HEPATIC ENZYME ACTIVITIES AND PLASMA INSULIN CONCENTRATIONS IN DIABETIC HERBIVOROUS VOLES

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ABSTRACT


The activities of the hepatic glycolytic enzymes glucokinase (GKase) and hexokinase (HKase) in herbivorous Microtus arvalis were very low and the hepatic fructose-1,6-diphosphatase (FDPase) activities were almost the same as those in C57BL/6J mice. Glycosuria was observed in over 50% of voles fed on a low fibre, high concentrate diet. Voles with a high incidence of glycosuria for over 6 weeks became insulin deficient. In these diabetic voles, the hepatic GKase, HKase and FDPase activities decreased considerably as a result of diminished insulin secretion and fatty degeneration of the hepatic cells. It was considered that M. arvalis would be a useful animal model in which to study disorders of glucose utilization in herbivora.

Keywords: diabetes, enzymes, insulin, liver, mice, Microtus, plasma, voles

INTRODUCTION

Herbivorous voles, Microtus arvalis Pallas, have the unusual nutritional characteristic that fermentation products in the digestive tract are used for energy (Kudo and Oki, 1982; 1984). The bacterial flora in the forestomach can easily be changed by changing the diet (Kudo and Oki, 1981). In voles supplied with a low fibre, high concentrate diet, alimentary diabetes was induced and the fermentation ability of the forestomach lowered (Arai and Oki, 1983; Arai et al., 1985). To clarify the metabolic characteristics of these voles as herbivores, the plasma insulin concentrations and hepatic glycolytic and gluconeogenic enzyme activities were measured in normal and diabetic voles and compared with those in normal mice. The histological changes in the liver and pancreas were also observed in the diabetic voles.

MATERIALS AND METHODS

M. arvalis were maintained in our laboratory. Normal control voles were fed on commercial pellets for herbivora (ZC, crude fibre 12.5%; Oriental Yeast Co., Tokyo, Japan) and cubed hay. Experimental voles were fed on pellets for mice or rats (CMF, crude fibre 3.5%; Oriental Yeast Co.) after weaning at 3 weeks of age, so as to induce diabetes. C57BL/6J mice fed on CMF pellets were also used for comparison.
Glycosuria was assessed using Tes Tape (Eli Lilly Co., Indianapolis, IN) weekly after
the animals were 4 weeks old. The animals were killed at 30 weeks of age by
decapitation under ether anaesthesia. Blood samples were collected from the jugular
veins into heparinized microfuge tubes and the pancreas and liver were removed
immediately after decapitation. The glucose concentration in whole blood was
measured by a glucose-oxidase method (Huggett and Nixon, 1957). The plasma
insulin concentration was measured by the micro ELISA sandwich method (Arai
et al., 1983). Guinea pig antibovine insulin (ICN ImmunoBiologicals, Lisle, Israel),
which had been diluted 2000 times in distilled water, was dispensed in 200 μl volumes
into each well of a polystyrene microtitre plate (Dynatech Laboratories Inc., Chantilly,
VA). The plate was stored overnight at 4°C. Plasma samples and insulin standard
solutions (Sigma Chemical Co., St. Louis, MO), which were diluted 1:5 with 0.01
mol/L phosphate buffer (pH 7.4) containing 0.15 mol/L NaCl (PBS) and 0.5% (w/v)
bovine serum albumin (diluent), were added in duplicate to the plate. After
incubation at 37°C for 3 h the plate was washed twice with PBS and 50 μl of
β-galactosidase conjugated guinea pig antiswine insulin (Mitsui Pharmaceutical Inc.,
Tokyo, Japan) diluted 1:1000 with diluent was dispensed into each well. After being
incubated again at 37°C for 2 h, the plate was washed twice, 50 μl of 0.1% (w/v)
2-nitrophenyl-β-D-galactopyranoside (Wako Pure Chemical Industries, Osaka, Japan)
as enzyme substrate was added and the plate was again incubated at 37°C for 1 h. The
reaction was terminated by the addition of 200 μl of 1% (w/v) sodium carbonate and
the absorbance at 410 nm of each well was determined.

The fresh liver was weighed and homogenized in a glass tube with two volumes of
buffer containing 0.05 mol/L tris-hydroxyethylaminomethane, Tris-HCl, 1 mmol/L
ethylenediaminetetra-acetic acid (EDTA) and 5 mmol/L 2-mercaptoethanol (pH
7.4). The homogenate was centrifuged at 100 000 g for 1 h and the resulting super-
natant was used for the assay of glucokinase (GKase), hexokinase (HKase) and
fructose-1,6-diphosphatase (FDPase). GKase and HKase activities were determined
according to the method of Bergmeyer et al. (1974). The reaction mixture contained
40 mmol/L triethanolamine, 0.91 mmol/L NADP, 0.64 mmol/L ATP, 8.0 mmol/L
MgCl₂ and 1.2 units of glucose-6-phosphate dehydrogenase in a final volume of
2.51 ml at pH 7.6. In addition to these ingredients, cuvette A contained 50 mmol/L
N-acetylglucosamine, cuvette B contained 0.5 mmol/L glucose and cuvette C
contained 200 mmol/L glucose. The reaction was started by the addition of 0.02 ml
freshly prepared enzyme extract (100 000 g supernatant) to each cuvette. The form-
ination of NADPH was recorded spectrophotometrically at 340 nm for 15 min at 25°C.
The difference between B and A in the rate at which NADPH was formed was a
measure of HKase activity and the difference between C and B was a measure of
GKase activity. The activities of GKase and HKase were expressed as nmol of
NADPH formed per min per mg protein.

The activity of FDPase was assayed by the method of Latzko and Gibbs (1974).
The reaction mixture contained 0.1 mol/L Tris-HCl, 10 mmol/L MgCl₂, 0.5 mmol/L
NADP, 3.5 units of phosphoglucone isomerase, 0.7 units of glucose-6-phosphate
dehydrogenase and 0.5 ml of freshly prepared enzyme extract at pH 8.8 in a final
volume of 2 ml. The reaction was initiated by the addition of 0.6 mmol/L of
fructose-1,6-diphosphate. The formation of NADPH at 25°C was measured
spectrophotometrically at 340 nm. The activity of FDPase was expressed as nmol of
NADPH formed per min per mg protein. The protein concentration was determined