hormone may indirectly affect the translocation 'in vivo' as it has been reported to stimulate lipolysis in adipose tissue leading to an increase in the circulating concentration of fatty acids [21,22]. Growth hormone secretion, as well as that of glucagon and glucocorticoids, is increased in conditions, such as stress, starvation or hypoglycaemia [23].

The present results indicate that growth hormone could increase the capacity of the liver to synthesise glycerolipids in stress conditions by increasing the total reservoir of phosphatidate phosphohydrolase activity. Expression of this activity will probably rely mainly on the net accumulation of fatty acids and their CoA esters in the liver [7].

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