Vol. 101, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 30, 1981 Pages 704-708

The Clearance of Glycoproteins in Diabetic Mice

Salvatore V. Pizzo, Mark A. Lehrman, Michael J. Imber, and C. Earl Guthrow

Departments of Pathology, Biochemistry and Medicine
Duke University Medical Center
Durham, North Carolina 27710

Received June 15, 1981

Summary: The clearances of N-acetyl-D-glucosaminyl-bovine serum albumin (GlcNAc-BSA), L-fucosyl-bovine serum albumin (Fuc-BSA) and asialoorosomucoid (ASOR) were evaluated in alloxan-induced diabetic mice and compared to non-diabetic litter mates. The clearances of Fuc-BSA and ASOR were identical in both groups. However, the clearance of GlcNAc-BSA was considerably slower in the diabetic animals. When GlcNAc-BSA was injected into normal mice in the presence of a blood glucose of 25 to 50 mM, competition was observed. No competition was seen with Fuc-BSA or D-galactosyl-BSA (Gal-BSA) in the presence of 50 mM glucose. In vitro binding studies with purified receptor for GlcNAc/mannose confirm that glucose competes for binding of GlcNAc-BSA to this receptor.

A number of carbohydrate recognition systems mediating endocytosis exist in mammals (1). Hepatocytes possess receptors which recognize terminal galactose (2) and fucose (3,4) while reticuloendothelial cells possess a receptor which recognizes both N-acetylglucosamine and mannose (5,6). These receptors appear to play a role in the in vivo catabolism of glycoproteins. Thus, IgG and IgM immune complexes clear via the galactose and GlcNAc/mannose receptors, respectively (7,8). This clearance is apparently mediated by previously sequestered antibody glycosyl residues which become exposed on binding of antigen.

The present study has examined these three receptor mediated pathways in diabetic mice. This study demonstrates that diabetic mice have a delayed clearance of GlcNAc-BSA but not Fuc-BSA or ASOR. Simultaneous injection of glucose and GlcNAc-BSA in normal mice also results in inhibition of clearance while glucose has no effect on the clearance of Fuc-BSA or Gal-BSA. In vitro studies of the GlcNAc/mannose receptor are consistent with these latter observations since, at high concentrations, glucose inhibits the binding of GlcNAc-BSA to the purified receptor. The competitive effect of glucose on the clearance of GlcNAc-BSA and

0006-291X/81/140704-05$01.00/0

on its in vitro binding to purified receptor suggests that elevated blood glucose concentration is the primary mediator of the delayed clearance of GlcNAc-BSA in diabetic mice.

Methods: Mice were made diabetic by five daily intravenous injections of alloxan (40 mg/kg) as previously described by Rossini et al (9). Blood glucose levels were monitored as described (10) over the course of eight weeks. Litter mates were maintained during this same period under the same conditions except that they did not receive alloxan. Clearance studies were performed with ^{125}I-labeled proteins in these animals as previously reported (3,4). The ligands studied were asialoorosomucoid (ASOR) prepared from orosomucoid by neuraminidase treatment (3), fucosyl-bovine serum albumin (Fuc-BSA) and N-acetylglucosaminyl-BSA (GlcNAc-BSA). The latter two ligands were prepared as described by Stowell and Lee (11). The mean carbohydrate valency of the neoglycoproteins was determined by titration with 2,4,6-trinitrobenzene sulfonic acid (12) and was Fuc-BSA, 50; GLcNAc-BSA, 47.

A second group of normal mice were used for clearance studies with Fuc-BSA, GlcNAc-BSA and in place of ASOR, galactosyl-BSA (Gal-BSA). The latter ligand had a carbohydrate valency of 42. In this series of studies the ligands were injected in the presence or absence of glucose at different concentrations such that the final blood glucose levels were 5, 25 or 50 mM as will be described in Results and Discussion. All sugars utilized in these studies were in the D configuration except for L-fucose.

In vitro binding assays were performed with soluble purified rat GlcNAc/mannose receptor as previously described (6), except that 300 ng ^{125}I-mannan was substituted with 10 ng ^{125}I-GlcNAc-BSA. Briefly, duplicate assays with and without 0.5 µg receptor were incubated 15 min at 23°C in .05 M Tris HCl, pH 7.8, .5% Triton X-100, .05 M $CaCl_2$, .4M KCl, 1% BSA and 0 to .1 M sugar (final volume .5 ml). At the end of 15 min, 10 ng ^{125}I-GlcNAc-BSA was added to each assay and the incubation continued for 15 min. The assay was terminated by the addition of 0.5 ml saturated $(NH_4)_2SO_4$ solution with .01 M $CaCl_2$, buffered at pH 7.2 with Tris base. The precipitate which formed after 10 min at 23° C was collected on Whatman GF/c filters under vacuum and washed with 10 ml of a 45% concentration of the saturated $(NH_4)_2$ SO_4 solution used in the previous step. Filters were counted for ^{125}I with a Scientific Products AW 14120 Gamma Counter. Nonspecific binding was determined by the addition of 10 µg GlcNAc BSA (unlabeled) and was 5% of the radiolabel in the assay.

Results and Discussion:

Fig. 1 shows the clearance of ASOR, Fuc-BSA and GlcNAc-BSA in diabetic mice. As previously described (9) these animals reached blood glucose levels of approximately 20 mM by two weeks. Clearance studies were performed with all three ligands at two and eight weeks after alloxan treatment. The data was essentially identical and the plots are the average of both groups. The clearance of ASOR and Fuc-BSA were nearly identical for diabetic mice and their control litter mates. However, GlcNAc-BSA clearance was consistently different. The clearance of GlcNAc-BSA in normal mice showed the disappearance of half the ligand in 1.8

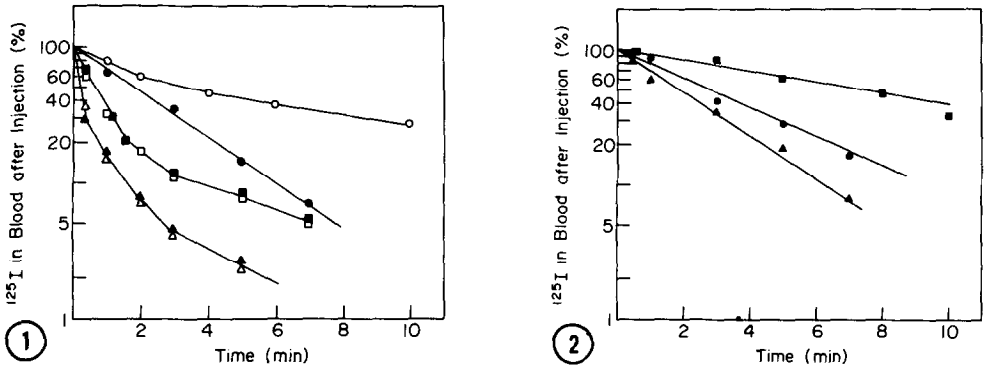


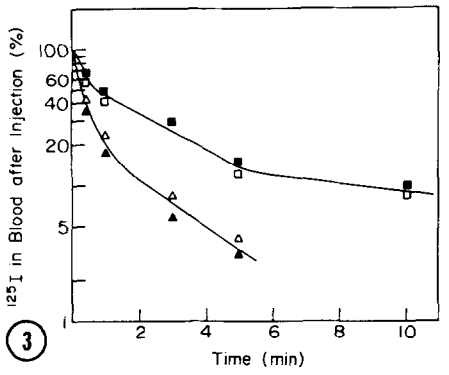
Fig. 1. The clearance of glycoproteins in diabetic and control mice. The filled symbols are controls (●, GlcNAc-BSA; ■, Fuc-BSA; and ▲, ASOR) while the open symbols are the diabetic animals (○, GlcNAc-BSA; □, Fuc-BSA; and △ ASOR).

Fig. 2. The clearance of GlcNAc-BSA in mice at differing blood glucose levels. Controls, 5 mM glucose, are represented by ▲; 25 mM glucose, ●; and 50 mM glucose, ■.

min while in diabetics it required 3.4 min for half the ligand to clear. Moreover, while in normal mice the ligand was rapidly removed from the circulation (7% remaining at 7 min) this was not the case in diabetic animals (27% at 10 min). The ligand continued to clear slowly from the diabetic circulation after this time (data not shown).

One possible explanation for these observations is that glucose directly competes for the clearance of GlcNAc-BSA but not the other ligands. This question was studied in a second series of mice in whom clearances were performed in the presence of exogenous glucose. The ligands studied were GlcNAc-BSA, Fuc-BSA and Gal-BSA in place of ASOR. Clearance of these ligands was determined in control animals (blood glucose, 5 mM) and in animals treated with exogenous glucose (blood glucose, 25 mM or 50 mM). A blood glucose of 25 mM corresponds to levels present in moderate diabetes while 50 mM represents levels present in severe diabetes.

Fig. 2 shows the effect of different blood glucose levels on the clearance of GlcNAc-BSA. The time for half the ligand to clear progressively increased as blood glucose increased. At 50 mM glucose the increase is from the control of 1.9 min to 7.4 min. By contrast, 50 mM glucose had little or no effect on the clearance of Gal-BSA or Fuc-BSA (Fig 3). These in vivo studies suggest diminished clearance of



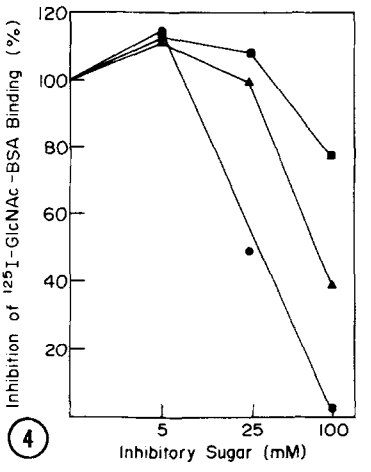


Fig. 3. The clearance of Gal-BSA and Fuc-BSA in mice at 5 and 50 mM glucose. Gal-BSA at 5 mM glucose is represented by ▲ and at 50 mM by △. Fuc-BSA at 5 mM glucose is represented by ■ and at 50 mM by □.

Fig. 4. Binding of ^{125}I-GlcNAc-BSA to purified rat GlcNAc/mannose receptor; the effect of GlcNAc, ●; glucose, ▲; and N acetylgalactosamine, ■.

ligands by the GlcNAc/mannose receptor in the presence of glucose concentrations observed in moderate to severe diabetes.

Since the data with varying glucose concentrations suggests a direct competition (Fig 2), the GlcNAc/mannose receptor was purified from rat and binding of ^{125}I-GlcNAc-BSA studied essentially as described (6). As is expected, GlcNAc is the best inhibitor of ^{125}I-GlcNAc-BSA binding, while N-acetylgalactosamine is a poor inhibitor (6). However, glucose becomes a reasonable inhibitor at high concentrations. It is of interest that both the purified gal and fuc receptors are also inhibited by glucose in vitro (13,14) but glucose apparently has little effect on the in vivo clearance of ligands by these receptors. It is difficult to directly compare in vitro and in vivo data because conditions are very different in these two situations. However, the in vitro data supports the suggestion that high glucose concentrations, as seen in diabetes, interfers with clearance of ligands by the GlcNAc/mannose receptor. The significance of such an effect is unclear. However, this receptor has been shown to clear IgM-antigen complexes (8). The effect of delayed clearance of such complexes, should it occur, is unknown at this time.

This study was supported by grants from the National Heart, Lung and Blood Institute (R01 HL 24066), the National Institute of Arthritis and Metabolism (R01 AM 26998) and the National Institute of General Medical Sciences (R01 GM 25766).

References

1. Neufeld, D.F. and Ashwell, G., (1980) in Lennarz, W. J. (ed) The Biochemistry of Glycoproteins and Proteoglycans pp. 241-266, Plenum Press, New York

2. Ashwell, G. and Morell, A.G. (1974) Adv. Enzymol. 41, 99-128

3. Prieels, J.-P., Pizzo, S.V., Glasgow, L.R., Paulsen, J.C., and Hill, R.L. (1978) Proc. Natl. Acad. Sci., USA 75, 2215-2219

4. Hill, R.L., Pizzo, S.V., Imber, M., Lehrman, M., Prieels, J.-P., Glasgow, L.R., Guthrow, C.E., and Paulson, J.C. (1980) Birth Defects: Original Articles Series, Vol XVI, pp 85-91

5. Stahl, P.D., Rodman, J.S., Miller, M.J., and Schlesinger, P.H. (1978) Proc. Natl. Acad. Sci., USA 75, 1399-1403

6. Kawasaki, T., Etoh, R., and Yamashina, I. (1978) Biochem. Biophys. Res. Comm. 81, 1018-1024

7. Thornburg, R.W., Day, J.F., Baynes, J.W., and Thorpe, S.R. (1980) J. Biol. Chem. 255, 6820-6825

8. Day, J.F., Thornburg, RW., Thorpe, S.R., and Baynes, J.W. (1980) J. Biol. Chem. 255, 2380-2365

9. Rossini, A.A., Like, A.A., Chicki, W.L., Appel, M.C., and Cahill, G.F. (1977) Proc. Natl. Acad. Sci. USA 74, 2485-2489

10. Like, A.A. and Rossini, A.A. (1976) Science 193, 415-417

11. Stowell, C. and Lee, Y.C. (1980) Biochemistry 19, 4899-4904

12. Fields, R. (1972) Meth. Enzym. 25, 464-468

13. Sarkar, M., Liao, J., Kabot, E.A., Tanabe, T., and Ashwell, G. (1979) J. Biol. Chem. 254, 3170-3174

14. Lehrman, M.A. and Hill, R.L. (1981) Fed. Proc. 40, 1820