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The Effects of Alloxan Diabetes, Insulin and Epinephrine on Glucose-6-phosphate Dehydrogenase from Rat Liver and Brain

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ABSTRACT

The control of glucose-6-phosphate dehydrogenase (G6PD) activity of the liver and brain were studied in normal and alloxan diabetic rats. Fasting decreased G6PD activity in brains and livers of normal rats significantly, but these decreases were reversed by placing the rats on a sucrose-rich diet. Injection of insulin into normal 48h fasted rats had no significant effect on G6PD activity after 15 min. However, epinephrine significantly decreased liver G6PD activity by 17%, 7.5 min. after injection. Epinephrine had no effect on brain G6PD activity.

In fed alloxan diabetic animals, the G6PD activity was found to be about 50% of that found in normal rats. Treatment of diabetic rats with protamine insulin partially reversed the decrease in G6PD activity caused by alloxan diabetes. It is concluded that insulin and epinephrine are important for the regulation of G6PD activity in vivo.

INTRODUCTION

Glucose-6-phosphate dehydrogenase catalyses the first dehydrogenation reaction of the hexose monophosphate shunt, resulting in the production of NADPH and 6-phosphogluconate lactone. Studies on the regulation of the rat liver enzyme have shown its activity to decrease with fasting and to increase beyond prefasting levels upon refeeding the rat with carbohydrate rich food (6,7). Considering the intimate connection between the nutritional status of the animal and the levels of circulating hormones like insulin (9) it may be postulated that glucose-6-phosphate dehydrogenase (G6PD) is under the control of hormones. The purpose of the present study was to examine the effects of insulin, alloxan diabetes and epinephrine on G6PD from rat brain and liver. The role of the nutritional status of the rats on G6PD activity was also studied.

MATERIALS AND METHODS

Porcine insulin and protamine-insulin were bought from Choay, France, glucose-6-phosphate, NADP, epinephrine and alloxan were bought from Sigma. Nozinan was from SPECIA, Paris. Male albino rats weighing 100-200 g were maintained on standard laboratory chow from Myog-Betsi Yaoundé, for at least one week before use in the experiments. The standard laboratory chow contained % w/w: protein 16.5%, fibre 6.5%, fat 3.7%, calcium 5% and water. In some experiments one part of the standard chow was mixed with four parts of sucrose and used for feeding the rats. For experiments with insulin, except where mentioned, the rats were fasted for 48h before injection of the hormone.

Diabetes was induced by injection of alloxan (40 mg/kg body weight) into the tail veins of rats after 24h of fasting (8). After three days, during which these animals were maintained on the standard laboratory chow and tap water, blood was taken for analysis of the glucose concentration of the blood.

Homogenates

In the experiments reported on Table 2 the rats were anaesthesized with ether followed by the injection of nozinan (16 mg per kg body wt). In all other experiments ether anaesthesia was used. Nozinan had the effect of reinforcing ether anaesthesia. After decapitation and exsanguination, livers and whole brains were rapidly excised, blotted, weighed and homogenized in 3 vol. 0.25 M sucrose, containing 1 mM EDTA (adjusted to pH 7.5 with 1 M tris). In experiments with epinephrine 0.20 M sucrose, containing 1 mM EDTA and 20 mM potassium phosphate, pH 7.5, was used to homogenize the tissues.

Enzyme assay

Glucose-6-phosphate dehydrogenase was assayed essentially according to the method of Langdon (4). It was confirmed that the substrate and co-factor concentrations were saturating for both the brain and the liver G6PD. One unit of G6PD activity is the amount of enzyme necessary to catalyse the reduction of 1 μmole NADP per minute at 25°C at the conditions described by Langdon (4). The results are expressed as the mean ± S.D. of G6PD units per g tissue. Statistical analysis of the results was performed using the students t-test. Glucose was determined as in (3).

RESULTS AND DISCUSSION

Effect of diet on G6PD activity

Previous investigators have shown that rat liver glucose-6-phosphate dehydrogenase decreases upon fasting and increases upon feeding of the animals (6,7).

However, the regulation of liver enzymes, e.g. pyruvate kinase may differ from that of isoenzymes from other tissues (11). Therefore we compared the effects of fasting and refeeding of rats on the activity of G6PD from rat liver and brain.

Table 1. Effects of diet on G6PD activity. Rats weighing 150-200 g were used. Fasting was for 48h during which the animals had free access to water. "Normally" fed animals were kept on the standard chow for 7 days before decapitation. After fasting for 48h animals were refed with sucrose-rich food for 72h before decapitation. Other details are given in the text. The number of animals is given in brackets.

Description	G6PD activity (Units/g tissue)	
	Liver	Brain
Normal (14)	1.15 ± 0.27^{a}	0.85 ± 0.50^{d}
Fasted (14)	0.66 ± 0.34^{b}	0.57 ± 0.34^{e}
Refed with sucrose-rich food (8)	2.11 ± 0.49^{c}	0.90 ± 0.10^{f}

The differences between a and b ($P < 0.01$), a and c ($P < 0.01$), d and e ($P < 0.05$) are statistically significant. The difference between d and f is insignificant.

Table 1 shows that upon fasting rat liver G6PD decreased to 57% of the normal levels. The effect of fasting was less pronounced on the levels of brain G6PD from the same rats, as the latter dropped only to 67% of the original levels. Refeeding of the fasted rats returned the brain G6PD activity to prefasting levels. With rat-liver, the addition of sucrose in the food increased G6PD activity up to 183% as compared with normal levels. The increases in the activities of liver and brain G6PD observed upon refeeding of the rats (Table 1) are smaller than those reported previously (7) for the liver enzyme. The reason for this apparent discrepancy is not known, but the use in the present work of a sucrose-rich food containing fat and a different strain of rats may explain the differences between the results on Table 1 and those of others (7). The above results are in agreement with the idea that a carbohydrate-rich diet enhances the glucose-6-phosphate dehydrogenase activity of liver as well as brain and presumably leads to the increased oxidation of glucose by the hexose monophosphate shunt.

Immediate effects of the administration of insulin and epinephrine on G6DP of rat liver and brain

The levels of circulating insulin are known to vary depending on the nutritional status of the rats (9). To investigate whether the changes observed in Table 1 were directly related to the levels of circulating insulin, the effect of intraperitoneal injection of insulin into fasted rats was tested after a

period of 15 mins.

Table 2. Immediate effects of insulin and epinephrine on G6PD activity of rat liver and brain. Rats weighing 120-150 g were used for the experiments. For experiments with insulin the animals were fasted for 48h during which they had free access to tap water. The animals were then anaesthesized with ether and nozinan as described under MATERIALS and METHODS. Insulin was diluted with 0.9% NaCl to 1.2 I.U. per ml and 1 ml was injected intraperitoneally per 100 g body weight. For experiments with epinephrine the animals were maintained with standard laboratory chow for 1 week before use in the experiments. Anaesthesia was performed with ether and nozinan as described above. Epinephrine, 8 µg per ml was dissolved in 0.9% NaCl and 1 ml injected per 100 g body weight of the animal. In the insulin and epinephrine experiments the rats were decapitated after 15 and 7.5 min., respectively. In parallel 0.9% NaCl was injected into 48h fasted (upper panel) and normally fed (lower panel) rats as controls for the respective treatments. In the epinephrine experiments (and saline controls - see lower panel), 30 mM phosphate buffer, pH 7.5, was used instead of tris/HCl in the enzyme assay (4). The number of animals is given in brackets.

Treatment of animals	G6PD activity (Units/g tissue)		Glucose (mM)
	Liver	Brain	
Insulin (9)	0.64 ± 0.18^{a}	0.54 ± 0.18^{c}	4.8 ± 1.2^{e}
Saline (9)	0.56 ± 0.11^{b}	0.48 ± 0.24^{d}	5.3 ± 0.3^{f}
Epinephrine (7)	1.20 ± 0.29^{l}	0.60 ± 0.31^{n}	7.6 ± 2.3^{q}
Saline (7)	1.44 ± 0.34^{m}	0.63 ± 0.49^{p}	6.2 ± 1.4^{r}

The differences between a and b, c and d, e and f and n and p are statistically insignificant. The differences between l and m and q and r are statistically significant, $P < 0.01$ in both cases.

The results presented in Table 2 show that insulin administration increased G6PD activity by 14% and 12% in liver and brain respectively. The changes are, however, statistically insignificant. This was also the case for the changes of the concentration of blood glucose. Apparently, these data could not tell whether the increases in G6PD activity upon refeeding (Table 1) could be attributable to an immediate effect of insulin

Some effects of insulin are opposite to those of epinephrine (2). Hence we examined the possible immediate effect of epinephrine on brain and liver G6PD (Table 2). Rats fed with standard laboratory chow for a week were injected with epinephrine intraperitoneally at 80 micrograms per kg body weight. After 7.5 min., the rats were decapitated and tissues removed for the analysis of G6PD activity. It can be seen from Table 2 that epinephrine significantly decreased the blood glucose concentration and the activity of rat liver G6PD by 17%, but had no apparent effect on the brain enzyme. The mechanism by which epinephrine

decreased G6PD activity is not known. The possibility that the hormone provoked a regulatory phosphorylation of the enzyme could not be confirmed by us, since incubation of homogenates with MgATP and cAMP did not minic the effect of epinephrine (c.f. references 5 and 10). However the possible regulation by phosphorylation of G6PD cannot be ruled out.

Effects of alloxan diabetes on liver and brain G6PD

The levels of circulating insulin in alloxan diabetic rats is very low as compared with that in fasting rats. In order to study the effects of insulin deficiency G6PD activity was measured in diabetic animals. Alloxan diabetes induced by intravenous injection of alloxan (40 mg per kg body weight (8)) was diagnosed three days after injection of alloxan, by elevated levels of blood glucose (Table 3).

Table 3. Effects of alloxan diabetes on G6PD of rat liver and brain. Alloxan diabetes was induced in rats weighing 80-100 g as described under MATERIALS and METHODS in the text. After the injection of alloxan the rats were fed with standard chow for 3 days. Diabetes was diagnosed by an increase in blood glucose using blood from decapitation or a tail vein. Four diabetic rats were treated by intraperitoneal injection of protamine insulin (under asceptic conditions) at a daily dosage of 1.2 units per rat for 7 days before decapitation. Other methods were described under MATERIALS and METHODS in the text. The number of animals used is shown in brackets.

Description	G6PD activity (Units/g tissue)		Glucose (mM)
	Liver	Brain	
Normal (14)	1.15 ± 0.27^{a}	0.85 ± 0.5^{d}	8.2 ± 2.0^{g}
Diabetic (9)	0.62 ± 0.24^{b}	0.43 ± 0.11^{e}	13.1 ± 4.3^{h}
Diabetic + Insulin (4)	0.86 ± 0.14^{c}	0.52 ± 0.08^{f}	5.8 ± 0.8^{i}

The differences between a and b ($P < 0.01$), d and e ($P < 0.05$), g and h ($P < 0.05$) are statistically significant; but differences between a and c, d and f, and g and i are insignificant.

With the development of diabetes, the liver and brain G6PD dropped to about 50% of the normal levels. In a separate group of diabetic animals the effects of insulin treatment was studied. It can be seen in Table 3 that the injection of insulin returned G6PD to 75% and 61% of the original levels in liver and brain respectively. The activity of insulin in these experiments was confirmed by the decrease in blood glucose concentration. It thus appears that insulin administration reverses at least in part, the decrease in G6PD activity observed in alloxan diabetic animals. These observations support the hypothesis that insulin plays an important part in regulating the activity of glucose-6-phosphate

dehydrogenase of both brain and liver of the rat. However, the effect of this hormone upon G6PD in the experiments of Table 3 was less pronounced than that of fasting and refeeding the rats (Table 1). This may indicate that the type of diet plays an important role in controlling glucose-6-phosphate dehydrogenase activity of the liver and brain.

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