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Sex- and Line-Dependent Differences of Renal α-Galactosidase and β-Galactosidase Activities in Two Highly Inbred Lines of Chinese Hamsters with Spontaneous Diabetes (40045)

A. Y. CHANG

Diabetes and Atherosclerosis Research, The Upjohn Company, Kalamazoo, Michigan 49001

Depression in the activities of several renal glycohydrolases has been reported in the streptozotocin-diabetic rats and postulated to be a contributory factor leading to microangiopathy (1). Thickening of capillary basement membrane is widely regarded as the basis of diabetic microangiopathy and sex-related difference has been observed in the width of muscle capillary basement membrane in humans (2). This investigation therefore was performed to determine if sex-dependent variation in the levels of renal glycohydrolases also exists in two highly inbred lines of Chinese hamsters, one with spontaneous glycosuria and hyperglycemia.

Materials and methods. The animals were selected from a Chinese hamster colony maintained for the production of genetically diabetic animals (3). The M line has produced only aglycosuric offspring in the last ten generations and the XA line contained over 90% glycosuric animals. The animals of the XA line used in this study, however, were all glycosuric.

After exsanguination through orbital sinus, the kidneys were removed and homogenized in 9 vol of chilled buffer containing 0.45 *M* sucrose, 0.68 m*M* EDTA adjusted to pH 7.0 with 0.5 *M* NaOH in Potter-Elvehjem vessel. The homogenates were centrifuged at 12,000*g* for 20 min. The pellets were washed once with the same buffer, suspended in 9 vol (tissue weight) of buffer, frozen and thawed thrice and homogenized in a Polytron homogenizer/disintegrator at a setting of seven for 15 sec.

p-Nitrophenyl derivatives were used as substrates for glycohydrolase assays at a concentration of 5.26 m*M* in a buffer made by adjusting 0.1 *M* Na_2HPO_4 to desired pH with 0.05 M citric acid. *N*-Acetyl-β-D-hexosaminidase, α-galactosidase and β-galactosidase were assayed at pH 4.5, α-glucosidase at 6.5 and β-glucosidase at 5.5. The assays followed the procedure of Felton *et al.* (4) and were run in a four point time course. One unit of enzyme was defined as the amount which hydrolyzed one nmole substrate per min. Blood sugar and protein were determined as described previously (3).

Column chromatography (0.9 × 30 cm) was carried out with Cellex D suspended in 0.01 *M* NaH_2PO_4, pH 6.0 and eluted with a linear gradient made by mixing 100 ml each of 0 and 0.25 *M* NaCl in the same buffer. Four ml kidney supernatant fraction from a M line female animal was chromatograph and 0.1 ml aliquots of 2.2 ml fraction were assayed for α-galactosidase and β-galactosidase activities in even-numbered tubes.

Results and discussion. Table I summarizes all data obtained in this study and they are expressed as means ± SEM of six animals in each group.

The animals were matched according to age and the males were significantly heavier than the females in the same line. The diabetic XA animals also tended to be slightly heavier that the M animals of the same sex. No significant difference, however, was observed in the kidney weight, expressed as % body weight in view of the difference in the body weights, in all four groups of animals. The female M animals showed significantly less soluble proteins in the kidney than the male counterparts and the significance of this observation remains unclear.

In both 12,000*g* supernatant and precipitate fractions, *N*-acetyl-β-D-hexosaminidase was present in the highest concentration and about 40% of its activity was recovered in the supernatant. Both males and females in the same inbred line showed similar activ-

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TABLE I. AGE, BODY AND KIDNEY WEIGHTS, BLOOD SUGAR AND RENAL GLYCOHYDROLASES IN MALE AND FEMALE CHINESE HAMSTERS OF M AND XA LINE.[a]

	M-Line			XA-Line		
	Male	Female	P^b	Male	Female	P^b
Age (weeks)	19.7 ± 3.6	20.0 ± 2.5	NS	20.2 ± 3.4	18.8 ± 2.0	NS
Body weight (g)	27.3 ± 1.5	18.9 ± 0.5	<0.001	29.2 ± 2.3	23.8 ± 1.0	<0.05
Blood sugar (mg/dl)	128 ± 5	125 ± 10	NS	362 ± 50	369 ± 50	NS
Kidney weight (% body weight)	1.00 ± 0.03	1.08 ± 0.03	NS	1.10 ± 0.04	1.13 ± 0.04	NS
12,000*g* supernatant						
protein concentration (mg/g)	91.7 ± 2.9	82.3 ± 1.7	<0.025	86.7 ± 2.2	88.9 ± 2.8	NS
N-acetyl-β-D-hexosaminidase (U/g)	1723 ± 85	1961 ± 150	NS	1607 ± 188	1898 ± 147	NS
α-glucosidase (U/g)	105 ± 6	97 ± 6	NS	112 ± 6	110 ± 5	NS
α-galactosidase (U/g)	702 ± 68	1176 ± 131	<0.005	392 ± 41	734 ± 82	<0.005
β-glucosidase (U/g)	998 ± 66	899 ± 26	NS	939 ± 54	830 ± 52	NS
β-galactosidase (U/g)	1186 ± 71	1837 ± 97	<0.001	810 ± 53	1304 ± 194	<0.05
12,000*g* precipitates						
protein concentration (mg/g)	82.8 ± 6.3	82.2 ± 4.0	NS	91.3 ± 5.1	86.6 ± 4.7	NS
N-acetyl-β-D-hexosaminidase (U/g)	2401 ± 221	2527 ± 142	NS	2409 ± 239	2749 ± 189	NS
α-glucosidase (U/g)	40 ±4	35 ± 2	NS	42 ± 3	38 ± 2	NS
α-galactosidase (U/g)	458 ± 43	438 ± 17	NS	385 ± 26	422 ± 26	NS
β-galactosidase (U/g)	809 ± 87	855 ± 49	NS	679 ±41	797 ± 60	NS

[a] All values are Mean ±SEM of six animals in each group.
[b] Student's *t* test. NS denotes "not significant", i.e. $P > 0.05$.

ities of this enzyme in the two subcellular fractions of the kidney extracts. Interestingly, no significant difference between M and XA line animals was observed in renal *N*-acetyl-β-D-hexosaminidase activity, in contrast to what was reported in the streptozotocin-diabetic rats which had significantly depressed level of this enzyme in the kidney (1). The cause for this discrepancy could arise from difference in species or between chemically-induced and hereditary diabetes.

Renal α-glucosidase activity was more prevalent in the 12,000*g* supernatant fraction, i.e. 73%, and no significant difference in its activity was observed between sexes or lines in either fraction. In preliminary experiments, β-glucosidase was found to reside mainly in the supernatant, i.e. 95%, and its activity was thus not measured in the precipitate fractions subsequently. Again, there was no significant difference in β-glucosidase activities between sexes or lines in the kidney.

Sex- and line-dependent differences in renal α-galactosidase and β-galactosidase activities, however, were highly significant in the supernatant fraction. Female animals showed higher activities in both enzymes than the male animals in either M or XA line. The diabetic XA animals also showed significantly less α-galactosidase and β-galactosidase in the kidney supernatant fractions than the corresponding M line animals with the following *P* values: <0.025 (α-galactosidase in female), <0.005 (α-galactosidase in male), <0.05 (β-galactosidase in male) and <0.005 (β-galactosidase in female). In the streptozotocin-diabetic rats, depression of renal β-galactosidase was also reported but the level of α-galactosidase has not been measured (1). The activities of these two enzymes in the 12,000*g* precipitate fractions were substantial but were not significantly different between sexes or lines.

Although the sex- and line-dependent variations in α-galactosidase and β-galactosidase appeared to be parallel, their activities were manifested by different proteins. When the 12,000*g* supernatant fraction of a M-line female animal was chromatogrammed on a Cellex-D column, β-galactosidase was eluted off in two early peaks,

whereas α-galactosidase was found to be more acidic at pH 6.0 and recovered in one major peak (Fig. 1). Therefore, the observed differences in α-galactosidase and β-galactosidase involved at least two to three protein entities. The observed sex- and line-dependent differences in these renal enzyme activities suggest that their levels may play significant roles in the development of diabetic microangiopathy in view of the fact that sex- and diabetes-related variations were reported in the width of muscle capillary basement membrane in humans (2). Studies to determine if the observed differences between the highly inbred lines were results of hyperglycemia or other genetic variants unrelated to hyperglycemia and if the differences between sexes or lines involved specific isozyme species are currently in progress.

Summary. Several glycohydrolase activities were measured in the 12,000*g* supernatant and precipitate fractions of kidney homogenates obtained from two highly inbred lines of male and female Chinese hamsters, one with hereditary diabetes. No difference was observed in *N*-acetyl-β-D-hexosaminidase, α-glucosidase and β-glucosidase whereas the activities of supernatant α-galactosidase and β-galactosidase were lower in the males and further depressed in the diabetic animals.

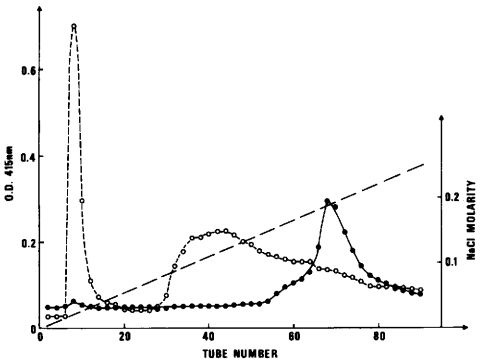

FIG. 1. Cellex D column chromatography of α-galactosidase and β-galactosidase in 12,000 *g* supernatant fraction of Chinese hamster kidney extracts. Size: 0.9 × 30 cm. Fraction: 2.2 ml. ●——● = α-galactosidase. ○·····○ = β-galactosidase. ----- NaCl concentration in the eluting buffer.

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