Plasma glycoproteins of diabetic and normal Chinese hamsters

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Summary. Plasma hexosamine levels were elevated in lines of diabetic Chinese hamsters when compared to nondiabetics. Plasma protein bound hexoses, a_2 macroglobulin and total lipoprotein levels were not significantly correlated with either animal line or levels of fasting blood glucose.

It has been advanced that the inordinate number of atherosclerotic lesions and other vascular complications in human diabetics is caused by increased glycosylation of proteins[2]. The major evidence for increased glycosylation of proteins in diabetics has been obtained on glycosylated hemoglobin[3]. Evidence for this increased glycosylation in diabetic plasma proteins is inconclusive[2,3]. The problems in measuring plasma glycoprotein concentrations in diabetics may be due to the genetic heterogenicity of humans. To eliminate this heterogenicity, we have analyzed plasma glycosylated proteins from highly inbred genetically diabetic Chinese hamster lines[4].

We report here on changes in glycoproteins among several lines of genetically diabetic and normal hamsters. Protein bound hexose measurements were determined in order to assess the level of nonenzymatic glycosylation while enzymatic glycosylation was assessed using the hexosamine analysis. In addition we examined the level of a_2 macroglobulin (a_2M) in these hamsters. a_2M is a major plasma glycoprotein which we have previously shown to have altered electrophoretic mobility in genetically diabetic and streptozoticin induced diabetic animals[5].

Materials and methods. Blood from Chinese hamsters was collected by EDTA from the orbital sinus and freed of erythrocytes by centrifugation[6]. The diabetic hamster lines (BE, BZ, L, and Z) resulted from many generations of inbreeding and selection for positive glucosuria[7]. Lines AV and M were not glucosuric and served as the controls[7].

Fasting blood glucose (FBG) was determined by autoanalyzer microglucose procedure and protein concentration was determined by the method of Lowry et al.[8]. Protein bound hexose was determined by the method of Kennedy[9] and the hexosamine by the method of Gatt and Berman[10]. a_2M concentrations were measured by using the artificial substrate N-benzoyl-DL-arginine-p-nitroanilide (BAP-NA)[11].

Lipids in each fraction were measured by precipitating 10 µl of sample with 0.5 ml of a 25 mg% Fat and Red 7B stain solution in methanol and 0.1 ml deionized H_2O. The fractions were centrifuged, the supernatant decanted, and the pellets washed with 50% methanol. The pellets were redissolved in 2.5 absolute methanol and read spectrophotometrically at 540 nm. Using human plasma as a standard, values obtained with the Fat red test gave equal results per mg protein for isolated VLDL and LDL and the total Fat red-values correlate well with the data obtained by scanning agarose gel lipoprotein profiles.

The data was computer analyzed using the SPSS system's 1 way least standard deviation method[12]. Plus and minus values in tables 1 and 2 represent standard error. Significance is at the 5% level (p 0.05) unless otherwise noted.

Results and discussion. Our results suggest that plasma glycoprotein concentrations are more dependent upon animal line rather than on an animal's FBG or urinary glucose level. Although plasma protein bound hexoses have been positively correlated with glycosylated hemoglobin levels in humans[8], we found no correlation of these plasma hexoses with either line, FBG or glucosuria (table 1). This lack of correlation with FBG in hamsters may indicate a low level of non-enzymatic glycosylation or a fast turnover rate of plasma proteins in these animals. Protein bound hexosamines (enzymatic glycosylations) were elevated in some lines of diabetics (table 1). This finding correlates with the date of Chang[13] who observed differences in N-acetyl-glucosaminidase activity among diabetic lines. He found line Z to be low in glucosaminidase activity. This Z line is related to the BZ line which we observed to have the highest hexosamine levels.

Table 1. Fasting blood glucose levels and some glycoprotein levels in diabetic and nondiabetic Chinese hamsters

Line	No. animals	Glucosuria	Fasting blood glucose mg/dl	Protein bound hexosamine mg/g protein	Protein bound hexose mg/g protein	a_2M activity mg trypsin activity/g protein
AV	23	–	107±7	34.2±0.1	27.7±2.5	1.9±0.4
M	10	–	88±4	35.7±5.0	16.0±6.9	2.5±0.5
L	6	+	125±12	54.3±9.1	35.1±13.8	3.0±0.2
Z	5	+	154±13	67.5±5.3	22.5±4.4	2.7±0.6
BE	19	+	255±20	46±4.0	30.7±3.6	2.7±0.06
BZ	7	+	173±19	76.9±6.1	23.6±1.3	–

Table 2. Comparison of a_2M with total glycoproteins and lipoproteins in plasma of diabetic and normal Chinese hamsters

	Hexosamine p < 0.07		Hexose p < 0.3		Lipid p < 0.03	
	No. animals	mg/g protein	No. animals	mg/g protein	No. animals	OD units/mg protein
Low a_2M 1.50–2.60*	10	56.1±4.3	6	19.9±3.3	10	0.21±0.2
High a_2M 2.61–5.00*	9	66.2±2.4	4	28.4±6.8	9	0.29±0.03

* mg trypsin activity per g protein.

A major plasma glycoprotein, α_2M, binds trypsin-like enzymes and prevents them from digesting large proteins. α_2M has been found to be in higher concentrations in human diabetics[14]. Brownlee[15] hypothesizes that in humans this increased α_2M inhibits the activity of leucocyte neutral proteases which aid in the digestion of basement membrane. This could result in the increased basement membrane thickness and microangiopathy of diabetics. Our data did not show a positive correlation of α_2M levels with diabetes (glucosuria or FBG) but α_2M levels were positively correlated with hexosamine and lipid levels (table 2).

We have, however, found that diabetes causes an increase in the charge and molecular weight of α_2M in hamsters[5], both of which could affect the binding and action of neutral protease and lead to the microvascular changes seen in diabetics[16].

Our data support the supposition that diabetes is a disorder of many etiologies. The great variety and diversity in glycoprotein observed in the highly inbred hamster lines emphasizes the complexity of establishing meaningful parameters in a heterogenous human population. We hope that by studying changes in the glycoproteins and lipoproteins in one of the diabetic lines we may be able to correlate the changes in plasma glycoproteins with a specific complication of diabetes.

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Biogenic amine acetylation: No detectable circadian rhythm in whole brain homogenates of the insect *Ostrinia nubilalis*

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Summary. With the biogenic amines tryptamine, dopamine, and octopamine as substrates, N-acetyltransferase activity shows no detectable circadian rhythm in homogenates of whole brains of the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae). The circadian clock of this insect may be fundamentally different from the N-acetyltransferase pacemaker in the pineal gland of vertebrates.

N-acetyltransferase (NAT) functions as a biological clock in the pineal gland of vertebrates[1]. NAT activity oscillates in a circadian rhythm, peaking at night in both diurnal and nocturnal mammals and in diurnal birds, and its activity controls the biosynthesis of pineal melatonin from the biogenic amine serotonin. NAT levels are also correlated with circadian activity of locomotion in many vertebrates[2]. NAT shows an endogenous circadian rhythm in vitro and responds to environmental light in cultured chicken pineal glands[3,4] and even in dispersed cell culture of the chick pineal[5]. In insects, circadian rhythms of locomotor activity, cuticle deposition, and various physiological processes are well established[6,7]. Evidence from transplantation and ablation experiments on the moths *Antheraea pernyi*[8,9], *Hyalophora cecropia*[9], and the cockroach *Leucophaea maderae*[10] suggest that the brain is the location of the photoperiodic clock. There is some evidence of circadian fluctuation of serotonin levels in *Drosophila melanogaster*[11], and Houk and Beck[12] have suggested that dopamine or a metabolite 'may be involved in an endogenous time measuring system and/or diapause induction-termination' in *Ostrinia nubilalis*. The biogenic amine dopamine is present in *O. nubilalis* brain[12] as is an NAT capable of acetylating dopamine and other amines[13]. This neural NAT is distinct from cuticular NAT participating in sclerotization on the basis of substrate specificities for a series of 8 amines[13]. Since a distinct neural NAT and amine substrate are present in an anatomical location which may contain the circadian center, we wished to determine if there was a rhythm to *O. nubilalis* brain NAT activity similar to that found in the pineal gland of vertebrates.

2 colonies of *O. nubilalis* were maintained on artificial diet in a long day, non-diapausing (LD 16:8) photoperiodic regime. One colony was 14 h out of synchronization to the other in its LD cycle to allow data for all time points to be obtained within a 12-h time span. Brains were dissected from cold-anesthetized live insects, homogenized, and assayed as previously described[13] within 15 min of the indicated clock time. All NAT assays were by a modification[13] of the radiochemical method of Deguchi and Axelrod[14] using ^{14}C-acetyl CoA and the unlabeled amines tryptamine, dopamine, and octopamine. Incubation times and tissue concentrations were chosen to ensure linear rates of product formation.

We were unable to demonstrate any circadian rhythm in NAT activity using either the phenylethylamines dopamine and octopamine or the indolealkylamine tryptamine as substrates (fig.). In the chick pineal, a 5–7-fold rhythmic