Vol. 104, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1982 Pages 84-88

THE TISSUE DISTRIBUTION OF FRUCTOSE-2,6-P_2 AND FRUCTOSE-6-P,2-KINASE IN RATS AND THE EFFECT OF STARVATION DIABETES AND HYPOGLYCEMIA ON HEPATIC FRUCTOSE-2,6-P_2 AND FRUCTOSE-6-P,2-kinase

Masamichi Kuwajima and Kosaku Uyeda

Veterans Administration Medical Center, Dallas, Texas 75216 and the University of Texas Health Science Center, Department of Biochemistry, Dallas, Texas 75235.

Received November 6, 1981

The tissue distribution of fructose-2,6-P_2 and fructose-6-P,2-kinase in rats was determined. The highest concentration of fructose-2,6-P_2 was found in liver, followed by brain, heart muscle, kidney, testis and skeletal muscle in decreasing order. Similar results were obtained with fructose-6-P,2-kinase activities in these tissues. Starvation, streptozotocin-induced diabetes or hypoglycemia lowers the fructose-2,6-P_2 levels and fructose-6-P,2-kinase activity in the liver.

Introduction

Phosphofructokinase, a key regulatory enzyme in glycolysis, is controlled by various metabolites (1). Among the effectors, βD-fructose-2,6-P_2 is the most potent activator of the enzyme (2-5). Fructose-2,6-P_2 is under hormonal control; its level decreases rapidly in hepatocytes in response to glucagon (6,7). More recently, fructose-6-P,2-kinase, the enzyme responsible for the synthesis of fructose-2,6-P_2 was discovered (8) and was also shown to be under hormonal control.

In this communication we determined (a) the level of fructose-2,6-P_2 and activity of fructose-6-P,2-kinase in various rat tissues, and (b) changes in

This work supported by the Veterans Administration and by the United States Public Health Service Grant AM 16194.

0006-291X/82/010084-05$01.00/0

fructose-2,6-P_2 level and fructose-6-P,2-kinase which occur in livers of starved, diabetic and hypoglycemic rats.

Materials and Methods

Muscle phosphofructokinase was prepared from rabbit muscle as before (11).

Male rats (100-200 g) were obtained from Charles Rivers and maintained on standard laboratory chow. Diabetic rats were prepared as follows: Rats were fasted for 24 hours and then streptozotocin (60 mg/kg) was injected intravenously; these rats were maintained on laboratory chow pellets. Urinal volume and blood glucose were determined to insure the rats were diabetic. Serum glucose concentrations of the diabetic rats was between 16 and 22 mM. Rats were made hypoglycemic by starving for 24 hours followed by two injections of insulin (120 units/kg) 1 hour apart and the rats were killed after 2 hrs (12). The serum glucose concentration in these rats was 0.7-1.3 mM. In all these experiments a minimum of 3 rats was used.

Rats were killed early in the morning either by decapitation using a guillotine or injection of 0.3 ml Nembutal. The tissues were immediately freeze-clamped and stored at -70°. The tissues were analyzed for fructose-2,6-P_2 and fructose-6-P,2-kinase activity on the same day. Fresh tissues were also analyzed for comparison, and there was no significant difference between fresh and freeze-clamped tissues.

Extracts for the determination of fructose-2,6-P_2 were prepared as follows: Freeze-clamped tissue (about 100 mg) was broken into small pieces by grinding with a glass rod in the presence of 3 ml 0.1 N NaOH and 0.01 M EGTA in liquid N_2. The mixture was allowed to thaw at 0° and the solution was homogenized with a Polytron homogenizer. The homogenate was centrifuged at 22,000 x g for 10 min. A buffer mixture (0.1 ml) containing 50 mM Tris/P (pH 8) and 5 mM dithiothreitol was added to the supernatant solution and fructose-2,6-P_2 was immediately assayed in the extract. A few preliminary experiments indicated that fructose-2,6-P_2 in these extracts did not change between 15 sec after the homogenization up to 2 hours. Extracts for fructose-6-P,2-kinase assay were prepared as follows: the tissue was homogenized using a Polytron homogenizer in 3 vol. of 50 mM Tris/P, pH 8, 5 mM EGTA, 150 mM NaF and 5 mM dithiothreitol and the homogenate was centrifuged at 20,000 x g for 10 min. The enzyme activity was determined in the supernatant solution as described previously (8).

Fructose-2,6-P_2 level was determined by its ability to relieve ATP inhibition of muscle phosphofructokinase and has been described previously (3,10). In order to insure that fructose-2,6-P_2 is specifically measured with this assay, each sample was incubated at pH 2 and 30° for 15 min under which conditions fructose-2,6-P_2 is completely hydrolyzed, and the acid-treated sample was assayed after neutralization. The fructose-2,6-P_2 concentration is reflected by the difference in activity before and after the acid treatment.

Results and Discussion

The level of fructose-2,6-P_2 in various tissues of rats is shown in Table I. Liver, brain and heart contain the highest concentration of fructose-2,6-P_2, while skeletal muscle contains the lowest concentration of this ester. The activity of fructose-6-P,2-kinase is also highest in liver and lowest in

Table I. Tissue Distribution of Fructose-2,6-P_2 and Fructose-6-P,2-kinase.

Tissue	Fru-2,6-P_2		Fruc-6-P,2-kinase
	units/g	nmoles/g	units/g
Liver			
Normal	10 ± 4	20 ± 3	1.8
Glucose*	17 ± 0.5	34 ± 1	3.2
Brain			
Normal	6.2 ± 1.0	12.4 ± 2.0	0.5
Hypoglycemic+	4.3 ± 0.5	8.6 ± 1.0	
Heart Muscle	6.6 ± 1.2	13.2 ± 2.4	0.45
Kidney	4.0 ± 1.0	8.0 ± 2.0	0.45
Adipose Tissue	3.0 ± 0.2	6.0 ± 0.4	0.28
Testis	1.9 ± 0.1	3.8 ± 0.5	1.0
Skeletal Muscle	1.3 ± 0.3	2.6 ± 0.6	0.19

The procedure for the preparation of tissue samples, extracts and the assay methods for fru-2,6-P_2 and fru-6-P,2-kinase are described under "Methods".

* Rats were starved for 24 hr and 3 ml of 40% glucose given by stomach tubing. The rats were killed 1 hr later.

\+ The serum glucose in the hypoglycemic rats was 0.7-1.3 mM.

skeletal muscle. Fructose-2,6-P_2 and fructose-6-P,2-kinase were not detectable in red cells. The enzyme activities in the other tissues, except testis, seem to correlate with the fructose-2,6-P_2 contents. The fructose-2,6-P_2 concentration in liver (as well as kidney) vary significantly. Administration of glucose results in 70% increase in both fructose-2,6-P_2 and fructose-6-P, 2-kinase activity. These results are in agreement with those obtained in vitro using isolated hepatocytes (6).

There appears to be no direct correlation between phosphofructokinase content or glycolytic activity of a tissue and its fructose-2,6-P_2 level. For example, the phosphofructokinase content and the glycolytic rate of skeletal muscle is several-fold higher than that of cardiac muscle, yet fructose-2,6-P_2

level and fructose-6-P,2-kinase activity is 4-fold lower in the skeletal muscle than heart muscle. Furthermore, the fructose-2,6-P_2 concentrations in the skeletal muscle and the heart of excercised rats is similar to that of the control. These results suggest that fructose-2,6-P_2 may not be an important effector of phosphofructokinase in skeletal muscle (and erythrocytes) and that the importance of fructose-2,6-P_2 in the control of this enzyme may depend upon the tissue.

The effect of starvation, hypoglycemia and diabetes on the fructose-2,6-P_2 level and fructose-6-P,2-kinase activity in liver is summarized in Table II. Starvation for 72 hours results in a loss of over 80% of fructose-2,6-P_2 and a similar decrease in fructose-6-P,2-kinase activity. Insulin induced hypoglycemia following a 24 hour fast results in the complete disappearance of fructose-2,6-P_2 in the liver and only 10% of fructose-6-P,2-kinase activity remaining compared to the control rats. The streptozotocin-induced diabetic rats also have 60% lower fructose-2,6-P_2 and 70% lower fructose-6-P,2-kinase

Table II. Effect of Starvation, Hypoglycemia and Diabetes on Fructose-2,6-P_2 and Fructose-6-P,2-kinase in Liver.

Treatment	Fru-2,6-P_2	Fru-6-P,2-kinase
	nmoles/g	units/g
Control	20 ± 3	1.8
Starvation		
24 hours	22 ± 1	1.2
48 hours	13 ± 0.3	0.84
72 hours	2.4 ± 0.4	0.43
Hypoglycemia	0	0.22
Diabetes	6.0 ± 0.8	0.44

The procedures for the preparation of these disease states are described under "Methods".

activity compared to the controls. These results are interesting since high concentrations (>10 mM) of glucose induce the synthesis of fructose-2,6-P_2 while glucagon effectively lowers its level (6). Under the diabetic conditions, high glucagon concentrations apparently overcome the effect of high concentrations of glucose in the circulating blood.

These observations suggest that the decrease in the level of fructose-2,6-P_2 is a reflection of the inactivation of fructose-6-P,2-kinase as suggested in the values found in different tissues of normal rats (Table I) as well as those in the livers of the diseased states of rat (Table II). Furthermore, the low fructose-2,6-P_2 levels and fructose-6-P,2-kinase activity in the starved, hypoglycemic and diabetic animals are consistent with our previous *in vitro* demonstration that fructose-6-P,2-kinase is inactivated by phosphorylation catalyzed by cAMP-dependent protein kinase (13) as well as *in vivo* data that glucagon stimulates fructose-6-P,2-kinase inactivation in isolated hepatocytes (9).

References

1. Uyeda, K. (1979) Adv. Enzymol. Relat. Areas Mol. Biol. 48, 193-244.
2. Furuya, E. and Uyeda, K. (1980) Proc. Natl. ACad. Sci. USA 77, 5861-5864.
3. Uyeda, K., Furuya, E. and Luby, L.J. (1981) J. Biol. Chem. 256, 8394-8399.
4. Van Schaftingen, E., Jett, M.F., Hue, L. and Hers, H.-G. (1981) Proc. Natl. Acad. Sci. USA 78, 3483-3486.
5. Pilkis, S.J., El-Maghrabi, M.R., Pilkis, J., Claus, T.H. and Cumming, D.A. (1981) J. Biol. Chem. 256, 3171-3174.
6. Richards, C.S. and Uyeda, K. (1980) Biochem. Biophys. Res. Comm. 97, 1535-1540.
7. Van Schaftingen, E., Hue, L. and Hers, H.-G. (1980) Biochem. J. 192, 881-895.
8. Furuya, E. and Uyeda, K. (1981) J. Biol. Chem. 256, 7109-7112.
9. Richards, C.S., Furuya, E. and Uyeda, K. (1981) Biochem. Biophys. Res. Comm. 100, 1673-1679.
10. Uyeda, K., Furuya, E. and Sherry, A.D. (1981) J. Biol. chem. 256, 8679-8684.
11. Uyeda, K. (1969) Biochemistry 8, 2366-2373.
12. Tarr, M. (1962) Am. J. Physiol. 203, 690-692.
13. Furuya, E., Yokoyama, M. and Uyeda, K. (1981) Proc. Natl. Acad. Sci. (in press).