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CELL SURFACE CHANGES IN DIABETIC RATS

STUDIES OF LECTIN BINDING TO LIVER CELL PLASMA MEMBRANES

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Summary

We have previously reported changes in the chemical composition of cell surface membranes in diabetic rats (Chandramoulis, V. and Carter, Jr., J.R. (1975) Diabetes 24, 257—262 [1]). To examine the possible implications of these changes for cell surface structures, we have measured the binding of labeled lectins and desialylated glycoproteins to plasma membranes prepared from the livers of streptozotocin-diabetic and control rats. Lectins were chosen which have affinities for different carbohydrate moieties. The binding of ricin and concanavalin A to liver cell membranes from the diabetic rats was significantly reduced, but no change in the binding of wheat germ agglutinin was noted. Binding of desialylated thyroxine-binding globulin, previously shown to be dependent on membrane sialic acid residues, was strikingly reduced in liver membranes from diabetic rats. These results strongly suggest that insulin deficiency leads to generalized changes in cell surface glycoproteins, at least in this animal model of diabetes.

Introduction

There have been numerous studies on the binding of lectins to normal and neoplastic mammalian cells [2]. However, measurements of lectin binding to isolated plasma membranes seem to be of recent origin. Concanavalin A has been shown to bind to plasma membranes from liver and mammary gland [3] and to inhibit 5′-nucleotidase from liver and mammary gland and activate Mg^{2+}-ATPase from liver and fat cells [4]. Concanavalin A and wheat germ agglutinin were found to be as effective as insulin in enhancing the rate of glucose trans-

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port and in inhibiting epinephrine-stimulated lipolysis in isolated adipocytes. In addition, concanavalin A and wheat germ agglutinin compete with insulin for binding to intact adipose cells and to insulin binding proteins solubilized from the membranes, suggesting that these lectins interact directly with insulin receptors [5].

Chang et al. [6] have recently suggested that, in at least two animal models of obesity (ob/ob and db/db mice), defects in insulin receptors may actually reflect a more generalized change in membrane glycoproteins. Such a change if present could have implications for a wide variety of cell functions associated with the surface membrane. We have recently reported [1] changes in the chemical composition of plasma membranes of liver and erythrocytes from streptozotocin diabetic rats. The present studies were conducted to see if these chemical changes were reflected by alterations in the lectin binding properties of cell surface membranes in these animals.

Materials and Methods

Materials

Concanavalin A (Grade IV), wheat germ agglutinin, α-methyl-D-mannopyranoside and *N*-acetyl-D-glucosamine were purchased from Sigma Chemical Co. Ovomucoid, Sephadex and CNBr-activated Sepharose 4B were products of Pharmacia Fine Chemicals. $Na^{125}I$ was from Amersham Searle. All other chemicals were reagent grade.

Animal and tissue preparation

Male Sprague-Dawley rats weighing 110—140 gm were made diabetic by the intravenous injection of streptozotocin (65 mg/kg body weight). Animals were used only if they had a blood sugar greater than 250 mg% on the third day after injection. Diabetic animals and comparably aged controls were maintained on ad libitum feeding and were sacrificed after four weeks. At this point, the average blood sugar of the diabetic rats was 575 mg/dl and of the controls 129 mg/dl. Body weight averaged 194 gm for the diabetics and 315 gm for the controls.

After sacrifice, livers were rapidly perfused in situ via the portal vein with 20—30 ml of chilled isotonic saline, excised and blotted dry. Livers were minced, and plasma membranes prepared by Ray's modification [7] of the method of Neville [8]. The final washed membrane preparation was diluted to a concentration of 1 mg of protein per ml in 1 mM sodium bicarbonate containing 0.5 mM $CaCl_2$ at pH 7.5 and stored at —60° C until assayed. Preliminary experiments indicated that membranes stored for as long as 6 months at —60° C showed no changes in lectin binding when compared to freshly prepared membranes.

Preparation of labeled lectins and desialylated thyroxine-binding globulin

Concanavalin A was iodinated with ^{125}I and purified by affinity chromatography on Sephadex G-100 as described by Cuatrecasas [9]. The labeled lectin had a specific activity of 1.6 μCi/μg. Greater than 99% of the radioactivity was precipitated by 7% trichloroacetic acid, and 96% adsorbed onto Sephadex

G-100. Chromatography of the unlabeled Concanavalin A on polyacrylamide gels in sodium dodecyl sulfate and dithiothreitol revealed three discrete and one diffuse band; 93% of the radioactivity of the I^{125}-labeled lectin co-migrated with these bands.

Wheat germ agglutinin was iodinated and purified by affinity chromatography on a small agarose-ovomucoid column as described by Cuatrecasas [9]. The labeled lectin had a specific activity of 2.6 μCi/μg protein. 96% of the radioactivity was precipitated by 7% trichloroacetic acid and 96% bound to a separate ovomucoid-agarose column. Polyacrylamide gels of the unlabeled wheat germ agglutinin in sodium dodecyl sulfate and dithiothreitol revealed a single band; 87% of the radioactivity of the I^{125}-labeled lectin co-migrated with this band, with the remaining 13% spread diffusely through the gel.

The 120 000 molecular weight lectin was isolated and purified from castor beans according to the method of Nicolson and Blaustein [10]. The purified ricin was iodinated with ^{125}I by the method of Levy et al. [11]. Specific activity of the ^{125}I-labeled ricin was 30 μCi/μg, and it showed a single radioactive band on polyacrylamide gel electrophoresis in sodium dodecyl sulfate and dithiothreitol.

Thyroxine-binding globulin was purified from pooled human serum, desialylated with *Vibrio cholerae* neuraminidase, and labeled with ^{125}I by previously described methods [12]. Specific activity was about 50 μCi/μg. Protein was measured by the method of Lowry et al. [13].

Binding and displacement assays

Specific binding of ^{125}I-labeled concanavalin A and wheat germ agglutinin to liver cell plasma membranes was measured by a method similar to that described by Cuatrecasas [9] for fat cells. Liver plasma membranes containing 50 μg of protein were suspended in 0.2 ml of Krebs-Ringer bicarbonate buffer with 0.1% (w/v) bovine albumin at a pH of 7.4. Incubations with the labeled lectins were carried out at 24°C for 60 min, after which the mixture was diluted with 3 ml of ice cold Krebs-Ringer bicarbonate buffer containing 0.1% (w/v) bovine albumin, filtered rapidly over a Millipore filter, and the filter washed with a further 10 ml of the same buffer. Several types of filters were tested and it was found, in agreement with Cuatrecasas [9], that Teflon filters (LSWP Millipore Corp.) gave the lowest background with concanavalin A and nylon filters (NRWP, Millipore Corp.) were best for wheat germ agglutinin. To determine "specific" binding, control tubes were incubated with 0.1 M α-methyl-D-mannopyranoside in the case of concanavalin A and excess lectin (200 μg per tube) in the case of wheat germ agglutinin. These gave respectively 91 and 96% displacement of tracer amounts of the labeled lectin after allowance was made for the counts bound to the filter in the absence of any membranes. In the case of concanavalin A the sugar was used, since unlabeled lectin even at 1 mg/ml displaced only 81% of a tracer amount of labeled lectin. In all binding studies, "non-specific" binding was corrected for by subtracting the counts bound to the filter in the presence of excess lectin or sugar.

To measure displacement of ^{125}I-labeled lectin by unlabeled lectin, two procedures were used. For wheat germ agglutinin, increasing amounts of unlabeled lectin were added to the membrane suspension and incubated for ten

minutes at 24°C, then a constant (tracer) amount of the ^{125}I-labeled wheat germ agglutinin added to all tubes and the incubation continued for another 50 min before dilution and washing [9]. To measure displacement of concanavalin A at 24°C, the labeled and unlabeled lectins were added together since preliminary experiments indicated that order of addition made no difference. In both cases, a correction was made for counts bound to the filter in the absence of plasma membranes.

Binding of ^{125}I-labeled ricin and displacement by unlabeled ricin was carried out with 50 μg of membrane protein in Krebs-Ringer-phosphate buffer containing 0.2% (w/v) albumin at 4°C for 60 min. This temperature was chosen since maximal binding was observed under these conditions. After the incubation, 3 ml of cold buffer containing albumin was added to the tubes and membranes were harvested by filtration and washing on Whatman GF/C (2.4 cm) glass fiber filters. Non-specific binding was measured in the presence of 0.1 M β-D-galactose.

For calculations of molar concentrations of the plant lectins, the following molecular weights were used: concanavalin A, 100 000 [14]; wheat germ agglutinin, 25 000 [15]; ricin, 120 000 [10].

The assay to measure binding of I^{125}-labeled desialylated thyroxine-binding globulin to liver membranes has been described in detail previously [16]. In this assay unlabeled desialylated thyroxine-binding globulin is first incubated with membranes after which labeled glycoprotein sufficient to saturate all binding sites is added. Binding was measured at 24°C.

In all assays, counting of the filters containing the membranes was done in a well-type gamma counter using disposable tubes.

Polyacrylamide gel electrophoresis

Analysis of membrane proteins on 5.6% polyacrylamide gels in sodium dodecyl sulfate and dithiothreitol and staining with Coomassie blue were done by the method of Fairbanks et al. [17].

Results

Binding and displacement of lectins

Concanavalin A. Concanavalin A binding to plasma membranes from the liver of a single normal rat is shown in Fig. 1. Saturation of "specific" binding sites was not observed at the highest concentration of the lectin tested, 200 μg/ml. Similar results have been reported by Cuatrecasas [9] for rat fat cells, where levels of 1 mg/ml were required to approach saturation. Under the assay conditions used here, approximately 15% of the added lectin bound at the lowest levels tested, declining to approximately 6% at 200 μg/ml.

Displacement of ^{125}I-labeled concanavalin A from liver membranes by unlabeled lectin (Fig. 2) shows a biphasic curve; this has been observed by others in fat cells [9] and liver plasma membranes [18]. With the addition of 10 μg/ml or less of the unlabeled lectin there is enhancement of the binding of the ^{125}I-labeled concanavalin A; at greater concentrations there is progressive displacement of the labeled lectin. However, even when 1000 μg/ml is added there is only 80% displacement of the labeled lectin, less than is displaced by 50 or

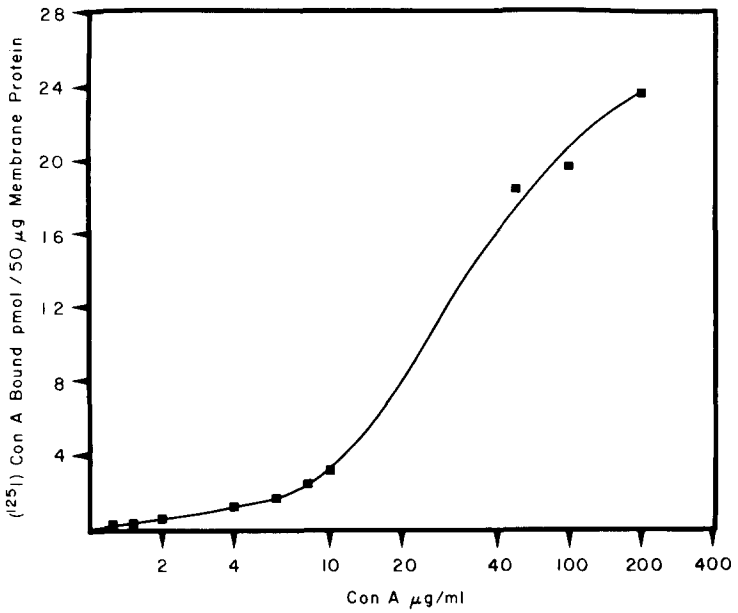

Fig. 1. Binding of ^{125}I-labeled concanavalin A (Con A) by liver plasma membranes from a normal rat. Specific binding was measured as described in Materials and Methods.

100 mM concentrations of the competing sugar α-methyl-D-mannopyranoside (Fig. 2).

Wheat germ agglutinin. Binding of wheat germ agglutinin to liver membranes is shown in Fig. 3. As with concanavalin A, saturation of the specific binding

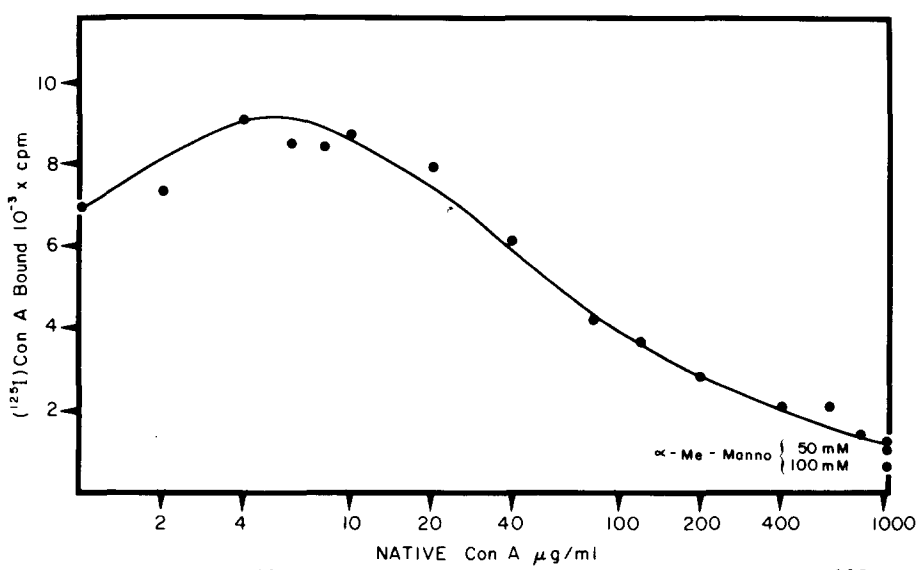

Fig. 2. Displacement of ^{125}I-labeled concanavalin A (Con A) by unlabeled lectin. ^{125}I-labeled concanavalin A (66.6 ng; 72 700 cpm) plus the indicated concentration of unlabeled lectin were added to 50 μg of liver plasma membranes in a total volume of 0.2 ml to determine displacement. See Materials and Methods for details.

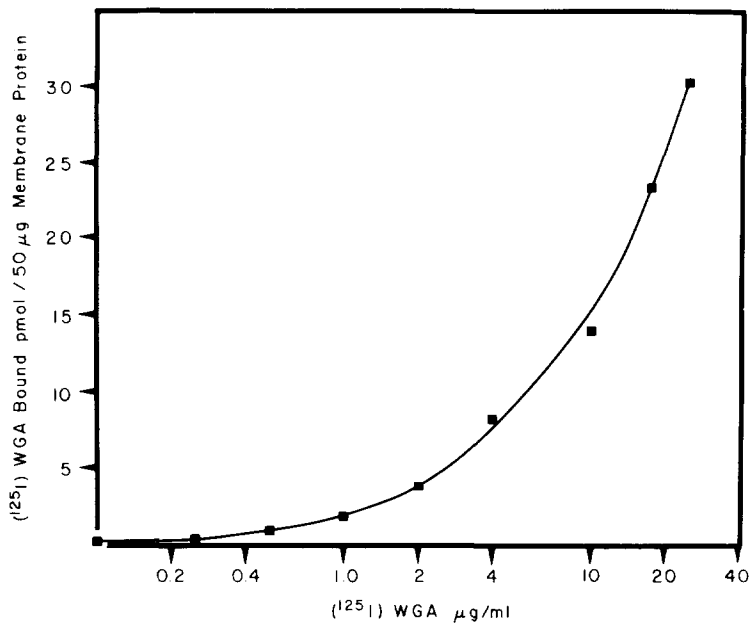

Fig. 3. Binding of ^{125}I-labeled wheat germ agglutinin (WGA) by liver plasma membranes from a normal rat.

sites was not obtained in the range of lectin concentration tested, up to 25 μg/ml. This corresponds to what has been reported for rat fat cells [9]. At very low concentrations, 50—60% of the radioactivity bound to the liver plasma membranes. In contrast to concanavalin A, displacement of ^{125}I-labeled wheat germ agglutinin by unlabeled lectin was observed at all concentrations tested (Fig. 4). The addition of 150 μg/ml of unlabeled wheat germ agglutinin led to almost complete (≈99%) displacement of the labeled lectin. Binding was also completely abolished (data not shown) when incubations were carried out in the presence of 100 mM *N*-acetyl-D-glucosamine.

Ricin. We studied only the 120 000 molecular weight protein obtained from castor beans. This lectin, with a specificity for galactosyl residues, has been less extensively studied than concanavalin A or wheat germ agglutinin; it was therefore necessary to determine optimal conditions to measure binding. A number of filters were tried; nylon and teflon Millipore filters and Whatman GF/c glass filters all gave acceptably low backgrounds; the glass filters were used in these experiments. Binding was higher at 4°C than at 24 or 37°C, in contrast to the other lectins.

Fig. 5 shows the time course of binding of ^{125}I-labeled ricin to liver membranes from a control rat at 4 and 24°C. Little further binding was observed after 60 min, and this time was routinely used in subsequent assays.

Binding of ricin to liver membranes as a function of lectin concentration is shown in Fig. 6. At low concentrations (20—100 ng/ml) binding is linear but becomes sigmoidal above 0.2 μg/ml. The binding sites on 50 μg of membrane protein were saturated at about 20 μg/ml of ^{125}I-labeled ricin.

When increasing amounts of unlabeled lectin were added to a tracer amount

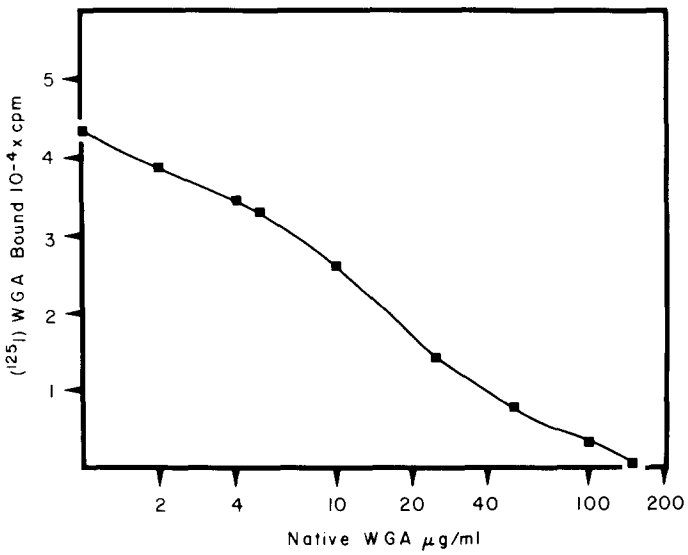

Fig. 4. Displacement of ^{125}I-labeled wheat germ agglutinin (WGA) by unlabeled lectin. ^{125}I-labeled wheat germ agglutinin (16 ng; 94 000 cpm) was added to 50 μg of membrane protein followed by the indicated concentration of unlabeled lectin to determine displacement. See Materials and Methods for details.

of ^{125}I-labeled ricin, a biphasic displacement curve was observed (Fig. 7) similar to that seen with concanavalin A (Fig. 2). Up to 0.4 μg/ml of unlabeled lectin increased the amount of labeled ricin bound; above 2 μg/ml, progressive displacement was observed. This suggests positive cooperativity at the lower levels of lectin tested. At 1 mg/ml of unlabeled ricin, 95% of the radioactivity was displaced; quantitative displacement (99%) was seen in the presence of 0.1 M β-D-galactose.

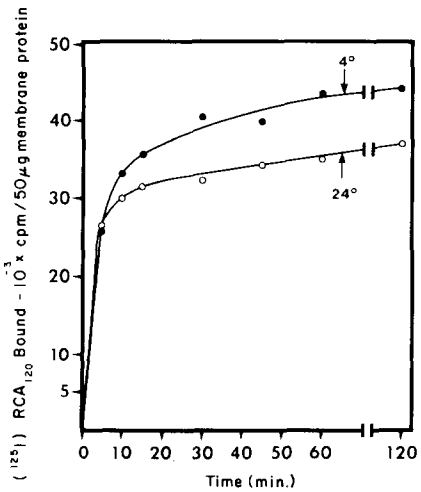

Fig. 5. Time course of binding of ^{125}I-labeled ricin (RCA_{120}) to liver plasma membranes from a normal rat. Binding to 50 μg of membrane protein was measured at 4 and 24°C as described in Materials and Methods.

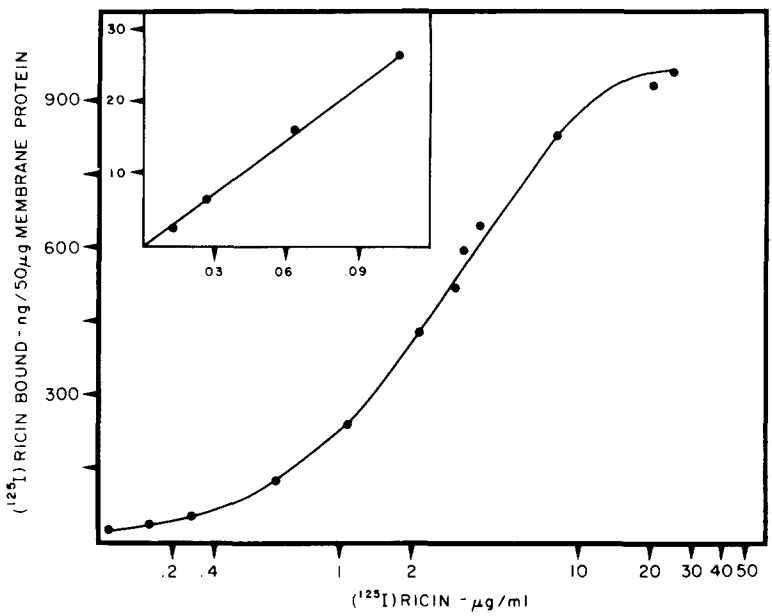

Fig. 6. Binding of ^{125}I-labeled ricin to liver plasma membranes. Measurements were made at 24°C. The insert shows linearity of binding at low levels of added ricin. Note arithmetic scale of insert abscissa.

Since little is known about the specificity of ricin binding, we carried out an experiment to see if wheat germ agglutinin or concanavalin A would competitively displace the bound ricin (Table I). Wheat germ agglutinin at 20 μg/ml displaced approximately 25% of the bound ricin and at 200 μg/ml 50% from both control and diabetic membranes; this inhibition was largely but not entirely reversed by adding 0.1 M *N*-acetyl-D-glucosamine. Concanavalin A at 100 μg/

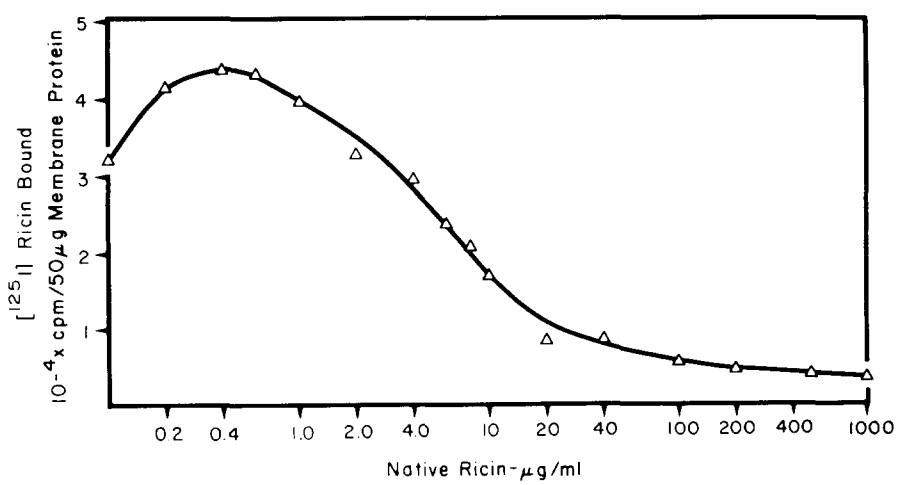

Fig. 7. Displacement of ^{125}I-labeled ricin by native lectin. ^{125}I-labeled ricin (4.5 ng, 72 120 cpm) plus the indicated concentration of unlabeled lectin was added to 50 μg of membrane protein in each tube to determine displacement. Incubation was at 4°C. See Materials and Methods for details.

TABLE I

EFFECT OF WHEAT GERM AGGLUTININ AND CONCANAVALIN A ON BINDING OF ^{125}I-LABELED RICIN BY LIVER MEMBRANES

Liver membranes (50 μg of protein) from diabetic and control animals were incubated in the presence or absence of the indicated lectins, with or without specific sugar, for 30 min at 4°C. At that point, ^{125}I-labeled ricin, (2.25 ng containing 39 600 cpm), was added to each tube and the incubation continued for another 60 min at 4°C. At the end of the incubation membranes were filtered, washed and counted as described in Materials and Methods.

Lectin added	Concentration (μg/ml)	Inhibitor	^{125}I-labeled ricin bound (cpm)	
			Control	Diabetic
None			25 167	20 425
None		0.1 M D-galactose	3 134	
Wheat germ agglutinin	20			15 035
Wheat germ agglutinin	20	0.1 M *N*-acetyl-D-glucosamine		18 926
Wheat germ agglutinin	200		13 854	10 305
Wheat germ agglutinin	200	0.1 M *N*-acetyl-D-glucosamine	21 861	16 533
Concanavalin A	100		21 296	18 088
Concanavalin A	100	0.1 M α-methyl-mannopyranoside	21 045	18 730

ml inhibited the binding of ricin only slightly, and this inhibition was not reversed by 0.1 M α-methyl-mannopyranoside.

Effect of diabetes upon lectin binding to liver cell membranes

We wished to see whether insulin deficiency resulted in changes in the binding properties of lectins on cell surfaces. For this purpose we chose three lectins with different carbohydrate specificities for binding. Because of the complexity of the dissociation curve for concanavalin A from liver membranes, we assessed binding directly. As shown in Fig. 8, plasma membranes prepared from the livers of diabetic rats showed a decrease of approximately 25% in the binding of concanavalin A at all concentrations of the lectin tested. Since we have found, as others have [9], that Scatchard analysis of concanavalin A binding gives uninterpretable curves, we are unable to say whether this reflects a change in affinity or number of one or more types of binding sites.

Since I^{125}-labeled wheat germ agglutinin is readily displaced by all concentrations of unlabeled protein, we studied the effect of diabetes on binding by displacement analysis. There was no significant difference between membranes from control and diabetic animals in the amount of ^{125}I-labeled lectin bound, and displacement by the unlabeled protein was identical at all concentrations tested (Fig. 9). These data give strong evidence that the differences observed in concanavalin A binding did not reflect a difference in the types of membranes isolated from livers of diabetic and control rats.

Results with ^{125}I-labeled ricin were similar to those seen with concanavalin A. Membranes from diabetic rats bound significantly less labeled lectin (15—25% decrease) than did membranes from control animals at all concentrations of lectin tested (Fig. 10).

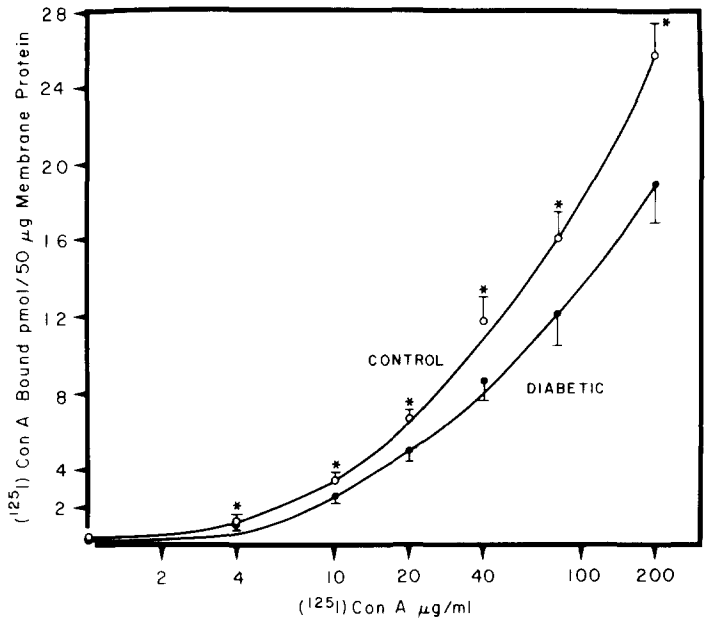

Fig. 8. Binding of ^{125}I-labeled concanavalin A (Con A) to liver membranes from control and diabetic rats. Membranes from 10 pairs of control and diabetic rats were tested as in Fig. 1. Results are given as the mean ± S.E.M. *, diabetic significantly different than control, $P < 0.05$.

Binding of desialylated glycoproteins

The characteristics of the binding of I^{125}-labeled desialylated thyroxine-binding globulin to liver plasma membranes from the rat have been described [12, 16]. This binding differs from the lectins studied above in being essentially

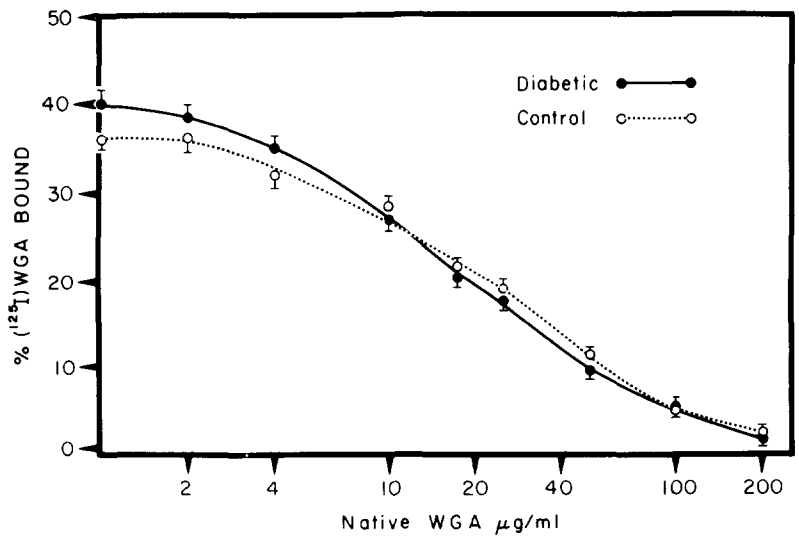

Fig. 9. Displacement of ^{125}I-labeled wheat germ agglutinin (WGA) by unlabeled lectin from membranes of control and diabetic rats. Liver membranes from 7 pairs of control and diabetic rats were tested as in Fig. 4. Results are the means ± S.E.M.

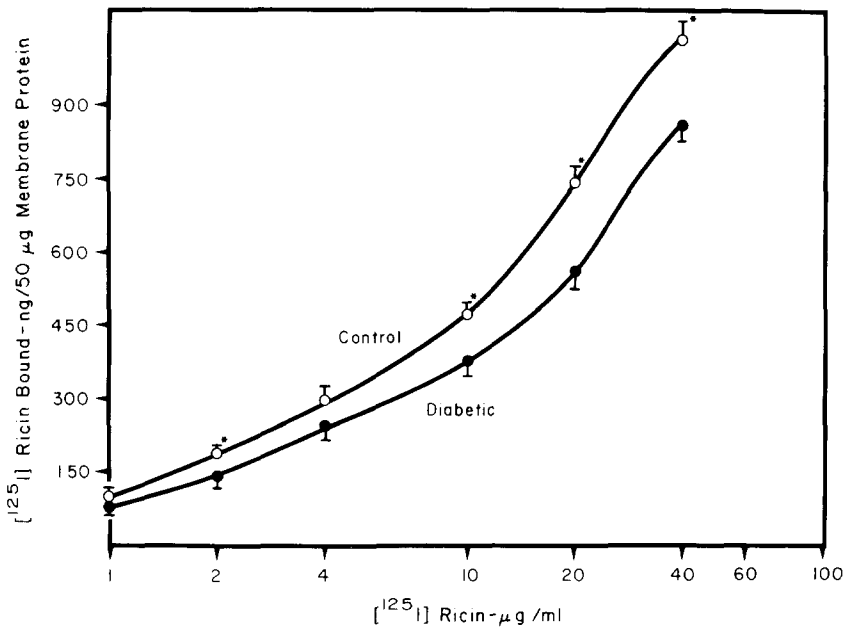

Fig. 10. Binding of ^{125}I-labeled ricin to liver plasma membranes from control and diabetic animals. The ability of membranes from 5 pairs of control and diabetic rats to bind ^{125}I-labeled ricin was tested as in Fig. 6. Results are means ± S.E.M. *, diabetic significantly different from control, $P < 0.05$.

irreversible [16]; it appears to depend on sialic acid residues in the membrane "receptor", since neuraminidase treatment of the membranes essentially abolishes binding [16]. We were particularly interested in the effects of chronic diabetes on the binding of desialylated glycoproteins to cell membranes, since we had previously shown [1] a small but significant decline in sialic acid con-

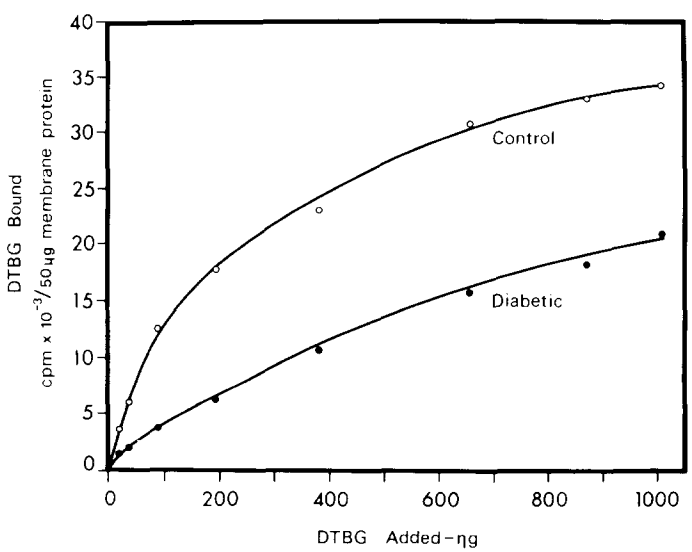

Fig. 11. Binding of ^{125}I-labeled desialylated thyroxine-binding globulin (DTBG) to liver plasma membranes from control and diabetic animals. Results are the mean values from 5 pairs of animals.

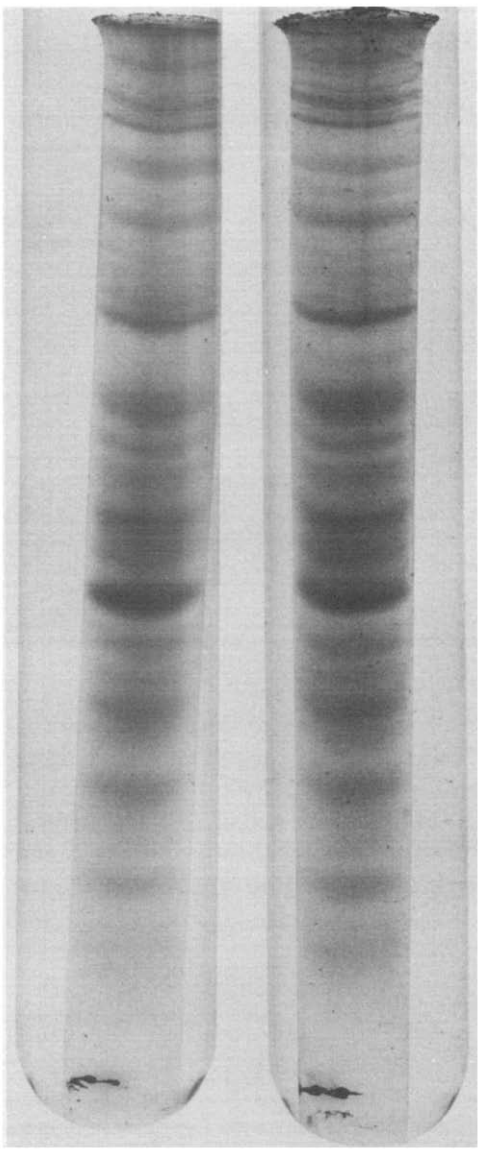

Fig. 12. Polyacrylamide gel patterns of liver plasma membranes from diabetic (left) and control (right) animals.

tent of both liver and red cell membranes obtained from diabetic animals. This change was reflected in a striking decrease in the binding of ^{125}I-labeled desialylated thyroxine-binding globulin to the plasma membranes of livers from diabetic rats as compared to controls (Fig. 11).

Effect of diabetes on liver receptors for insulin

Davidson and Kaplan [19] have reported that liver plasma membranes prepared from streptozotocin-diabetic rats bind more insulin than membranes

from control animals. We have confirmed this observation in our present studies. Membranes from four pairs of control and diabetic animals were tested for their ability to bind ^{125}I-labeled insulin over the range of 4—20 ng/ml of the hormone. Binding was approximately twice as great to the liver membranes of diabetic animals (data not shown) over the whole concentration range tested.

Protein composition of plasma membrane fractions

It appeared possible that our results could in part be caused by contamination of the liver plasma membrane fraction from diabetic rats by intracellular membranes. To assess this possibility, we examined representative membrane preparations from control and diabetic animals by polyacrylamide gel analysis (Fig. 12). No differences were observed in the protein patterns of these two preparations. Since the gel patterns of both rough and smooth endoplasmic reticulum fractions (not shown) differ significantly from that of plasma membranes, it is highly unlikely that there is a significant difference in the composition of the plasma membranes prepared from control and diabetic rats.

Discussion

Our initial interest in possible changes in cell surface membranes in diabetes was the result of a chance observation. Normally, liver plasma membranes prepared by the Neville method [8] are obtained as a discrete, thin sheet between the 37 and 41% sucrose layers on a discontinuous gradient. In the course of preparing membranes from diabetic animals for other studies, we observed that these membranes were isolated as a rather diffuse, flocculant band extending 1 or 2 mm into each layer. This suggested a rather significant change in some physical property of the membrane such as density or surface charge. Our initial studies on the chemical composition of these plasma membranes [1] showed small but reproducible changes in both cholesterol and sialic acid content. Lectins seemed to offer ideal probes to look further at possible changes in cell surface structure. While there has been great interest in changes in hormone "receptors" in states of obesity, insulin resistance and diabetes [20], we were interested in the possibility of finding more generalized changes in cell surface structures. While these studies were in progress, Chang et al. [6] reported that binding of concanavalin A and wheat germ agglutinin to liver membranes, kidney cells and cultured lymphocytes was decreased in two animal models of obesity and diabetes, ob/ob and db/db mice.

Our studies have confirmed and extended the results of Chang et al. [6] in another animal model of diabetes. Our streptozotocin-treated animals develop moderate disease but do not show severe ketosis, and the majority can be maintained without insulin for periods of 4—8 weeks. We chose three lectins with different carbohydrate specificities as probes to look for changes in glycoprotein and/or membrane structure induced by insulin deficiency. In the case of concanavalin A and wheat germ agglutinin, the lectins appear to recognize predominantly separate "sites" on the membrane. Our own studies (Table I) indicate some competition between ricin and wheat germ agglutinin for binding sites but essentially none between ricin and concanavalin A.

The initial studies of binding and displacement of concanavalin A and wheat

germ agglutinin to control plasma membranes (Figs. 1—4) gave results comparable to those reported by others in liver membranes and fat cells [9,18]; in particular, we noted the enhanced binding ("positive cooperativity") of ^{125}I-labeled concanavalin A induced by low concentrations of unlabeled lectin (Fig. 2). This is not observed with wheat germ agglutinin. Since we could not find comparable studies on ricin, we characterized the binding of this lectin somewhat more extensively. Binding to membranes is rapid even at 4°C (Fig. 5) and essentially complete by 60 min; binding is somewhat greater at 4°C than at 24 or 37°C. Binding is completely reversed by high concentrations of the sugar D-galactose and more than 90% reversed by sufficiently high concentrations of unlabeled lectin (0.5—1.0 mg/ml). Like concanavalin A, low concentrations of the lectin (<1 μg/ml) increase the binding of ^{125}I-labeled ricin (Fig. 7), suggesting positive cooperativity of at least one class of high affinity receptors. As with wheat germ agglutinin [9], Scatchard plots of this data gave complex curves that were difficult to interpret. In view of the likelihood that multiple glycoproteins are involved in the binding of a single lectin [21,22], attempts at extensive analysis of binding data would appear to be fruitless until single, purified glycoprotein species can be studied.

In view of the changes already observed in sialic acid content in liver membranes from diabetic animals [1], we were anxious to test binding of a protein with specificity for this terminal residue. For this purpose we chose desialylated thyroxine-binding globulin. Binding of this and other desialylated glycoproteins is completely inhibited by prior treatment of liver membranes with purified neuraminidase [16] and thus is dependent on the integrity of membrane sialic acid residues. Unlike the lectins studied, binding of desialylated thyroxine-binding globulin is not readily reversible [16].

Diabetes induced significant changes in the binding of all proteins studied except wheat germ agglutinin. First, we were able to confirm (data not shown) the observation of Davidson and Kaplan [19] that insulin binding was increased in liver membranes from these animals. The binding of concanavalin A and ricin was reduced 20—25% in liver membranes of diabetic animals as compared to controls. Since binding of wheat germ agglutinin was not significantly changed, this suggests separate receptors for these lectins. Since concanavalin A and ricin do not compete for the same receptors (Table I), these data presumably reflect changes in at least two, and possibly more, cell surface receptors. The changes observed in the binding of desialylated thyroxine-binding globulin were even more striking (Fig. 11). The 50% decrease represented a much greater reduction than we observed [1] in total sialic acid content of these membranes, which declined about 10% in diabetic animals. This suggests either a selective reduction in the sialic acid content of the specific receptor for desialylated glycoproteins, or some change in the receptor beyond simple decreases in terminal sialyl residues.

One possible explanation for the above findings would be contamination of the membrane preparations from diabetic rats with intracellular membranes. This seems unlikely for a number of reasons. First, contamination would have to be of the order of 25—50% to explain the observed differences between controls and diabetics. Second, wheat germ agglutinin binding was unchanged and insulin binding significantly increased; the latter in particular would be unlikely

if the membranes from diabetic animals were significantly more contaminated with intracellular structures than control membranes. Third, in a previous study [1] we found no change in either total membrane phospholipids or individual classes of phospholipids in liver membranes from diabetic animals; since the phospholipid composition of each membrane is relatively specific, it is likely that we would have seen changes if such contamination had occurred. Finally, gel analysis (Fig. 12) showed absolute identity of the proteins in the plasma membranes from diabetic and control livers, making significant contamination highly unlikely.

Taken together, these experiments suggest significant and widespread changes in cell surface receptors in the streptozotocin-diabetic rat. It is not clear at present whether these represent structural changes in the carbohydrate sidechains or the basic protein skeleton of the membrane glycoproteins, or perhaps some more subtle aspect of membrane structure affecting receptor mobility or availability to ligands.

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