# EFFECT OF CYCLIC AMP ON HEXOKINASE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE ISOZYMES IN ALBINO RAT TISSUES

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The study of regulation of hexokinase (HK), the limiting enzyme of glycolysis, and of glucose-6-phosphate dehydrogenase (G6PDH), the limiting enzyme of the pentose phosphate pathway of carbohydrate oxidation, is of exceptional interest: Changes in the activity of these two enzymes determine the state of the two most important metabolic pathways in carbohydrate metabolism. The role of substrates, products, coenzymes, and various metabolites in the regulation of both HK and G6PDH is well known [3, 4]. The hormonal mechanisms of regulation of these enzymes have received less study. When the organism is exposed to subextremal and extremal factors, an important place in the regulation of HK and G6PDH is assumed by glucocorticoids, catecholamines, and cyclic AMP, as an intracellular regulator in the action of adaptive hormones. Both enzymes are known to be present in various tissues as different isozymes, each with its own kinetic characteristics and features of regulation [1, 2]. This must be taken into account when any mechanisms of regulation are analyzed: isosteric, allosteric, etc.

In the investigation described below an attempt was made to analyze the effect of cyclic AMP on changes in total activity and the isozyme spectrum of HK and G6PDH, in order to establish the pattern of hormonal regulation of the two enzymes in tissues with different functional specialization.

## EXPERIMENTAL METHOD

Female Wistar albino rats weighing 150-200 g were used. The animals were decapitated and tissue of the liver, gastrocnemius muscle, and adrenals was quickly removed, washed with cold 0.15 M KCl to remove blood, and homogenized in 4 volumes (the liver in 2 volumes) of 0.1 M glycyl-glycine buffer, pH 7.4, containing 5 mM EDTA, 5 mM mercaptoethanol, and 10 mM glucose. Total HK [6] and G6PDH [7] activity and the isozyme spectra of both enzymes [5] were determined spectrophotometrically in the cytosol. To reveal HK isozymes the gel was incubated in a mixture of the following composition: 75 mM glycyl-glycine buffer, pH 8.2, 5 mM ATP, 10 mM $MgCl_2$, 1 mM NADP, 2 mM $NaN_3$, 10 mM glucose (to reveal glucokinase 100 mM), 0.4 mg/ml nitro-BT, 0.04 mg/ml phenazine metasulfate in final concentration. To reveal G6PDH isozymes the same incubation mixture was used without ATP and glucose, but with the addition of glucose-6-phosphate in a concentration of 2 mM. Relative activity of isozymes in the gel was determined by means of a Chromoscan densitometer (Joyce Lobel, England). When data on total activity of the enzymes was used, the absolute activity of the isozymes also was calculated and expressed in nmoles NADPH/min/mg protein.

## EXPERIMENTAL RESULTS

Total HK activity in the muscles and adrenals was relatively high, but much lower in the liver (Fig. 1). On electrophoresis, when the glucose concentration in the incubation medium was 10 mM, three isozymes were detected in the liver: HK-1, HK-2, and HK-3. If the glucose concentration in the incubation medium was 100 mM glucokinase also was revealed. The ratio between activities of the HK isozymes depended on the glucose concentration in the incubation medium. If it was low the ratio was optimal for HK, if high it was optimal for glucokinase. Only two types of enzyme were present in the muscles — HK-1 and HK-2. Activity

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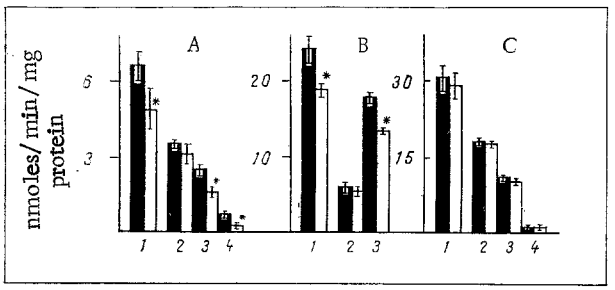


Fig. 1

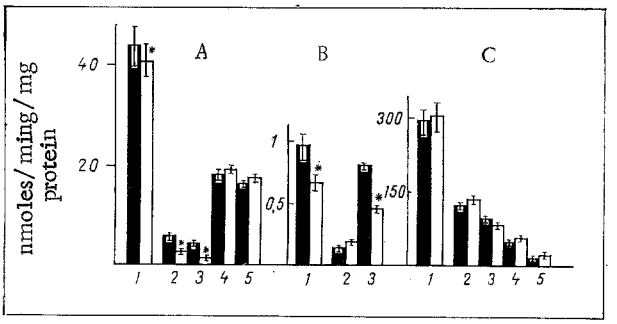


Fig. 2

Fig. 1. Changes in total HK activity and isozyme spectrum under the influence of cyclic AMP. A) Liver, B) muscle, C) adrenal. 1) Total HK activity, 2-4) HK-1, HK-2, and HK-3 respectively. Black columns — control (nothing added), white columns — with addition of cyclic AMP. *) Differences from control significant. Glucose concentration in incubation medium 10 mM.

Fig. 2. Changes in total G6PDH activity and isozyme spectrum under the influence of cyclic AMP. 1) Total G6PDH activity, 2-5) activities of isozymes of types 1, 2, 3, and 4, respectively. Remainder of legend as to Fig. 1.

of HK-2 was the highest, accounting for 75% of the total. The most active isozyme in the adrenals was HK-1 (60%), HK-2 was less active (37%), and only very low activity of HK-3 was found (3-4% of total HK activity). Preincubation of the liver cytosol with cyclic AMP, ATP, and $MgCl_2$ in concentrations of $10^{-6}$-$10^{-5}$, $10^{-3}$, and 0.05 M respectively, led to a reduction in total activity of the enzyme because of inhibition of HK-2 and HK-3. Activity of HK-1 and HK-4 (glucokinase) was unchanged. A similar result was obtained for muscles also: In this case preincubation with cyclic AMP led to a decrease in total HK activity because of inhibition of HK-2 only. The $MgCl_2$ in the incubation medium for muscles could be replaced by $CaCl_2$. Preincubation of the cytosol of the adrenal glands with cyclic AMP, ATP, and $MgCl_2$ did not change the ratio of activities of HK isozymes (Fig. 1).

The results evidently indicate that under the influence of cyclic AMP the kinase of HK, which phosphorylates the enzyme with the $\gamma$-phosphorus of ATP, is activated. This process is accompanied by a decrease in activity of the enzyme. Only HK-2 and HK-3 undergo phosphorylation. Cyclic AMP-dependent protein kinase is probably not present in the adrenals, and that is why preincubation of the cytosol with cyclic AMP did not lead to inhibition of HK or of its isozymes. HK kinase evidently has high selectivity, for in the presence of cyclic AMP neither HK-1 nor HK-4 is inhibited. This fact is most interesting. In those organs (kidneys, heart) where HK is present mainly as HK-1, under stress conditions, when the cyclic AMP concentration in the tissues rises, total activity of the enzyme changes by a lesser degree. In the brain mainly HK-1 is present [5]. This helps to explain the well-known fact that during exposure of the organism to extremal factors the rate of glycolysis in the brain is not reduced. Total activity of the enzyme in muscles, on the other hand, is due mainly to HK-2. It is not by accident that under stress conditions glycolysis in muscles is inhibited to a considerable degree. Since muscles account for about 70% of the total body weight, this provides a basis for economy in glucose utilization in the body as a whole. The brain thus is privileged before other tissues in the struggle for glucose.

G6PDH activity also differs in different tissues. In the adrenals, for example, activity of this enzyme is very high. This is due to the importance of the pentose phosphate pathway, which supplies reduced equivalents (NADPH) for steroid production. Activity of the enzyme is much lower in the liver and very low in muscles (Fig. 2). Activity of G6PDH of types 1, 2, 3, and 4 was revealed in the liver by electrophoresis in polyacrylamide gel. Distribution of their activities corresponded to a ratio of 12.9, 9.4, 41.1, and 36.5%. In the adrenals isozymes of types 3 and 4 and minor fractions — isozymes of types 5 and 6 were found. The ratio between their activities was 42.4, 35.0, 17.4, and 5.1%. Specific activity in the muscles corresponded to isozymes of types 1 and 2. Preincubation of the liver cytosol with cyclic AMP, ATP, and $MgCl_2$ led to inhibition of activity of G6PDH of types 1 and 2. Activity of the types 3 and 4 isozymes was unchanged (Fig. 2). Because of low total enzyme activity in the muscles, the inhibitory effect of cyclic AMP naturally was weak. In

the adrenals, preincubation of the cytosol with cyclic AMP did not change the ratio between activities of G6PDH isozymes. In the writers' view, inhibition of G6PDH in the presence of cyclic AMP, ATP, and $MgCl_2$ is mediated through cyclic AMP-dependent protein kinase, which phosphorylates isozymes of types 1 and 2 and thus reduces their activity. The same mechanism probably acts in this case as with HK. This protein kinase does not act on isozymes of other types: 3 and 4 or the minor fractions. This provides an explanation of many well-known facts. For example, during exposure of the organism to subextremal and extremal factors G6PDH activity in the liver falls, although not so substantially as HK activity. The reason is that mainly isozymes of types 3 and 4, which are not susceptible to the action of cyclic AMP-dependent protein kinase, are present in the liver. The contribution of isozymes of types 1 and 2 to the decrease in total activity of the enzyme is not so important. The absence of G6PDH isozymes of types 1 and 2 in the adrenals also makes regulation of the enzyme through cyclic AMP-dependent protein kinase impossible. Conversely, the presence of isozymes of only types 1 and 2 in muscles may lead to a high degree of phosphorylation of the enzyme and to a considerable reduction in its activity. This may be connected with the very low activity of the enzyme in normal muscles. Further research is required for a closer analysis of this problem.

The results of the present experiments are evidence that HK and G6PDH are enzymes whose hormonal regulation takes place by a mechanism of phosphorylation — dephosphorylation. This mechanism is realized through an increase in the cyclic AMP concentration in the tissues under the influence of adaptive hormones (adrenalin, hydrocortisone) and activation of the corresponding cyclic AMP-dependent protein kinases. Isozymes of both enzymes react unequally to cyclic AMP, thus demonstrating the selectivity of action of protein kinases. Differences in the isozyme spectrum of HK and G6PDH and also, probably, differences in the distribution of protein kinases, lie at the basis of differences in the action of hormones on enzyme activity in tissues with different functional specialization.

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