Hormonal regulation of phosphorylase phosphatase activity in rat liver

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The effect of glucagon and insulin on rat liver phosphorylase phosphatase activity in vivo was investigated. The activity of phosphatase was found to decrease following the administration of glucagon and increase with insulin in a reversible manner. No change was detected in the activity of heat-stable phosphatase inhibitors in the hormone-treated samples. Liver protein kinases (regulatory subunit of cAMP-dependent protein kinase and/or Ca^{2+}-dependent phosphorylase kinase) are suggested to regulate the activity of hepatic phosphorylase phosphatase (type 1 and 2A).

Glycogen metabolism *Hormonal regulation* *Protein phosphatase*

1. INTRODUCTION

Reversible protein phosphorylation involving the action of protein kinases and phosphatases is the major mechanism by which hormonal control of glycogen metabolism is mediated [1]. Activation of protein kinase, phosphorylase kinase and phosphorylase, reflecting an increase in cAMP concentration in the liver, was reported by glucagon [2]. The concentration of phosphorylase *a* may be increased due to the concerted inhibition of phosphorylase phosphatase (type 1 and 2A) with the activation of phosphorylase kinase. At present no such evidence is available [3].

Insulin is capable of decreasing the level of cAMP in vivo provided it has previously been raised by a cAMP-elevating hormone, enabling it to antagonize the action of glucagon in the liver [4,5]. Administration of insulin to rabbits results in the inactivation of phosphorylase kinase and phosphorylase in the liver without any change in the concentration of cAMP [6].

Inhibitor-1 is a powerful inhibitor of protein phosphatase-1 only after being phosphorylated by cAMP-dependent protein kinase. Phosphorylation/dephosphorylation of inhibitor-1 was demonstrated in skeletal muscle (references, see [7]) and in rat liver [8].

Here we investigated the effect of glucagon and insulin on rat liver phosphorylase phosphatase activity in vivo.

2. MATERIALS AND METHODS

2.1. *Chemicals, buffers and enzymes*

Glucagon and insulin (from bovine pancreas) were obtained from Sigma, Sephadex G-25 was supplied by Pharmacia. Other chemicals used were of analytical grade.

Buffer A contained 250 mM sucrose, 50 mM imidazole (pH 7.4), 4 mM EDTA, 10 mM β-mercaptoethanol and protease inhibitors as in [9]. Buffer B contained 50 mM β-glycerophosphate (pH 6.3), 4 mM EDTA, 10 mM β-mercaptoethanol and 100 mM NaF.

Liver phosphorylase *a* and rabbit muscle ^{32}P-labelled phosphorylase *a* were prepared as in [10] and [11], respectively. The catalytic subunit of protein phosphatase-1 was prepared from rabbit skeletal muscle [12] as modified in [13].

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2.2. *Handling of animals and livers*

Experiments were performed by liver biopsy on fed female Wistar rats anaesthetized as in [2]. The abdomen was opened and the liver exposed. The first biopsy was taken 20 min after the beginning of anaesthesia. Bleeding was controlled by hemostatic plugs. At 5 min after the first biopsy was taken 0.9% NaCl or glucagon (10 μg/kg) or insulin (10 U/kg) plus glucose (1 g/kg) were injected intravenously and additional samples were cut off from different lobes.

For the assay of phosphorylase kinase and phosphatase, biopsies were immediately homogenized in a Potter-Elvehjem tube in 2 vols ice-cold buffer A. All subsequent steps were carried out at 0–4°C. The homogenate was centrifuged for 10 min at 8000 × *g* and 1 ml of supernatant was filtered through a column of Sephadex G-25 (20 × 1.5 cm) equilibrated in buffer A.

For the assay of phosphorylase *a* the samples were homogenized in 10 vols ice-cold buffer B, centrifuged and the supernatants were used.

2.3. *Assays*

Phosphorylase *a* was assayed as in [14]. Phosphorylase phosphatase was determined with 5 U/ml rabbit liver phosphorylase *a* in the presence of 1 mM caffeine at 37°C. One unit of activity of phosphatase inactivates 1 nmol phosphorylase *a* per min under the assay condition [12].

Heat-stable inhibitors were assayed in the supernatants of boiled liver extracts with ^{32}P-labelled phosphorylase *a* by their ability to inhibit muscle phosphatase-1. One unit of inhibitor is that amount which inhibits 0.02 mU of phosphatase-1 by 50% [15].

Phosphorylase kinase was measured as in [16] using 1 mM ATP and 2 mM Mg acetate.

3. RESULTS

In control experiments with rats treated with 0.9% NaCl no significant changes were detected in liver phosphorylase phosphatase activity (table 1). The increase in phosphatase activity after gel filtration and dilution is – at least in part – due to the decrease in the concentration of phosphatase inhibitors. In the experiments below gel-filtered liver extracts of 25 mg/ml protein concentration were used. We have previously demonstrated that rabbit liver extract contained 30% type 1 and 70% type 2A phosphatase [17]. The distribution of phosphatase-1 and -2A, catalysing the dephosphorylation of phosphorylase *a*, was the same in rat liver extract (not documented).

Table 1

Phosphorylase phosphatase activity in rat liver following the administration of 0.9% NaCl

Time of biopsy (min)	Phosphatase activity (U/mg protein)		
	Liver extract [25 mg/ml]	Sephadex G-25 filtrate	
		[25 mg/ml]	[2.5 mg/ml]
0	20 ± 5	21 ± 5	65 ± 13
7	22 ± 7	23 ± 6	68 ± 11
15	18 ± 6	20 ± 5	73 ± 12
45	23 ± 5	23 ± 5	69 ± 13

Liver extracts were diluted with buffer A to attain similar protein concentrations, and values in brackets indicate the protein concentration of the assay mixture. Activities are expressed as means ± SE of 5 experiments

As the method seemed to be suitable for detecting phosphatase activity, the effects of glucagon and insulin in vivo were probed. To avoid hypoglycemia initiating an intense glycogenolysis in the liver, insulin was administered together with glucose [18]. The activity of phosphorylase kinase and endogenous phosphorylase *a* was detected parallel with the phosphatase assay. In agreement with previous works [2,6,16] the administration of glucagon caused a rapid increase in the activity of phosphorylase kinase and insulin inactivated it transiently (fig.1A). Phosphorylase *a* activity was increased by glucagon and decreased by insulin administered with glucose, respectively (fig.1B).

According to our results phosphorylase phosphatase activity is also controlled by these hormones. Phosphatase activity was found to decrease by 20–40% following the administration of glucagon and to return to the original value after 30–45 min. On the other hand, phosphatase was activated by 30–50% with insulin in a reversible manner (fig.1C). Maximal effects were obtained 7 min after the addition of the hormone.

No change was detected in the activity of heat-stable phosphatase inhibitors in the hormone-treated samples (table 2).

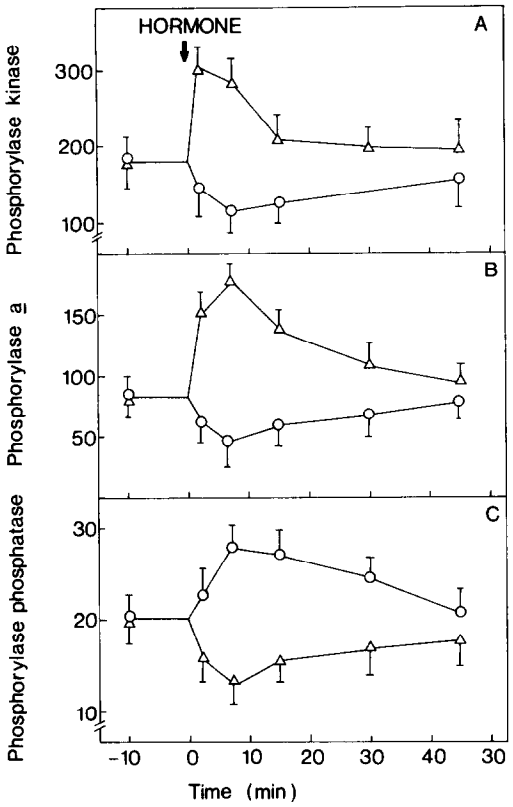

Fig.1. Effect of glucagon (△) and insulin plus glucose (○) on the activity of phosphorylase kinase (A), phosphorylase *a* (B) and phosphorylase phosphatase (C). Activities are expressed in units/g of protein. Values shown are means ± SE for 4–6 experiments.

Table 2

Effect of glucagon and insulin plus glucose on the activity of heat-stable inhibitors

Time of biopsy (min)	Activity of heat-stable inhibitors (U/mg protein) in response to	
	Glucagon	Insulin + glucose
0	179 ± 22	179 ± 22
2	178 ± 23	184 ± 28
7	180 ± 27	189 ± 24
15	173 ± 25	179 ± 26
30	187 ± 27	171 ± 28
45	173 ± 24	187 ± 30

Values are means ± SE for 4 and 5 experiments, respectively

4. DISCUSSION

Glucagon caused a transient inactivation of phosphatase parallel with the activation of phosphorylase kinase and phosphorylase. On the other hand, phosphatase activity was increased in response to insulin administered with glucose in concert and the inactivation of phosphorylase kinase, resulting in a transient inactivation of phosphorylase. Although glucose alone can also inactivate phosphorylase [18], it is not supposed to mask the effect of insulin, since gel filtration removed the low molecular mass metabolites.

It was previously demonstrated that insulin increased phosphorylase phosphatase activity of hepatocytes and anaesthetized rats. Liver lobes were freeze-clamped and tissue extracts were pulverized in liquid nitrogen in these experiments [19]. The hypotonic media and frozen liver can disrupt the native structure of phosphatase with a concomitant increase in the specific activity of the enzyme by several-fold. The latter possibility was clearly shown by Doperé and Stalmans [9]. Our experimental approach favours the preservation of the cellular conditions thereby the change in phosphorylase phosphatase activity upon hormonal treatment may reflect a physiologically relevant control mechanism.

The changes of phosphatase-1 activity in response to glucagon and insulin could not be attributed to the phosphorylation-dephosphorylation of inhibitor-1 as no change was detected in the activity of heat-stable inhibitors. On the other hand, the degree of phosphorylation of inhibitor-1, as judged by the degree of immunofluorescence, was changed by glucagon or insulin in rat liver [8]. Therefore the regulatory role of inhibitor-1 cannot be ruled out.

Another regulatory device for type 1 and 2A phosphatases is offered by protein kinases. The regulatory subunits of type I and II cAMP-dependent protein kinase inhibit the dephosphorylation of phosphorylase *a* catalysed by various phosphatases [20–23]. Glucagon raises the cAMP-concentration modulating the amount and the degree of phosphorylation of the regulatory subunit, and insulin is also capable of decreasing the level of cAMP. It seems that the regulatory subunit may control the activity of liver phosphorylase phosphatase-1 and -2A. Phos-

phorylase kinase isolated from skeletal muscle also inhibits protein phosphatase-1 and -2A, and the inhibitory effect is several-fold larger when kinase is phosphorylated [24,25]. The reversible activation of Ca^{2+}-dependent phosphorylase kinase occurs upon hormonal treatment in the liver. This process may also regulate the activity of hepatic phosphorylase phosphatase.

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