Metabolism
Clinical and Experimental

VOL 47, NO 6 JUNE 1998

PRELIMINARY REPORT

Glucose-6-Phosphatase Activity in the Hypothalamus of the ob/ob Mouse

Akhtar Khan, Ling Zong-Chao, Suad Efendic, and Bernard R. Landau

The hypothalamus and cortex from ob/ob mice and their lean littermates were sonicated and then incubated with glucose-6-phosphate (glucose-6-P) and glycerol phosphate (glycerol-P). The difference between the rates of hydrolysis of glucose-6-P and glycerol-P was taken as the measure of glucose-6-phosphatase activity. The activity was much higher in the hypothalamus from ob/ob mice versus their lean littermates. Activity was undetected in the cortex. These findings raise the possibility that a defect in the regulation of glucose-6-phosphatase activity in a portion of the hypothalamus may relate to the mechanism underlying obesity in the ob/ob mouse. However, obese gene product administration to ob/ob mice, while reducing the body weight, did not alter the glucose-6-phosphatase activity.

GLUCOSE-6-PHOSPHATASE activity, although low in comparison to that in the liver and kidney, is present in the brain.[1-3] The activity appears to have characteristics of classic microsomal glucose-6-phosphatase as evidenced by latency, membrane association, and pH dependence.[3] Activity in histochemical and cytochemical preparations was localized mainly to neuronal cell bodies and dendritic stems,[4] but localization solely to astrocytes using an immunohistochemical technique has been reported.[3] Quantitative assessments of the enzyme's regional distribution are few, but no region appears to have particular activity.[4] The activity in rat brain has been reported to change in response to glucocorticoid and sex hormone administration.[5,6] A futile cycle of glucose phosphorylation to glucose-6-phosphate (glucose-6-P) and simultaneous hydrolysis of glucose-6-P catalyzed by the phosphatase, called glucose cycling, was concluded to be of considerable magnitude in rat brain.[7,8] However, other studies report the contrary.[9,10]

The ob/ob mouse provides an animal model of non–insulin-dependent diabetes mellitus, exhibiting both hyperglycemia in the presence of functioning pancreatic β cells and obesity. We found glucose cycling in islets of the ob/ob mouse such that 30% to 40% of the phosphorylated glucose was dephosphorylated. In islets from lean littermates, cycling was only 4%.[11] Glucose-6-phosphatase activity was correspondingly much higher in islets of the ob/ob mouse compared with lean littermates, offering a possible explanation for the hyperglycemia in ob/ob mice.[12]

Hunger is a consequence of a decrease in the blood glucose concentration. Hunger has been related to changes in electrical activity and glucose uptake in the nuclei of the hypothalamus, the so-called center of satiety and feeding.[13] We hypothesized that glucose cycling might be increased in sites within the hypothalamus of the ob/ob mouse, decreasing glucose utilization in those portions, resulting in increased appetite, and thus providing a possible explanation for the obesity. Accordingly, we assessed glucose-6-phosphatase activity in hypothalami from ob/ob mice and their lean littermates. The cortex was used as an additional control.

MATERIALS AND METHODS

Materials

[1-^{14}C]glucose-6-P (60 mCi/mmol) and [2-^{3}H]glycerol-3-P (15 Ci/mmol) were purchased from DuPont NEN Research Products (Boston, MA). Glucose-6-P, β-glycerol-P, and HEPES were purchased from Sigma Chemical (St Louis, MO), and Amberlite MB-3 monobed resin was from BDH (Poole, UK). Glucose oxidase was obtained from

From the Department of Molecular Medicine, Karolinska Hospital, Stockholm, Sweden; and the Departments of Medicine and Biochemistry, Case Western Reserve University, Cleveland, OH.

Submitted August 6, 1997; accepted December 23, 1997.

Supported by grants from the Swedish Medical Research Council (00034), Eli Lilly & Co (Indianapolis, IN), and the National Institutes of Health (DK-14507), and a Fogarty International Center Senior Fellowship Award (TW 01986).

Address reprint requests to Akhtar Khan, PhD, Department of Molecular Medicine, Endocrine and Diabetic Unit, Karolinska Hospital, 171 76 Stockholm, Sweden.

0026-0495/98/4706-0001$03.00/0

Boehringer (Mannheim, Germany). Biosynthetic mature human obese gene product (hoB) was a gift from Eli Lilly & Co (Indianapolis, IN).[14]

The ob/ob mice aged 10 to 12 months and weighing 50 to 85 g and their lean littermates weighing 18 to 36 g were killed by decapitation. The brains were split at the midline, and the hemihypothalami were dissected, as well as portions of the cortex. Anatomical limits for dissection of the hypothalamus were from the beginning of the anterior commissure to the end of the mammillary body, the border between the thalamus and the hypothalamus, and a distance of about 1 mm from the midline.[15] All procedures were performed at 0° to 4°C.

Before killing, three ob/ob mice were injected daily for 1 week intraperitoneally with 100 μg hoB in 0.1 mL phosphate-buffered saline (PBS), while three other ob/ob mice were injected daily with only 0.1 mL PBS. Three other ob/ob mice and simultaneously three lean mice were injected daily for 1 week intraperitoneally with 100 μg hoB in 0.1 mL PBS.

Incubations

Glucose-6-phosphatase activity was measured by the hydrolysis of glucose-6-P to glucose. Between 5 and 15 mg hypothalamus or cortex was placed in a tube containing 0.2 mL 50-mmol/L HEPES (pH 7.4) and sonicated for 5 to 10 seconds. Then, 0.8 μCi [1-^{14}C]glucose-6-P (4mmol/L) was added and incubation was performed for 30 minutes at 37°C. The incubation was stopped by placing the tubes in ice water and adding 0.2 mL 0.3-mol/L $ZnSO_4$ and after mixing 0.2 mL of a saturated solution of $Ba(OH)_2$. An identical incubation was performed, but without the addition of tissue. To assess the contribution of nonspecific phosphatase(s) to hydrolysis of glucose-6-P, the hydrolysis of glycerol-P to glycerol was measured. Incubation was as described before, except that 1 μCi [2-^{3}H]glycerol-P (4 mmol/L) was added instead of [1-^{14}C]glucose-6-P.

Measurement of Activity

After adding 2 mg glucose to each tube containing labeled glucose-6-P and 0.9 mg glycerol to each tube containing labeled glycerol-P, the contents of each tube were mixed by vortexing and centrifuged for 2 minutes in an Eppendorf centrifuge (10,000 × *g*). The supernatant was passed through a column of the MB-3 resin, and the effluent was evaporated to dryness. The residue remaining after evaporation of the effluent was dissolved in 1 mL H_2O, and 0.5 mL was counted for radioactivity. The dpm in the residue was taken as the dpm in glucose or glycerol. The residue on high-performance liquid chromatography (HPLC) using a Bio-Rad 87C column (Bio-Rad, Hercules, CA) showed single radioactive peaks with the mobility of glucose and glycerol. A mean of 75% of the 2 mg glucose measured using glucose oxidase (model 23A Glucose Analyzer; Yellow Springs Instrument, Yellow Springs, OH) and an average of 73% of the 0.9 mg glycerol measured using glycerol dehydrogenase[16] were recovered in the residue. Correction was made for these losses. When the HPLC fraction with the mobility of glucose was incubated with glucose oxidase and the resulting reaction mixture was deionized and concentrated and the concentrate chromatographed, there was no longer radioactivity with the mobility of glucose. An average of 95% of labeled glucose and glycerol added to the HPLC column was recovered in the peaks.

For the radioactivity of [1-^{14}C]glucose-6-P incubated with hypothalami from ob/ob and lean mice, the recovery in glucose was, respectively, 5.2% ± 0.2% and 3.5% ± 0.2%. Recovery in incubations with the cortex from ob/ob mice was 2.9% ± 0.5% and from lean mice 2.6% ± 0.4%. In the incubations without tissue, 0.14% ± 0.02% of the radioactivity added was recovered in glucose. For the radioactivity of [2-^{3}H]glycerol-3-P incubated with hypothalami from ob/ob and lean mice, recovery in glycerol was, respectively, 3.6% ± 0.4% and 3.0% ± 0.8%. Recovery in incubations of cortex from ob/ob mice was 3.6% ± 0.5% and from lean mice 3.1% ± 0.3%. In the incubations without tissue, 0.8% ± 0.4% was recovered in glycerol. Phosphatase activity is expressed in nanomoles per milligram wet weight per 30 minutes by dividing the radioactivity in glucose and glycerol in the presence minus the absence of tissue at completion of 30 minutes of incubation, by the specific activity in dpm per nanomole of incubated [1-^{14}C]glucose-6-P and [2-^{3}H]glycerol-P, and by the wet weight of tissue.

Table 1. Rates of Hydrolysis of Glucose-6-P and Glycerol-P (nmol/mg wet weight/30 min) by Sonicated Hypothalamus and Cortex From ob/ob and Lean Mice

Tissue	Glucose-6-P	Glycerol-P	Difference
Hypothalamus			
ob/ob (n = 9)	3.17 ± 0.20*	1.71 ± 0.10	1.46 ± 0.24
Lean (n = 7)	1.86 ± 0.11	1.72 ± 0.14	0.14 ± 0.16
Cortex			
ob/ob (n = 5)	1.55 ± 0.13	1.54 ± 0.21	0.01 ± 0.19
Lean (n = 3)	1.41 ± 0.23	1.40 ± 0.34	0.01 ± 0.31

*$P < .001$ *v* lean.

Statistics

Results are recorded as the mean ± SEM. The significance of differences was determined by a paired *t* test.

RESULTS

Specific glucose-6-phosphatase activity is taken as the difference between the rates of glucose-6-P and glycerol-P (nonspecific) hydrolysis (Table 1).[17] The difference was significant, a mean of 48.8 nmol/mg/min, in the hypothalamus from ob/ob mice. There was no significant difference, a mean less than one tenth as much, ie, 4.6 nmol/mg/min, in the hypothalamus from lean mice. In the cortex from ob/ob and lean mice, the rates of hydrolysis of [^{14}C]glucose-6-P to [^{14}C]glucose and of [^{3}H]glycerol-3-P to [^{3}H]glycerol were the same. Although hoB treatment reduced the weight of ob/ob mice ($P < .05$), it did not affect glucose-6-phosphatase activity (Table 2).

Table 2. Rates of Hydrolysis of Glucose-6-P and Glycerol-P (nmol/mg wet weight/30 min) by Sonicated Hypothalamus From ob/ob and Lean Mice Treated With hoB

Experiment No.	hoB	Initial Weight (g)	Weight Change (%)	Glucose-6-P	Glycerol-P	Difference
1						
ob/ob (n = 3)	+	62 ± 4	−9.3 ± 0.9	4.41 ± 0.48	2.70 ± 0.36	1.71 ± 0.36
ob/ob (n = 3)	−	65 ± 7	+0.7 ± 0.4	4.68 ± 0.30	2.76 ± 0.15	1.92 ± 0.29
2						
ob/ob (n = 3)	+	61 ± 2	−7.7 ± 0.6	4.74 ± 0.42*	2.43 ± 0.24	2.31 ± 0.40
Lean (n = 3)	+	23 ± 4	+2.1 ± 3.8	2.34 ± 0.18	2.40 ± 0.24	0.06 ± 0.13

*$P < .05$ *v* lean.

DISCUSSION

The finding of the same extent of hydrolysis of glucose-6-P and glycerol-P by the hypothalamus from lean mice and by the cortex supports the notion that the hydrolysis is due to nonspecific phosphatase(s), ie, phosphatase(s) not differentiating between glucose-6-P and glycerol-P. This finding supports the use of the difference between glucose-6-P and glycerol-P hydrolysis as a measure of specific glucose-6-phosphatase activity.[17] The activity may or may not be due to a microsomal glucose-6-phosphatase as found in the liver. Glucose-6-phosphatase activity in the hypothalamus from ob/ob mice was much higher than in the hypothalamus from lean mice (Table 1). The rate of hydrolysis of [^{3}H]glycerol-3-P was similar in the ob/ob and lean mice hypothalamus, so the activity of nonspecific phosphatase(s) appears to be the about the same in both. There was no difference and hence no detectable glucose-6-phosphatase activity in the cortex.

The increased hydrolysis of glucose-6-P in the ob/ob hypothalamus and not in the hypothalamus from lean littermates or in the cortex is an intriguing observation whose importance, if any, is unknown. It became particularly intriguing because of the finding that a deficiency in the obese gene product appears to be the cause of obesity in the ob/ob mouse, acting at the level of the hypothalamus.[18-19] Hence, the possibility was considered that it acts through regulation of glucose-6-phosphatase activity. However, administration of hoB to the ob/ob mouse for 1 week, while producing weight loss, did not alter the activity.

REFERENCES

1. Karnovsky ML, Anchors JM, Zoccoli MA: Glucose-6-phosphatase from cerebrum. Methods Enzymol 90:396-402, 1982
2. Colilla W, Jorgenson RA, Nordlie RC: Mammalian carbamyl phosphate: Glucose phosphotransferase and glucose-6-phosphate phosphohydrolase: Extended tissue distribution. Biochim Biophys Acta 377:117-125, 1975
3. Forsyth RJ, Bartlett K, Burchell A, et al: Astrocytic glucose-6-phosphatase and the permeability of brain microsomes to glucose-6-phosphate. Biochem J 294:145-151, 1993
4. Pertsch M, Duncan GE, Stumpf WE, et al: A histochemical study of the regional distribution in the rat brain of enzymatic activity hydrolyzing glucose- and 2-deoxyglucose-6-phosphate. Histochemistry 88:257-262, 1988
5. Garcia MV, Cabezas JA, Perez-Gonzalez MN: Alterations in the activities of subcellular fraction marker enzymes in rat liver and brain by hydrocortisone and corticosterone treatment. Int J Biochem 17:203-205, 1985
6. Garcia MV, Cabezas JA, Perez-Gonzalez MN: Effect of oestradiol, testosterone and medroxyprogesterone on subcellular fraction marker enzyme activities from rat liver and brain. Comp Biochem Physiol 80:347-354, 1985
7. Huang MT, Veech RL: The quantitative determination of the in vivo dephosphorylation of glucose-6-phosphate in rat brain. J Biol Chem 257:11358-11363, 1982
8. Huang MT, Veech RL: Metabolic fluxes between [^{14}C]2-deoxy-D-glucose and [^{14}C]2-deoxy-D-glucose-6-phosphate in brain in vivo. J Neurochem 44:567-573, 1985
9. Nelson T, Lucignani G, Atlas S, et al: Reexamination of glucose-6-phosphatase activity in the brain in vivo: No evidence for a futile cycle. Science 229:60-62, 1985
10. Nelson T, Lucignani G, Goochee J: Invalidity of criticisms of the deoxy-glucose method based on alleged glucose-6-phosphatase activity in brain. J Neurochem 46:905-919, 1986
11. Khan A, Chandramouli V, Östenson C-G, et al: Glucose cycling is markedly enhanced in pancreatic islets of obese hyperglycemic mice. Endocrinology 126:2413-2416, 1990
12. Khan A, Hong-Lie C, Landau BR: Glucose-6-phosphatase activity in islets from ob/ob and lean mice and the effect of dexamethasone. Endocrinology 136:1934-1938, 1995
13. Bray GA, Fisler J, York DA: Neuroendocrine control of the development of obesity: Understanding gained from studies of experimental animal models. Front Neuroendocrinol 11:128-181, 1990
14. Stephens TW, Basinski M, Bristow PK, et al: The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 377:530-532, 1995
15. Slotnick BM, Leonard CM: A Stereotaxic Atlas of the Albino Mouse Forebrain. Rockville, MD, US Department of Health, Education and Welfare, Alcohol, Drug Abuse and Mental Health Administration, 1975
16. Wieland O: Glycerol UV-method, in Bergmeyer HU (ed): Methods of Enzymatic Analysis, vol 3. Deerfield Beach, FL, Verlag Chemie, 1974, pp 1404-1409
17. Täljedal I-B: Presence, induction and possible role of glucose-6-phosphatase in mammalian pancreatic islets. Biochem J 114:387-394, 1969
18. Zhang Y, Proenca R, Maffei M, et al: Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432, 1994
19. Schwartz MW, Seeley RJ, Campfield LA, et al: Identification of targets of leptin action in rat hypothalamus. J Clin Invest 98:1101-1106, 1996