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ACID GLYCOHYDROLASE IN CHINESE HAMSTER WITH SPONTANEOUS DIABETES

III. LINE-DEPENDENT VARIANCE

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Summary

The activities of 5 acid glycohydrolases were measured in the 0.5% Triton X-100 extracts of the kidneys of 10 highly-inbred lines of Chinese hamsters. The diabetic lines showed enlarged kidneys and, in general, depressed levels of renal α-galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22), β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) and *N*-acetyl-β-D-hexosaminidase (2-acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30). Correlations between blood sugar and renal enzyme levels were also significant with the following coefficients: α-galactosidase (—0.5634), β-galactosidase (—0.6120) and *N*-acetyl-β-D-hexosaminidase (—0.4212). However, exceptions were also observed. For instance, the AC line animals showed high levels of renal α-galactosidase and β-galactosidase and the XA line animals had normal levels of *N*-acetyl-β-D-hexosaminidase despite the fact that these animals had prolonged hyperglycemia. Considerable line-dependent variations in these renal enzyme levels were also observed in the diabetic and nondiabetic groups. It appears that the depression of renal α-galactosidase, β-galactosidase and *N*-acetyl-β-D-hexosaminidase generally accompanied or followed the manifestation of diabetes, but certain genetic determinants may obliterate their depressions to discernibly abnormal levels.

Introduction

Studies on the tissue levels of acid glycohydrolases in two highly inbred lines of Chinese hamsters with different phenotypes revealed significant differences in renal α-galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22) and β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) activities

between the diabetic XA and the nondiabetic M lines [1]. These renal enzymes were partially purified and characterized and similar properties were observed between the XA and M line animals. Furthermore, these enzymes were present at similar or less different levels in the prediabetic XA and their age- and sex-matched M line animals than in the mature animals. It was thus concluded that the depression of these enzyme contents in the kidney of the XA animals was a consequence of the development of hyperglycemia [1].

Previously, it has been demonstrated that lactate dehydrogenase (L-lactate: NAD^+ oxidoreductase, EC 1.1.1.27) level and isozyme pattern in tissue were highly variable among the lines of the Chinese hamster colony and that the line-specific variations arose from genetic traits unrelated to the manifestation of glycosuria [2]. Therefore, this study was carried out to determine if the levels of acid glycohydrolases in the kidney were also dictated by genetic traits in a manner analogous to that of lactate dehydrogenase isozyme pattern in the Chinese hamster colony. Such a study will also furnish further information as to the roles of acid glycohydrolases in and their relationship to the manifestation of diabetes. The acid glycohydrolases studied here include α-galactosidase, β-galactosidase, α-glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20), β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) and *N*-acetyl-β-D-hexosaminidase (2-acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30).

Materials and Methods

The animals were selected from the same Chinese hamster colony as described previously [1,2]. Fig. 1 shows the origin of each line. The diabetic lines were L, XA, X, AH, Z, AC, and ZM and the non-diabetic lines were AA, M, and AV. It is apparent that all lines were derived from the L line in one way

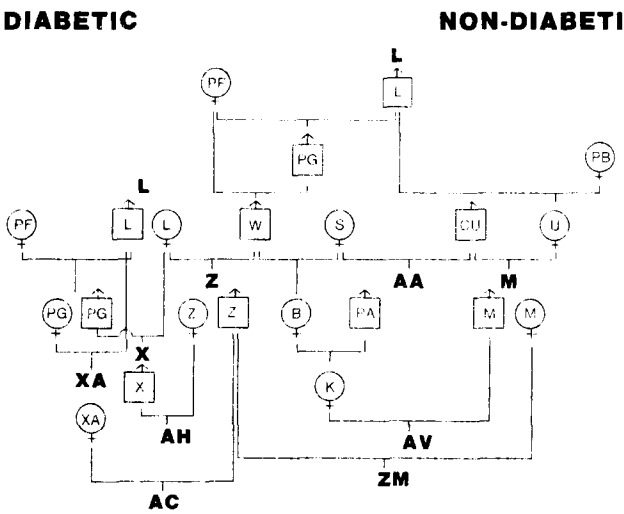

Fig. 1. Origin of the lines in the Chinese hamster colony. ♀ depicts the dams and ♂ the sires in the original crosses.

or another. The lines were propagated by sister-brother matings using glycosuria or aglycosuria as the selection trait. The numbers of inbreeding generations for the animals used in this study are indicated in the parentheses as follows: M (15th), AV (6th—7th), AA (14th—16th), AC (14th), ZM (3rd—4th), XA (16th—17th), X (17th—18th), AH (13th—15th), Z (15th), and L (29th—31st). All animals were male and ranged in age from 7 to 18 months.

The animals were exsanguinated through the orbital sinus. A 20 μl aliquot of blood was diluted with 1 ml 0.6 M NaF and kept frozen for sugar determination [3] later. The kidneys were excised, rinsed in cold saline, blotted, halved, decapsulated, weighed and homogenized in 9 vols. (tissue weight) of chilled solution containing 0.5% Triton X-100, 0.45 M sucrose, 0.68 mM EDTA, pH 7.0 with Polytron® homogenizer/disintegrator (30 s at setting 7). The supernatants obtained after centrifugation at 12 000 $\times g$ for 20 min were used to measure glycohydrolase activity. Enzyme assays were described as before using *p*-nitrophenyl derivatives as substrates [1,4]. One unit was defined as one nmol substrate turnover per min. Statistical analyses were carried out with analysis of variance techniques and Duncan's multiple range test [5].

Results

Table I summarizes the blood sugar levels, kidney weights and glycohydrolase activities in the animals of the diabetic and nondiabetic lines. The mean blood sugar value of the Chinese hamsters in the diabetic lines (331 ± 13.4 mg/100 ml) was significantly greater than that of the non-diabetics (88 ± 9.7 mg/100 ml). Because of the variation in age, the body weights of these animals were not analyzed and the kidney weights were calculated in terms of % body weight. The diabetic line animals showed significantly enlarged kidneys as compared to the nondiabetics. The mean values of the activities of renal α-galactosidase, β-galactosidase and *N*-acetyl-β-D-hexosaminidase in the diabetic animals were significantly lower than those in the nondiabetics. No difference, however, was observed in renal α-glucosidase and β-glucosidase activities between the two groups of animals.

The mean values of the variables listed in Table I and the duration of glyco-

TABLE I

BLOOD SUGAR, KIDNEY WEIGHT AND ACID GLYCOHYDROLASE LEVELS IN DIABETIC AND NON-DIABETIC CHINESE HAMSTERS

Means ± S.E. (18 animals for non-diabetic and 41 for diabetic). *P* values are from Duncan's multiple range test, ns denotes not significant, i.e. $P > 0.05$.

Measurements	Non-diabetic	Diabetic	*P*
Blood sugar (mg/dl)	88 ± 4.7	331 ± 13.4	<0.01
Kidney wt. (% body wt.)	0.89 ± 0.012	1.25 ± 0.028	<0.01
N-Acetyl-β-D-hexosaminidase (units/g)	4117 ± 170	3147 ± 81	<0.01
α-Galactosidase (units/g)	1351 ± 64	997 ± 57	<0.01
β-Galactosidase (units/g)	1761 ± 88	1318 ± 52	<0.01
α-Glucosidase (units/g)	122 ± 3.8	116 ± 4.3	ns
β-Glucosidase (units/g)	680 ± 13	679 ± 14	ns

TABLE II

BLOOD SUGAR, DURATION OF GLYCOSURIA AND RENAL ACID GLYCOHYDROLASE LEVELS IN TEN DIFFERENT HIGHLY INBRED LINES OF CHINESE HAMSTERS

Means ± S.E. superscripted with the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range tests.

Line and number of animals	Blood sugar (mg/dl)	Duration of glycosuria (d)	Kidney wt. (% body wt.)	*N*-Ac-β-D-hexosaminidase (units/g)	α-Galactosidase (units/g)	β-Galactosidase (units/g)	α-Glucosidase (units/g)	β-Glucosidase (units/g)
M (6)	69.0 ± 4.8 ^{c}	—	0.91 ± 0.02 ^{d}	3881 ± 145 ^{b}	1394 ± 76 ^{a,b}	2063 ± 75 ^{a}	121 ± 12 ^{b,c}	685 ± 27 ^{b,c}
AV (6)	88.0 ± 2.9 ^{c}	—	0.89 ± 0.02 ^{d}	3653 ± 217 ^{b,c}	1174 ± 69 ^{b,c}	1416 ± 86 ^{b,c}	128 ± 4 ^{b}	669 ± 18 ^{b,c}
AA (6)	106 ± 7 ^{c}	—	0.89 ± 0.02 ^{d}	4778 ± 248 ^{a}	1520 ± 138 ^{a}	1856 ± 136 ^{a}	115 ± 4 ^{b,c}	688 ± 27 ^{b,c}
AC (6)	276 ± 24 ^{b}	479 ± 6 ^{a}	1.39 ± 0.06 ^{a}	2830 ± 68 ^{d}	1643 ± 99 ^{a}	1877 ± 99 ^{a}	152 ± 9 ^{a}	790 ± 23 ^{a}
ZM (6)	289 ± 38 ^{b}	413 ± 30 ^{b,c}	1.10 ± 0.06 ^{c}	3291 ± 210 ^{c,d}	1130 ± 147 ^{c}	1538 ± 103 ^{b}	82 ± 1.3 ^{e}	614 ± 10 ^{c}
XA (6)	310 ± 45 ^{a,b}	364 ± 36 ^{c,d}	1.13 ± 0.03 ^{b}	3959 ± 266 ^{b}	699 ± 47 ^{e}	1217 ± 65 ^{c,d}	103 ± 4.6 ^{d,c}	617 ± 61 ^{c}
X (6)	331 ± 35 ^{a,b}	324 ± 14 ^{d}	1.50 ± 0.06 ^{a}	2872 ± 138 ^{d}	974 ± 61 ^{c,d}	1272 ± 60 ^{c,d}	127 ± 3 ^{b}	735 ± 18 ^{a,b}
AH (6)	344 ± 34 ^{a,b}	179 ± 33 ^{e}	1.19 ± 0.03 ^{b}	3044 ± 122 ^{d}	824 ± 18 ^{d,e}	1081 ± 28 ^{d,e}	134 ± 8 ^{b}	678 ± 14 ^{b,c}
Z (5)	383 ± ± 29 ^{a}	377 ± 23 ^{b,c,d}	1.21 ± 0.05 ^{b,c}	2904 ± 71 ^{d}	1006 ± 64 ^{c,d}	1340 ± 46 ^{b,c}	88 ± 2.3 ^{d,e}	699 ± 16 ^{b,c}
L (6)	387 ± 28 ^{a}	435 ± 21 ^{a,b}	1.25 ± 0.08 ^{b}	3129 ± 174 ^{c,d}	641 ± 47 ^{e}	905 ± 66 ^{e}	123 ± 5 ^{b}	651 ± 26 ^{b,c}

suria in each individual line are summarized in Table II. The lines are arranged in an increasing order according to their blood sugar levels. The superscripted letters indicate the results of statistical analyses by Duncan's multiple range test [5]. For instance, the blood sugar levels of lines M, AV, and AA were not significantly different at the 0.05 alpha level and they are superscripted with the same letter, c; whereas, the blood sugar levels of lines AA and AC were significantly different and they are superscripted with different letters, b and c. Henceforth, the animals in lines Z and L studied here were more hyperglycemic than those in lines AC and ZM.

The duration of glycosuria in the diabetic line animals varied and are listed in Table II; the difference was due to variation in age of the animals rather than in age of onset. The mean age of the AC line animals was 17.2 ± 0.17 months and the AH line 10.5 ± 1.34 months; the other diabetic lines had values between lines AC and AH. The kidney weights were greater in the diabetic animals but they also varied among the animals in the diabetic lines. AC and X line Chinese hamsters had the most enlarged kidneys, whereas ZM, XA and AH lines the least.

The activities of *N*-acetyl-β-D-hexosaminidase in the kidney extracts varied considerably among the lines. In general, the nondiabetic lines showed higher levels than the diabetic lines. However, exception was found in the hyperglycemic XA animals which showed a level of renal *N*-acetyl-β-D-hexosaminidase in the range of the nondiabetic animals. Among the nondiabetic lines, the AA line showed an exceptionally high level of this enzyme, which was significantly different from all other lines, whereas the AV line had relatively low activity of *N*-acetyl-β-D-hexosaminidase which was almost in the range of the diabetic lines. Among the diabetic lines, except line XA, relatively constant activities of this enzyme were observed, ranging from 2830 ± 68 units/g in AC to 3921 ± 210 units/g in ZM (Table II).

The diabetic lines showed less renal α-galactosidase activity than the nondiabetic lines AA, M, and AV; however, AC line animals stood out as glaring exceptions and had higher α-galactosidase activity than animals in other lines (Table II). Within the diabetic and nondiabetic groups, highly variable activities of α-galactosidase was found among the lines. A similar situation was observed in the activity of renal β-galactosidase. The diabetic lines tended to have low levels of β-galactosidase. Exceptions again were found in the AC line animals which had a high level of β-galactosidase and were hyperglycemic, and in the AV line animals which had relatively low level of β-galactosidase and normoglycemia (Table II).

Although renal α-glucosidase activity also varied considerably among the lines, no obvious relationship could be discerned between its level and the manifestations of hyperglycemia. AC line, diabetic, showed the highest level of α-glucosidase and ZM and Z lines, also diabetic, the lowest. The nondiabetic M, AV, and AA lines showed levels interspersed between the diabetic lines. Similarly, the level of renal β-glucosidase also lacked any apparent relationship to the condition of hyperglycemia. Furthermore, relatively constant levels of β-glucosidase were obtained for all the lines, unlike the other glycohydrolases (Table II).

The correlation coefficients between the above discussed variables are given

TABLE III

CORRELATION COEFFICIENTS BETWEEN VARIABLES IN TABLE II

Total numbers of observations were 59 for each variable. Minus sign indicates inverse relationship.

	Duration of glycosuria	Kidney wt.	*N*-Acetyl-β-D-hexosaminidase	α-Galactosidase	β-Galactosidase	α-Glucosidase	β-Glucosidase
Blood sugar	0.7126	0.6725	−0.4212	−0.5634	−0.6120	−0.1486	0.0903
Duration of glycosuria	—	0.6812	−0.5027	−0.2387	−0.3206	−0.1082	0.0092
Kidney weight	—	—	−0.5511	−0.2959	−0.4016	0.2245	0.2745
N-Acetyl-β-D-hexosaminidase	—	—	—	0.2672	0.3689	−0.0818	−0.2057
α-Galactosidase	—	—	—	—	0.8930	0.2735	0.2729
β-Galactosidase	—	—	—	—	—	0.1207	0.2374
α-Glucosidase	—	—	—	—	—	—	0.5515

in Table III. Significant positive correlation was observed between blood sugar level and duration of glycosuria (0.7126), between blood sugar and kidney weight (0.6725), between α-galactosidase and β-galactosidase levels (0.8930), between α-glucosidase and β-glucosidase levels (0.5515), and between duration of glycosuria and kidney weight (0.6812). Inverse correlation was also found between blood sugar level and α-galactosidase activity (—0.5634), between blood sugar and β-galactosidase (—0.6120), and between *N*-acetyl-β-D-hexosaminidase and kidney weight (—0.5511), duration of glycosuria (—0.5027) and blood sugar (—0.4214).

Discussion

In a previous study on XA and M line Chinese hamsters, it was concluded that, as a consequence of hyperglycemia, renal α-galactosidase and β-galactosidase levels were depressed [1]. The present study yields data showing that, although the thesis holds true in general, exceptions do arise. Among the ten lines of animals studied here, the AC line emerged as a glaring oddity. The AC line animals used in this study showed a long history of glycosuria and hyperglycemia, but they also showed the highest level of α-galactosidase and the second highest of β-galactosidase among the 10 lines of animals. The inordinately high levels of these enzymes in the AC line could arise from the following causes. The mechanism by which hyperglycemia induces the depression of renal α-galactosidase and β-galactosidase failed to operate in the AC line. Alternatively, the AC line was genetically predisposed to produce excessively high levels of these enzymes which simply masked the hyperglycemia-dependent depression of the enzyme levels. A study on the young animals in the AC and other lines before the onset of glycosuria will provide some clues to the cause of the unusual observations in the AC line.

In the study on renal *N*-acetyl-β-D-hexosaminidase levels of XA and M line animals, it was concluded that the level of this enzyme in the kidney was not governed by the blood sugar level since similar levels were obtained in the two lines [4]. The present study confirms the previous data but also reveals that the previous conclusion was premature and maybe erroneous [4]. It is apparent from this study that the diabetic lines excepting the XA showed less renal *N*-acetyl-β-D-hexosaminidase than the nondiabetic lines. Furthermore, a significant correlation ($P < 0.01$) of —0.4212 was found between renal *N*-acetyl-β-D-hexosaminidase and blood sugar levels in the 59 animals studied here. It, therefore, appears that the anomaly associated with *N*-acetyl-β-D-hexosaminidase in the XA line was analogous to that of α-galactosidase and β-galactosidase in the AC line and that it could arise from one or more of the possible routes described above.

Although line-specific variations were also found in renal α-glucosidase and β-glucosidase levels in the colony, no significant correlation was evident between the levels of these enzymes and the manifestations of diabetes. This confirms our previous conclusion based on the studies on M and XA lines [1]. Nevertheless, the considerable divergence in the levels of these five acid glycohydrolases reported here and the previous observation on the highly variable activity and isozyme pattern of lactate dehydrogenase [2] indicate that signi-

ficant variability may exist in most gene products in these highly inbred lines of the Chinese hamster colony. These observations also bear the pitfall of comparative studies on biochemical entities in two highly inbred lines of experimental animals serving as models of hereditary human diseases. Caution must be taken in correlating changes in biochemical parameters to the disease process in such animal models. In particular, the possible obliteration of actual changes by predisposed genetic factors must be considered.

The unpredictable nature of the occurrence of various forms of diabetic complications has long puzzled diabetologists, and predisposed genetic factors are likely to play roles in causing such unpredictability. Our findings that renal acid glycohydrolase levels were highly variable in the Chinese hamster colony with spontaneous diabetes and their obvious roles in glycoprotein degradation offer possible candidates for these predisposed genetic factors. It would be interesting to see if AC or XA line animals are less prone to develop kidney lesions than lines L, AH, or Z. Studies correlating the levels of renal acid glycohydrolases and the thickness of glomerular capillary loop basement membrane [6] in individual animals from different lines are currently in progress.

Several other lines of significant experimental evidence also emerged from this study. Enlargement of kidneys in the diabetic animals shows positive correlation to the degree of severity and the duration of glycosuria. However, other factors, most likely genetically determined, also affect diabetic nephromegaly. For example, lines AC and ZM, comparable in blood sugar level and duration of glycosuria, showed significantly different degree of nephromegaly. The difference in kidney weight could not account for the depression of renal α-galactosidase and β-galactosidase activity in the diabetic animals, although a significant inverse correlation was observed between kidney weight and renal *N*-acetyl-β-D-hexosaminidase level. Finally, highly significant correlations between α- and β-glucosidase levels, and, particularly, between α and β-galactosidase suggest that the regulations of the two glucosidases or the two galactosidases in the kidney might be coupled since the activities of the two galactosidases could be easily separated by ion-exchange chromatography [1].

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