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# Effect of Streptozotocin Diabetes on Acid Phosphatase and Selected Glycosidase Activities of Serum and Various Rat Organs (37786)

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The vascular lesions associated with *diabetes mellitus* may be related to alterations in the amounts of saccharides normally bound in mucopolysaccharides and glycoproteins. Glycoproteins from serum and some tissues from experimentally diabetic rats contain less bound carbohydrate than normal controls, while there is an increase in the serum concentration of free monosaccharides (1–2). Lowered protein-bound carbohydrate levels in some tissues of experimentally diabetic animals could reflect either an impaired synthesis of glycoproteins or an increased rate of glycoprotein catabolism. Evidence favoring the latter concept is the detection of elevated activities of β-D-glucuronidase,[1] and *N*-acetyl-β-D-glucosaminidase in sera from diabetic patients (3–6) and in sera and lens (with other glycosidases) of alloxan–diabetic rabbits (7, 8). These enzymes are of lysosomal origin and are reported to be involved in mucopolysaccharide and glycoprotein degradation (9). Accordingly, it is of interest to compare the activities of additional lysosomal glycosidases thought to be involved in glycoprotein catabolism in serum and selected tissues from normal and diabetic animals. In this paper, we report that significant elevations of the glycosidases α-L-fucosidase, α-D-mannosidase, β-D-galactosidase, and *N*-acetyl-β-D-glucosaminidase occur in serum and epididymal fat tissue from streptozotocin-diabetic rats, as compared to normal controls. Levels of these enzymes in other organs of streptozotocin-diabetic animals were normal.

*Experimental Procedures. Chemicals.* The *p*-nitrophenyl glycosidases were obtained from the Sigma Chemical Co. *p*-Nitrophenyl phosphate was a product of Calbiochem. Streptozotocin was a generous gift from Dr. George Garritson of the Upjohn Co. Other reagents used were commercial products of the highest available purity.

*Tissue preparations.* Male Sprague–Dawley rats (Holtzman) weighing 100–300 g were made diabetic by injection of 60 mg/kg streptozotocin (in 0.1 *N* citrate, pH 4.5) administered via a tail vein. These animals, and age-matched controls, had free access to water and to Purina Rat Chow. Diabetic rats, together with their controls, were sacrificed 1 to 38 days after injection of the drug under ether anesthesia, the abdomen was cut, and blood samples were collected by puncture of the aorta. Tissues were removed and immediately homogenized in ice-cold 0.25 *M* sucrose (20% wt tissue/v) using a glass–Teflon homogenizer. An aliquot

[1] The Enzyme Commission nomenclature for the enzymes discussed in this paper are as follows: *N*-acetyl-β-D-glucosaminidase [β-2-acetylamino-2-deoxy-D-glucoside acetylaminodeoxyglucohydrolase (EC 3.2.1.30)]; acid phosphatase [orthophosphate monoester phosphohydrolase (EC 3.1.3.2)]; α-L-fucosidase [α-L-fucoside fucohydrolase (EC 3.2.1.--)]; β-D-galactosidase [β-D-galactoside galactohydrolase (EC 3.2.1.23)]; glucose-6-phosphatase [D-glucose-6-phosphate phosphohydrolase (EC 3.1.3.9)]; β-D-glucuronidase [β-D-glucuronide glucuronohydrolase (EC 3.2.1.31)]; α-D-mannosidase [α-D-mannoside mannohydrolase (EC 3.2.1.24)].

of the homogenate was taken and diluted 1:11 (v/v) with ice-cold distilled water in order to shock the lysosomal membranes osmotically. After further homogenization in the glass–Teflon apparatus, aliquots of this preparation were used in the determination of enzymic activities.

*Assays.* Blood sugar levels were estimated by the *o*-toluidine method (10). Data obtained from diabetic rats having blood sugar values less than 300 mg/100 ml were not used. Protein levels were estimated using the biuret method (11).

Acid phosphatase[1] and glycosidase activities were determined by the liberation of *p*-nitrophenol from the appropriate *p*-nitrophenyl substrate. The assay mixture contained 0.1 ml of serum or of tissue homogenate and 1.0 ml of 2 m*M* substrate dissolved in a buffer of pH yielding maximum activity. The buffers employed were as follows: 0.2 *M* phosphate – 0.1 *M* citrate (pH 5.0) for α-L-fucosidase and acid phosphatase; 0.2 *M* phosphate – 0.1 *M* citrate (pH 4.6) for α-D-mannosidase; 1.0 m*M* acetate (pH 5.0) for β-D-galactosidase; and 0.1 *M* citrate (pH 4.5) for *N*-acetyl-β-D-glucosaminidase.

The mixtures were incubated in a shaker water bath for 30 min at 37°. The reaction was terminated by addition of 1.0 ml of ice-cold 10% trichloroacetic acid and the tubes were centrifuged to remove denatured protein. Blank preparations in which 0.1 ml of water replaced the homogenate were prepared in the same manner. Aliquots (1.5 ml) of the clear supernatant were taken and added to 1.5 ml of 0.5 *M* NaOH, and the absorbance of the *p*-nitrophenolate anion read at 420 nm. Readings were compared to a standard curve prepared with *p*-nitrophenol and are presented in units of micromoles of *p*-nitrophenol produced per minute.

*Results.* As shown in Table I, the activities of the glycosidases α-L-fucosidase, α-D-mannosidase, β-D-galactosidase, and *N*-acetyl-β-D-glucosaminidase were significantly increased in serum and epididymal fat pads from streptozotocin-diabetic rats. The average increases were 1.6 and 1.7 times the control values, respectively. The elevation of the glycosidase activities was evident regardless of whether data were presented in terms of units per gram of tissue, as in Table I, or as specific activity (units/mg protein). The duration of streptozotocin-diabetes did not affect the results obtained. No significant differences in glycosidase activities between streptozotocin-diabetic and normal rats were found in liver, kidney, epididymus, testis, or spleen. In contrast, the hepatic glycosidase activities of rats fasted 72 hr were elevated an average of 2 times over control values. The sera from fasted rats were about 1.5 times higher in glycosidase activities than normal controls. Kidney glycosidase activities did not seem affected by a 72-hr fast.

Acid phosphatase activity was increased 1.2 times compared to normal control value in liver from streptozotocin-diabetic rats. It was elevated 1.9 times in epididymal fat pads from the diabetic animals. No significant alteration of acid phosphatase activity levels was observed in serum, kidney, epididymus, spleen, or testis preparations from streptozotocin-diabetic rats as compared to normal controls. Acid phosphatase activity in livers from 72-hr fasted rats was 1.4-fold higher than that of fed rats. Serum acid phosphatase activity was about 1.5-fold higher in 72-hr fasted rats than in controls. Kidney acid phosphatase levels did not appear to be affected by starvation. It should be noted that some of the *p*-nitrophenylphosphate hydrolysis catalyzed by liver preparations might be due to microsomal glucose-6-phosphatase[1] activity, which increases in starvation and diabetes (12). We could not differentiate between the activities of these two phosphatases with the homogenates employed in this study. However, kidney is also a rich source of glucose-6-phosphatase (12), but we found no effect of starvation or diabetes on the rate of substrate hydrolysis catalyzed by kidney homogenates.

*Discussion.* An elevation over normal control values of lysosomal glycosidase and acid phosphatase activities occurred only in epididymal fat tissue from streptozotocin-diabetic rats. The levels of glycosidase activities were also increased in sera from diabetic rats compared to controls. Similar results have been obtained with some enzyme activities studied in sera from human patients and alloxan-

TABLE I. Comparison of Selected Hydrolase Activities of Normal and Streptozotocin-Diabetic Rats.[a]

| Tissue | α-L-Fucosidase | α-D-Mannosidase | β-D-Galactosidase | N-acetyl-β-D-Glucosaminidase | Acid Phosphatase |
|---|---|---|---|---|---|
| Liver | (μmoles PNP/min/g wet tissue) | | | | |
| Normal (28) | 0.26 ± 0.02 | 0.36 ± 0.03 | 0.35 ± 0.02 | 3.8 ± 0.2 | 1.7 ± 0.04 |
| Diabetic (28) | 0.27 ± 0.02 | 0.31 ± 0.03 | 0.35 ± 0.03 | 4.2 ± 0.2 | 2.0 ± 0.06[b] |
| Fasted (5) | 0.46 ± 0.04[b] | 0.38 ± 0.02 | 0.92 ± 0.13[b] | 5.6 ± 0.4[b] | 2.3 ± 0.05[b] |
| Serum | (mμmoles PNP/min/ml serum) | | | | mumoles PNP |
| Normal (28) | 6.9 ± 0.6 | 4.9 ± 0.7 | 2.6 ± 0.3 | 21.6 ± .8 | 20.8 ± 1.2 |
| Diabetic (28) | 10.0 ± 0.8[b] | 8.3 ± 1.0[b] | 3.9 ± 0.5[c] | 34.2 ± 2.2[b] | 20.5 ± 1.4 |
| Fasted (5) | 8.4 ± 1.0 | 9.6 ± 0.3[b] | 3.8 ± 0.8 | 30.6 ± 2.9[b] | 28.7 ± 2.5[c] |
| Kidney | (μmoles PNP/min/g wet tissue) | | | | |
| Normal (15) | 0.99 ± 0.05 | 0.50 ± 0.06 | 0.96 ± 0.03 | 7.6 ± 0.4 | 2.7 ± 0.1 |
| Diabetic (15) | 0.95 ± 0.04 | 0.37 ± 0.08 | 0.87 ± 0.04 | 7.7 ± 0.4 | 2.7 ± 0.1 |
| Fasted (4) | 1.08 + 0.05[c] | 0.53 + 0.04 | 0.95 + 0.09 | 6.8 + 0.8[b] | 3.8 ± 0.36[c] |
| Epididymal fat pad | (μmoles PNP/min/g wet tissue) | | | | |
| Normal (28) | 0.51 ± 0.05 | 0.06 ± 0.02 | 0.10 ± 0.02 | 0.24 ± 0.03 | 0.15 ± 0.02 |
| Diabetic (28) | 1.20 ± 0.08[b] | 0.12 ± 0.02[c] | 0.20 ± 0.04[c] | 0.55 ± 0.01[c] | 0.29 ± 0.04[b] |
| Epidydymus | (μmoles PNP/min/g fresh tissue) | | | | |
| Normal (9) | 1.8 ± 0.2 | 3.0 ± 0.3 | 2.3 ± 0.2 | 8.6 ± 0.6 | 1.1 ± 0.07 |
| Diabetic (9) | 2.1 ± 0.3 | 2.8 ± 0.3 | 2.4 ± 0.3 | 9.1 ± 0.6 | 1.2 ± 0.09 |
| Testis | (μmoles PNP/min/g fresh tissue) | | | | |
| Normal (9) | 1.0 ± 0.07 | 0.16 ± 0.03 | 0.17 ± 0.02 | 1.1 ± 0.07 | 0.73 ± 0.04 |
| Diabetic (9) | 1.2 ± 0.1 | 0.17 ± 0.03 | 0.16 ± 0.02 | 1.2 ± 0.11 | 0.75 ± 0.05 |
| Spleen | (μmoles PNP/min/g fresh tissue) | | | | |
| Normal (9) | 0.36 ± 0.05 | 0.11 ± 0.02 | 0.60 ± 0.08 | 2.5 ± 0.2 | 3.3 ± 0.3 |
| Diabetic (9) | 0.39 ± 0.06 | 0.11 ± 0.02 | 0.54 ± 0.06 | 2.1 ± 0.2 | 2.7 ± 0.1 |

[a]Values given are the mean of the number of determinations shown in parentheses ± standard error of the mean. Differences are not significant unless noted as follows:

[b]$P < 0.01$.

[c]$P < 0.05$.

diabetic rabbits (4—7). The level of acid phosphatase activity in rat serum was unchanged from normal by streptozotocin-diabetes, but it was slightly elevated in the diabetic liver compared to controls. Neither epididymal fat tissue nor liver from diabetic animals yielded the same alterations in lysosomal hydrolase activities as that found in the serum from diabetic rats. However, epididymal fat tissue cannot be ruled out completely as a source of serum glycosidases since it is possible that acid phosphatase activity may be more tightly bound to the membranes of the ruptured lysosomes than the glycosidases (13) and hence may not be released rapidly into the serum. Alternatively, it should be noted that the lysosomal fraction is heterogenous; several fractions of lysosomes, each fraction apparently with a different distribution of acid hydrolases, may be obtained from rat liver (14). Hence, diabetes could affect some classes of lysosomes differently than others in the organs affected, and thus lead to an unusual pattern of elevated serum hydrolase activities.

Compared to control values, rats fasted for 72 hr possessed elevated activities of acid phosphatase and α-L-fucosidase, α-D-mannosidase, β-D-galactosidase and N-acetyl-β-D-glucosaminidase in their livers and sera, but not kidneys. Desai (15) also showed that fasting leads to increased acid phosphatase and β-glucuronidase activities in rat liver, but not rat kidney. Although the number of animals used in our studies of fasted animals is small, it is clear that the tissue distribution of

the elevated glycosidase activities and acid phosphatase activity differs greatly in fasted animals compared to streptozotocin-diabetic rats.

The metabolic significance of the increased lysosomal glycosidase activity in diabetic epididymal fat tissue and serum is not known. It is tempting to try to correlate the increased serum glycosidase activities with the decreased levels of protein-bound carbohydrate observed in some tissues from diabetic animals (1, 2). However, enzymes which catalyze the hydrolysis of *p*-nitrophenyl substrates do not necessarily catalyze the release of sugars from naturally occurring glycoproteins and glycolipids (16, 17). Therefore, assays employing appropriate glycolipid and glycoprotein substrates need to be made before we may postulate any correlation between the levels of glycosidases in serum and the amount of protein-bound carbohydrate in various tissues.

Finally, we should note that the diabetic state is not the only disorder in which elevated serum glycosidase activities have been noted. Increased levels of various lysosomal glycosidase and other acid hydrolase activities have been found in the liver and serum of patients with hepatitis (18), rats with experimental hepatic fibrosis, cirrhosis, and hepatoma (18), urine from rats with chemically induced kidney damage (19), and in sera from pregnant women (5), patients with myocardial infarction (5), various mucopolysaccharidoses (4, 20), rheumatoid arthritis (21), and inflammatory disorders of muscle and connective tissue (22). Levels of serum glycosidase activities increase as a function of the age of the subject (5). Obviously, if the elevation of lysosomal acid hydrolase activities in serum is to have any diagnostic significance, a unique pattern of ratios of free serum acid hydrolase activities characteristic for a given pathological state must be discovered.

*Summary.* The activities of the lysosomal hydrolases α-L-fucosidase, α-D-mannosidase, β-D-galactosidase, and *N*-acetyl-β-D-glucosaminidase are significantly elevated in epididymal fat tissue and in serum from streptozotocin-diabetic rats compared with those from age-matched normal controls. Acid phosphatase activity is significantly raised over control values in epididymal fat tissue and, perhaps, liver from streptozotocin-diabetic rats. In contrast, rats fasted 72 hr show increased activities of all the glycosidases and acid phosphatase in their liver and serum. Glycosidase and acid phosphatase activities of epididymus, kidney, spleen and testis are not affected by streptozotocin-diabetes.

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