Vol. 106, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 14, 1982 Pages 175-178

Effects of Fasting and Insulin on Hepatic Phosphofructokinase (PFK)

Francis Binkley and Susan M. Richardson

Department of Biochemistry, Emory University, School of Medicine

Atlanta, GA 30322

Received March 29, 1982

Summary: Fasting of susceptible mice for 24 hr led to a sharp decrease of apparent hepatic PFK activity which was increased to near fed levels by incubation of cytosolic fractions with GSH. Incubation of the cytosols with GSSG reduced the PFK to a lower level-(GSSG insensitive activity). The action of insulin was to increase total PFK with a marked increase of the GSSG insensitive activity.

Introduction: Phosphofructokinase (PFK) of muscle appears to be controlled by large changes in substrate and metabolite levels depending upon the activity of the muscle. The evidence for such substrate and metabolite control of hepatic PFK is not convincing and other mechanisms of control are probably required to explain the marked shift to gluconeogenesis with fasting. Isaacs and Binkley (1,2) have shown that fasting of lean animals for 24 hr leads to the formation of mixed disulfides of cytosolic proteins with GSH and have postulated that such formation could have a marked effect on hepatic metabolism.

This study was concerned with the effects of fasting on the PFK activities of hepatic cytosols, the effects of preincubation with GSH (to reverse the formation of mixed disulfides) and with GSSG (to promote formation of mixed sulfides). The effects of insulin were investigated to determine the effects, if any, on the sensitivity of hepatic PFK to GSH and GSSG.

Materials and Methods: Animals used in the study were 7-8 week old males from a local CD-1 inbred colony (original stock from Charles River), and C57Bl/6J purchased from Jackson Laboratories. Average weights of the animals were 31 g for the CD-1 and 22 g for the C57Bl/6J. Average weight loss on fasting was 2 g for the CD-1 and 3 g for the C57Bl/6J. With fasting the liver weights for the CD-1 animals decreased from 1.9 to 1.4 g compared to from 1.1 to 0.9 g for the C57Bl/6J. The animals were maintained on a 12-hour, light-dark cycle and Purina mouse chow and water were furnished ad libitum. "Fasted mice" were removed to clean cages (to prevent scavenging from the cage floor) and fasted for 24 hr with access to water only. All procedures were initiated at 9:00 A.M. The mice were anesthetized with CO_2, decapitated and blood was collected in heparinized tubes for determination of glucose in the plasma by the method of Goodwin

0006-291X/82/090175-04$01.00/0

(3). The livers were perfused in situ with 1 ml or more of physiological saline to produce total blanching. The livers were then removed, blotted dry and homogenized in a Potter-Elvehjem homogenizer with cold (4°) 0.25 M sucrose, 0.04 M Tris buffer, pH 7.4, 9 ml per g wet weight. The homogenates were centrifuged at 15,000 g for 30 min at 0° in a Sorvall centrifuge and the supernatant was the "cytosol" used in these studies. Results differed very little when all solutions were saturated with N_2. PFK was assayed at 25° by the method of Dunaway and Weber (4) (without added thiols) with reagents from Sigma and daily verification of the method with PFK from rabbit muscle (Sigma). Protein was determined by the method of Lowry et al. (5) with bovine serum albumin as a standard. In the preincubation studies, the cytosols were incubated at 25° with 0.005 M GSH or 0.0025 M GSSG (neutralized) in the assay buffer (less substrates and enzymes) for 30 min and appropriate dilutions were assayed for PFK in the linear range; the final concentration of GSH and GSSG in the assay was 10^{-4} M. "Iced", 0°, controls did not differ from controls preincubated at 25° for 30 min. Preincubation with 0.005 M AMP, ADP, citrate or inorganic phosphate had little or no effect with the final concentration of 10^{-4} M in the assay mixture. Insulin (Iletin) was given IP, 0.1 unit per Kg, and the animals were sacrificed after 30 min. The average lowering of plasma glucose was about 30 mg dl^{-1}.

Results and Discussion

GSH and GSSG (5 mM) preincubated with the rabbit muscle PFK or mouse muscle cytosols did not alter the activity nor did 50 μM added to the assay mixture alter the results with the commercial muscle PFK or the cytosols of skeletal muscle of the mice. Other controls included the reactivation of the GSSG-decreased activity of liver cytosols by the addition of 5 mM dithiothreitol and by addition of 5 mM glucose-6-phosphate and 1mM NADP and the inactivation to the GSSG-treated level by 5 mM t-butyl hydroperoxide with reactivation with 5 mM glucose-6-phosphate and 1 mM NADP (2).

Thirty min was chosen as the time of preincubation after the finding that 20 min was sufficient for maximal changes (2-3 fold greater than the effects at 5 min). This does not apply to cytosols of renal tissue. After 5 min or less activation of renal PFK by GSH, the GSSG sensitive activity was rapidly destroyed and could not be reactivated with glucose-6-phosphate and NADP.

The results in Table I indicate that the mouse livers contain at least two isozymes, one sensitive and the other insensitive to treatment with GSSG. Pretreatment of cytosols with GSH significantly increased the PFK activity of only the C57Bl/6J mice (small and lean) and had little effect with cytosols of the CD-1 mouse (heavier and fatter). The effect of pretreatment with GSSG was very similar so that the difference between the GSH and GSSG treated cytosols were essentially identical with both strains.

Feeding habits of the two strains vary significantly in that the lean mice feed only early in the dark period, whereas the heavier obese mice feed throughout the day.

Table I

Effects of Fasting and Insulin on Hepatic PFK of Two Strains of Mice

	C57B1/6J Mice			
			Insulin	
Cytosol treatment	Fed	Fast	Fed	Fast
Control	14 ± 3	7 ± 2	39 ± 4	18 ± 5
GSH	24 ± 2	21 ± 3	40 ± 2	24 ± 5
GSSG	5 ± 2	5 ± 2	21 ± 3	13 ± 2
(GSH-GSSG)	19	16	19	11
Blood glucose	144 ± 18	96 ± 9	94 ± 8	59 ± 6
	CD-1 Mice			
Control	25 ± 6	22 ± 3	39 ± 5	22 ± 4
GSH	27 ± 5	27 ± 4	38 ± 6	40 ± 7
GSSG	6 ± 2	8 ± 2	20 ± 3	21 ± 3
(GSH-GSSG)	21	19	18	19
Blood glucose	194 ± 16	179 ± 12	127 ± 9	103 ± 7

PFK values are expressed as μmol min^{-1} mg protein $^{-1}$ x 10^3. Glucose is expressed as mg dl^{-1}. There were six or more animals in each group. Means and SEM are given. GSH - GSSG is a measure of the GSSG sensitive form(s) of PFK.

The ob+/ob+ mouse, derived from the C57B1 mouse, has a high level of GSSG insensitive PFK that is little altered by additional insulin (data not given); these animals are reported to be hyperinsulinemic (6). Dunaway and Weber (4) have described two isozymes in rat liver, L_1 and L_2, with L_1 as the major isozyme (about 85 per cent). Dithiothreitol was present in their extraction and assay solutions and not in ours. Tanaka, Au and Sakane (7) did not mention the use of thiols in their preparations and described rat liver as having a single isozyme. Gonzalez, Tsai and Kemp (8) utilized dithriothreitol in extraction and in electrophoresis and described three isozymes in the liver of the CBA/J mouse; they labeled these isozymes as B_4, AB_3 and A_2B_2 and the single enzyme of rat liver as A_4. Kurata, Matsushima and Sugimura (9) described a single isozyme of rat liver and reported two different isozymes in the kidney.

The effect of insulin was to induce or activate a GSSG insensitive form of PFK with no apparent effect on the GSSG sensitive form. This would provide the liver with increased glycolysis even with mixed disulfides present or with a lowered level of the sensitive form. Similar effects of insulin on hepatic PFK of rats has been reported by others (10,11). Dunaway and Weber reported that the increase was with the major isozyme whereas the increase in our studies was with a normally minor form. However, the animals and criteria differ and it is not possible to make any direct comparison.

These results indicate that the mixed disulfide mechanism may be an important control of hepatic carbohydrate metabolism. The inactivation of phosphorylase phosphatase as the mixed disulfide with GSH (12) is another example pointing toward this type of control as is the activation of eIF-2 by GSSG (13). The relationship of this mechanism to the protective peptide (with a high cysteine content) (14) and to the newly discovered activator of PFK (15) is not apparent.

We are indebted to Dr. D.M. Ziegler for helpful criticism.

References

1. Isaacs, J.T. and Binkley, F. (1977) Biochim. Biophys. Acta 497, 192-204
2. Isaacs, J.T. and Binkley, F. (1977) Biochim. Biophys. Acta 498, 29-38
3. Goodwin, J.F. (1970) Clin. Chem. 16, 85-91
4. Dunaway, G.A. and Weber, G. (1974) Arch. Biochem. Biophys. 162, 620-628
5. Lowry, O.H., Rosebrough, V.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
6. Herberg, L. and Coleman, D.L. (1977) Metabolism 26, 59-99
7. Tanaka, T., Au, T. and Sakane, Y. (1971) J. Biochem. 69, 609-612
8. Gonzalez, F., Tsai, M.Y., Kemp, R.G. (1975) Comp. Biochem. Physiol. 52, 315-319
9. Kurata, N., Matsushima, T. and Sugimura, T. (1972) Biophys. Biochem. Res. Commun. 48, 473-479
10. Dunaway, G.A. and Weber, G. (1974) Arch. Biochem. Biophys. 162, 629-637
11. Taunton, D.O., Stifel, F.B., Greene, H.L. and Herman, R.H. (1974) J. Biol. Chem. 249, 7228-7239
12. Shimazu, T. Tokutake, S. and Usami, M. (1978) J. Biol. Chem. 253, 7376-7382
13. Clemens, M.J., Safer, B., Merrick, W.C., Anderson, W.F. and London, I.M. (1975) Proc. Natl. Acad. Sci. USA 72, 1286-1290
14. Dunaway, G.A., Jr., and Segal, H.L. (1976) J. Biol. Chem. 251, 2323-2329
15. Van Schaftingen, E. and Hers, H.G. (1980) Biochem. Biophys. Res. Commun. 96, 1524-1531