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HORMONE-INDUCED EFFECTS ON THE RAT LIVER MICROSOMAL GLUCOSE-6-PHOSPHATASE SYSTEM IN VITRO

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SUMMARY: We studied the effects of various glucocorticoids, glucagon and insulin on the activity of rat liver microsomal glucose-6-phosphatase. Preincubation of microsomes with corticosterone, cortisone, cortisol and dexamethasone as well as glucagon increased the rate of glucose-6-phosphate hydrolysis by about 1.5 fold relative to the controls. The maximum activation occurred at about 10 nM steroids and 0.3 nM glucagon, respectively. On the other hand, increasing concentrations (8.3 - 50 nM) of insulin progressively inhibited glucose-6-phosphatase up to 26%; the activity of which, however, remains completely in a latent state within the microsomal membrane and can be released from it by Triton treatment. In terms of the substrate transport hypothesis, the results are interpreted as evidence that regulation of glucose-6-phosphate hydrolysis is achieved by direct interactions either of the hormones themselves or of a possible second messenger with the carrier moiety of the rat liver microsomal glucose-6-phosphatase system.

Several lines of evidence suggest that the functional glucose-6-phosphatase of rat liver endoplasmic membranes is a two-component system consisting of a glucose-6-phosphate-specific transporter and a nonspecific phosphohydrolase localized within the luminal surface of the membrane (1,2). Furthermore, the substrate transport across the microsomal membrane from the cytoplasmic side to the hydrolase has been demonstrated to be the rate-controlling process of microsomal glucose-6-phosphate hydrolysis and in terms of the substrate transport hypothesis this kinetic feature is assumed to be the reason of the considerable latency of glucose-6-phosphatase activity which is released by treatment of microsomes with detergents (1).

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In accordance with this view,Arion et al.(3) have postulated that glucocorticoid treatment of rats increases the concentration of the proposed translocase.However,it would be expected that changes in the enzymatic activity of glucose-6-phosphatase in response to various metabolic and hormonal regulations should be caused,at first,by direct effector-dependent modulations of the transport capacity before inductive adaptation of the amounts of glucose-6-phosphatase compounds could occur.

In this study,we have therefore investigated the influence of various steroids,glucagon and insulin on the activity of rat liver microsomal glucose-6-phosphatase in vitro.The results indicate that glucocorticoids and glucagon produce a stimulation of the glucose-6-phosphate hydrolysis in general,comparable to that produced by detergents,whereas insulin inhibits the glucose-6-phosphatase activity which has remained essentially latent within the microsomal membrane,however.

MATERIALS AND METHODS

Glucose-6-phosphate was obtained from Boehringer Mannheim GmbH (FRG);cortisone,cortisol,11-deoxycorticosterone,11-deoxycortisol,progesterone and aldosterone from Merck,Darmstadt (FRG); 5ß-dihydrocortisone,3α,5ß-tetrahydrocorticosterone and 5ß-dihydrocortisol from Ikapharm,Ramat-Gan (Israel);corticosterone and 17α-hydroxyprogesterone from Serva,Heidelberg (FRG); dexamethasone from Hoechst AG,Frankfurt (FRG);rat insulin (lyophilized with 1 mg of human albumin per 0.1 mg insulin) and porcine glucagon (lyophilized;containing 0.001% insulin) from Novo Research Institute,Bagsvaerd (Denmark).All the other reagents were of analytical grade.

Male Wistar rats (AF/Han.) weighing about 200 g and fasted for 14 h were used.Microsomes were prepared by differential centrifugation in the presence of 1 mM EDTA,washed in 1.18% KCl and resuspended in 0.1 M Tris-HCl pH 7.4 (4).

Detergent-modified microsomes were prepared,if not otherwise stated,at 0°C by the addition of 0.05 vol. of 1% (w/v) Triton X-114 in 0.1 M Tris-HCl pH 7.4 to 0.95 vol. of untreated (intact) microsomes at a protein concentration of 2 mg/ml to give a Triton/microsomal protein ratio of 0.25 and a final Triton concentration of 0.05% (2).

The activity of glucose-6-phosphatase (EC 3.1.3.9) was measured as described by Harper (5) using 0.2 M imidazole-HCl

Table 1. The latency of glucose-6-phosphatase as a function of preincubation.

Time of preincubation	Enzyme activity in % of untreated microsomes			
	Triton X-114		Cortisone	
min	0°C	37°C	0°C	37°C
5	105	58	125	101
10	115	22	130	94
30	135	4	136	100
60	155	-	137	101
180	170	2	147	103
300	176	-	148	104

Microsomes were treated with Triton X-114 as described in Materials and Methods and with cortisone (50 nmol/2 mg protein in a final volume of 1 ml 0.1 M Tris-HCl pH 7.4), respectively, then preincubated at 0°C or 37°C for times given in the Table and the enzyme activities were subsequently measured (10 min, 37°C). The assays were initiated by adding aliquots of the preincubation mixtures to the test medium. Glucose-6-phosphatase activity of untreated microsomes was 452-543 mU/mg protein. Typical results representative of 4 similar experiments.

buffer pH 6.5. The enzyme activity (mU/mg protein) is expressed as inorganic phosphorus released. Protein was assayed by the biuret method of Bode et al. (6).

RESULTS AND DISCUSSION

We first investigated the conditions which provide maximal stimulation of glucose-6-phosphate hydrolysis by steroids. The results of typical experiments are summarized in Table 1 and 2, where effects of Triton X-114 on glucose-6-phosphatase are also included for comparison. It can be seen that prolonged preincubation of rat liver microsomes with cortisone at 0°C following a short term test incubation at 37°C released most of the latent glucose-6-phosphatase activity even though detergent-induced modifications of the microsomal membranes liberated much more latent activity, especially if the subsequent test incubation was carried out at lower temperatures. The time course studies

Table 2. The latency of glucose-6-phosphatase as a function of temperature during the test incubation.

Temperatue of the test incubation mixture °C	Enzyme activity in % of untreated microsomes	
	Triton X-114	Cortisone
8	271	105
15	230	108
30	196	129
37	183	151

Microsomes were treated with Triton X-114 or cortisone as described in the legend of Table 1, then preincubated at 0°C for 3 h and the enzyme activities were subsequently measured (10 min of test incubation) for temperatures indicated in the Table. Further details are given in the legend of Table 1. Typical results which are representative of 3 similar experiments.

of preincubation at 37°C indicate that latency could no longer be observed; without substrate protection Triton X-114 denatured the glucose-6-phosphatase very quickly. On the other hand, cortisone is presumably bound under these conditions to the proposed nuclear acceptor (7) which is assumed to contaminate our microsomal preparation.

The latency of glucose-6-phosphatase as a function of C_{21}-steroids and dexamethasone concentration is illustrated and compared in Fig. 1. Half-maximal activation occurred at about 1 nM corticosterone, cortisol and cortisone as well and almost no activation was present at 0.1 nM; 10 nM was near saturation. This value shows the largest stimulation by glucocorticoids observed in the course of these experiments and relative to the controls the maximum latency released was in the range of 40 - 50%, respectively. Preincubation of rat liver microsomes with 11-deoxycorticosterone and 11-deoxycortisol enhanced the rate of glucose-6-phosphate hydrolysis by about 30 - 40% (relative to the controls), whereas progesterone and 17α-hydroxyprogesterone

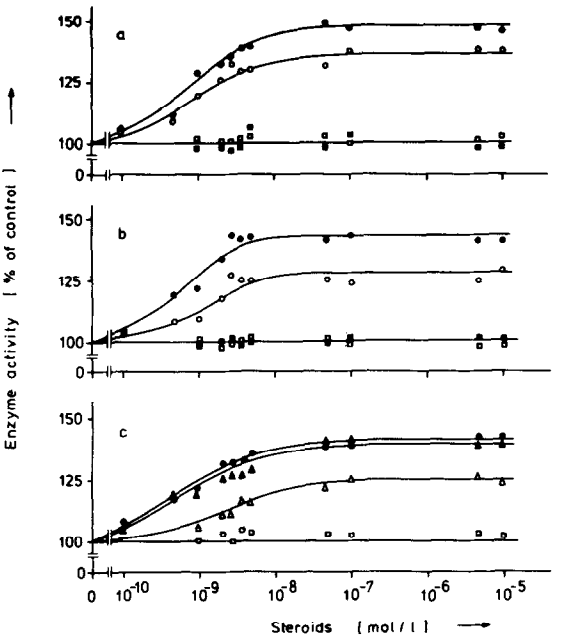

Fig.1. Effects of steroids on the latency of rat liver microsomal glucose-6-phosphatase.Microsomes (2 mg protein) were preincubated (3 h at 0°C) in 1 ml 0.1 M Tris-HCl pH 7.4 with steroid concentrations indicated in the Figure and then the enzyme activities were measured (10 min, 37°C).The assays were initiated by adding 0.05 ml of the preincubation mixtures to the test medium.Glucose-6-phosphatase activity of control microsomes (treated identically as the test,but without steroids) was 472-554 mU/mg protein.Typical results representing 3 similar experiments,respectively. a) corticosterone (●),11-deoxycorticosterone (○),progesterone (■),3α,5β-tetrahydrocorticosterone (□); b) cortisol (●),11-deoxycortisol (○),17α-hydroxyprogesterone (■),5β-dihydrocortisol (□); c) cortisone (●),5β-dihydrocortisone (□),dexamethasone (▲),aldosterone (△).

as well as the 5β-reduced steroids were completely ineffective in releasing any latency of the glucose-6-phosphatase activity, even if large amounts of these compounds were added under the conditions employed.Aldosterone and dexamethasone again stimulated glucose-6-phosphate hydrolysis and together with the results presented above,this could suggest that both the Δ^4-structure of ring A and the 21-hydroxy group are essential for the effects of steroid hormones on the microsomal glucose-6-phosphate transport system.

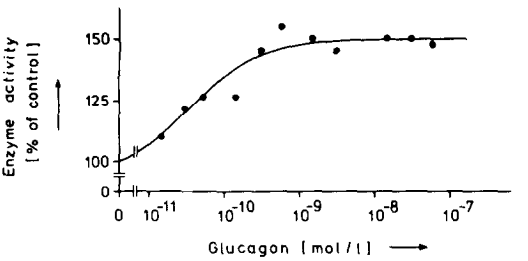

Fig.2. Effects of glucagon on the latency of rat liver microsomal glucose-6-phosphatase. Experimental details as for Fig.1, except that glucagon was used. Typical results representing 3 similar experiments.

The dose response curve depicted in Fig.2 shows the influence of glucagon concentration on microsomal glucose-6-phosphatase activity. As expected and providing some evidence for the hypothesis that glucagon could lead to a short-term functional activation of glucose-6-phosphatase in perfused liver of fetal guinea pigs (8), the stimulatory effect of this hormone is comparable to that of glucocorticoids. The maximum release of latency is achieved with 0.3 nM glucagon.

Fig.3 shows the results of typical experiments in which rat liver microsomes were first preincubated with varying amounts of rat insulin and then treated with or without Triton X-114 as described under Materials and Methods. It can be seen that insulin drastically decreased the glucose-6-phosphate hydrolysis and that the subsequent Triton treatment released the full activity which had remained in a completely latent state within the hormone-specific altered membrane.

Taken together, the experiments described here clearly indicate that regulation of glucose-6-phosphate hydrolysis occurs by direct interaction of hormonal effectors with a substrate transport system and thus our results provide additional support for the carrier model of rat liver microsomal glucose-6-phosphatase developed by Arion et al. (1) and recently confirmed again by Schulze and Speth (2). The apparent inhibition of glucose-6-phosphatase by insulin and the subsequent detergent-induced release

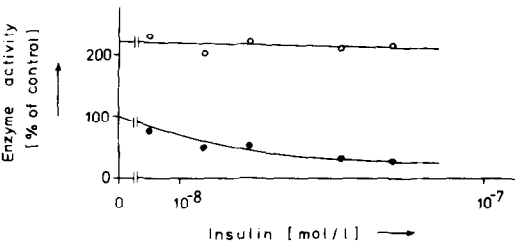

Fig.3. Effects of rat insulin on the latency of rat liver microsomal glucose-6-phosphatase. Microsomes (2 mg protein) were preincubated (15 min at 0°C) in 1 ml 0.1 M Tris-HCl pH 7.4 with rat insulin concentrations indicated in the Figure and then the enzyme activities were measured (15 min, 15°C) (2) on preparations both without (•) and with (o) applying a Triton X-114 treatment (15-16 h at 0°C) according to Materials and Methods. The assays were initiated by adding 0.1 ml of the preincubation mixtures to the test medium. Glucose-6-phosphatase activity of control microsomes (treated identically as the test, but without insulin and/or Triton) was 64-85 mU/mg protein. Typical results representing 3 similar experiments.

of the full latency strongly demonstrate that insulin influences the carrier moiety rather than the hydrolase itself. Furthermore, it shows that substrate transport is indeed the rate-limiting process in microsomal glucose-6-phosphate hydrolysis, as required by the model proposed (1). Consequently, glucocorticoids and glucagon should raise the transport capacity by activation of the preformed carrier in vitro. Assuming that this conclusion is valid, activation and induction of the catalytic component alone without concomitant enhancement of the substrate transport potential cannot stimulate glucose-6-phosphate hydrolysis in vivo, as suggested for glucocorticoid administration (9,10) and illustrated for fasted and alloxan-diabetic rats (1,3), respectively.

However, whether glucocorticoids, glucagon and insulin acting in amounts comparable to physiological concentrations (11-15) are themselves capable of causing the effects on the glucose-6-phosphate carrier described in this paper or whether these effects are caused by a second messenger released in response to interactions between those hormones and a specific receptor of the plasma membranes (16) present in our microsomal preparation remains to be investigated.

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