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DIABETES-INDUCED VARIATION IN HEPATIC BINDING PROTEIN

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The total capacity of hepatocytes to bind asialoorosomucoid was measured on normal and streptozotocin diabetic rats. 4 days after the streptozotocin injection, a slight decrease of total receptor concentration was observed while a more marked reduction of cell surface receptor occurred. In animals sacrified 11 days after the streptozotocin injection, the total capacity of hepatocytes to bind asialoorosomucoid was about 70 % of the normal level.

Modifications of the glycoconjugate content of different cells have been observed in human and experimental diabetes mellitus (1, 2). Furthermore in isolated hepatocytes from streptozotocin diabetic rats, the endocytosis of desialylated (3) serum glycoproteins was markedly decreased (4, 5). These alterations have been related to a decrease in the number of cell surface specific receptors (5) and shown to be reversible by insulin treatment of the diabetic animals.

Since the receptors of serum asialoglycoproteins are present on the sinusoidal surface of hepatic parenchymal cells and are also located intracellularly (6, 8) this study was undertaken in order to see if, in the case of diabetes, the decrease in the number of cell surface receptors is accompanied by a decrease of total receptor concentration.

MATERIALS AND METHODS

Human orosomucoid, a generous gift from Doctor Wickerhauser (Plasma Derivatives Laboratory of American Red Cross) was desialylated and labelled as described previously (5). Male Sprague-Dawley rats (220-250 g) were obtained from Charles Rivers (France). Collagenase type III was from Worthington. Scintillation fluid (aqueous combining system) was from Amersham. Other chemicals were reagent grade.

Treatment of rats and cell preparation

After an overnight fast (with free access to water) rats were randomly divided into two groups. Animals from one group received streptozotocin (65 mg/kg body weight, dissolved in isotonic saline acidified to pH 4.5 with citric acid) through the tail vein (diabetic rats). Rats from control group were injected with medium alone (normal rats). The general characteristics of streptozotocin diabetic rats were shown in Table I.

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TABLE I : GENERAL CHARACTERISTICS OF STREPTOZOTOCIN DIABETIC RATS

	Animal number	Body weight variation (g)	Blood Glucose (g/l)	Serum Insulin (μU/ml)
4 days after the streptozotocin injection	8	no	3.5 ± 0.5	3.4 ± 2
11 days after the streptozotocin injection	8	30 ± 7	5 ± 0.5	1.7 ± 0.8
20 days after the streptozotocin injection	8	50 ± 10	7 ± 0.3	1 ± 0.5

Blood glucose was measured by glucose oxidase method (9) and serum insulin by radioimmuno-assay (10)

Hepatocytes were prepared using the collagenase perfusion procedure of Berry and Friend (11). Routinely 70-85 % of single cells and 80-90 % of viable cells, as judged by either trypan blue exclusion or release of lactate dehydrogenase (12) were obtained from normal or diabetic rats.

Solubilization-Precipitation assay for determination of total receptor number

The cell pellets containing 5 x 10^6 cells were solubilized in medium (A) 1.33 % Triton x 100 (Packard)/20 mM Tris H Cl pH 7.6/150 mM Na Cl/50 mM Ca Cl_2. Different concentrations of $|^3H|$ asialoorosomucoid were then added in a final volume of 0.5 ml (A). The mixtures were incubated at 4° C for 2 hours in a giratory shaker.

After incubation, the samples were precipitated according to the method of Hudgin et al (13) 0.5 ml of satured ammonium sulfate adjusted to pH 7.6 with Tris was added to the mixture. Following an additional 30 min on ice, each sample was diluted by adding 10 ml of 50 % saturated ammonium sulfate/20 mM Ca Cl_2 adjusted to pH 7.6 with Tris (B) and was filtered through GF/C Filter (Whatman) that had been soaked previously in the solution B added of 0.5 % bovine serum albumin (Sigma). Precipitated materiel on the filters was washed three times with 5 ml of solution B added 0.5 % bovine serum albumin. The filters were eluted by 2 ml of water for an overnight, 15 ml of scintillation fluid was then added and the radioactivity was counted.

Non specifically bound $|^3H|$ asialoorosomucoid was determined either by dissolving the cells in the solution A containing 2 M galactose (Sigma) or by incubating the solubilized cells in presence of an excess (1000 x) of unlabelled asialoorosomucoid. Routinely aspecific binding was about 3 ± 1 % of $|^3H|$ asialoorosomucoid added.

Determination of cell surface receptor number

The binding assays were performed in triplicate flat bottomed tubes by incubating at 4° C 3 x 10^6 cells/ml of Krebs buffer with 4 μg/ml of $|^3H|$ asialoorosomucoid/ml for 2 hours as previously reported (5, 14).

RESULTS AND DISCUSSION

The ability of soluble hepatocytes freshly isolated from normal and diabetic rats to bind $|^3H|$ asialoorosomucoid is shown in Fig.1. The number of total receptors was estimated at the point of saturation. In normal rats,

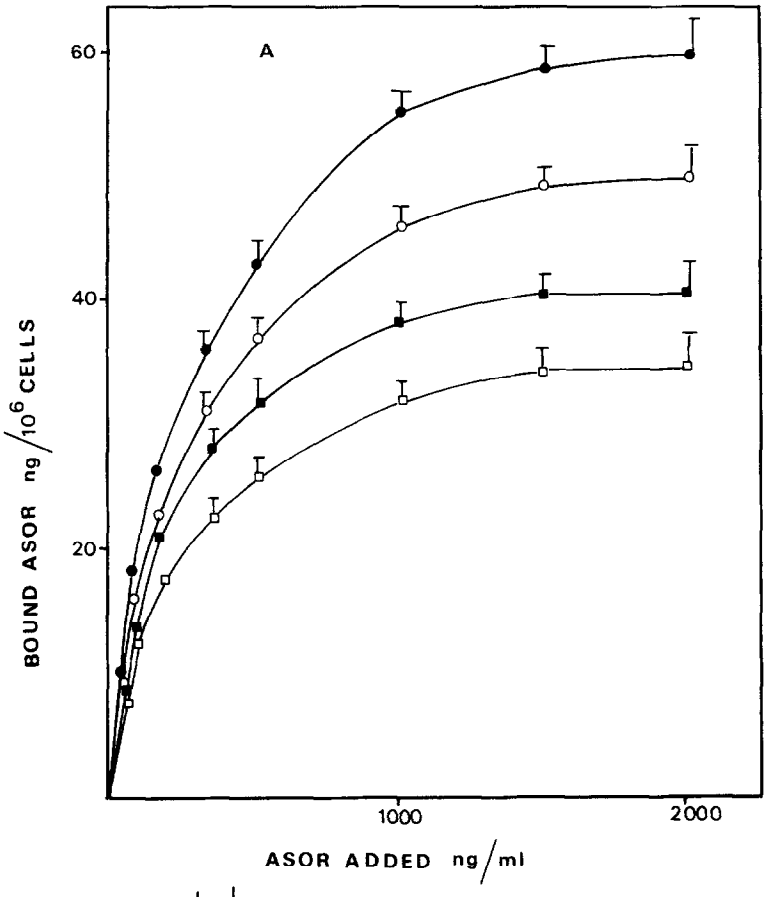

Fig 1 : Saturation of $|^3H|$ asialoorosomucoid binding by solubilized freshly isolated hepatocytes from normal and diabetic rats.
Solubilized hepatocytes (0.5 x 10^6 cells/ml) were incubated in triplicate with different concentrations of $|^3H|$ asialoorosomucoid (50 - 2000 ng/ml) at 4° C for 2 h. Each point gives the average value obtained from six separate experiments using six rats of each group.

●——● normal rats
○——○ diabetic rats (4 days after streptozotocin injection)
■——■ diabetic rats (11 days after streptozotocin injection)
□——□ diabetic rats (20 days after streptozotocin injection)

60 ± 4 ng of $|^3H|$ asialoorosomucoid were bound by 10^6 hepatocytes, which corresponds to 800 000 ± 53 000 receptors/cell. This value is close to that reported by Steer and Ashwell (8). In diabetic hepatocytes (Fig 1 and table II) binding capacity is reduced to an extent varying with the localization of the receptors.

The decrease in the number of total receptors is progressive : 85 ± 5 % of normal in 4-day diabetic rats, 70 ± 5 % in 11-day ones and

TABLE II : CELL SURFACE RECEPTOR AND TOTAL RECEPTOR NUMBER IN NORMAL AND DIABETIC RAT HEPATOCYTES

RATS	CELL SURFACE RECEPTOR / CELL	TOTAL RECEPTOR / CELL
Normal rat	150 000 ± 21 000	800 000 ± 50 000
Diabetic (4 days after the streptozotocin injection	70 000 ± 20 000	680 000 ± 40 000
Diabetic (11 days after the streptozotocin injection	67 000 ± 15 000	540 000 ± 30 000
Diabetic (20 days after the streptozotocin injection	65 000 ± 15 000	480 000 ± 28 000

Each point gives the average value obtained from six separate experiments using six rats of each group.

60 ± 5 % in 20-day ones. By contrast, the number of cell surface receptors was already dramatically reduced in 4-day diabetic hepatocytes and did not change after a prolongation of the diabetic state (table II).

Thus our observations evidenced two different patterns of alteration in this diabetic model. They differ by the degree of deterioration, depending on the relevant process, plasma membrane in one case and intracellular in the other. The marked early decrease in the number of receptors at the cell surface might be explained by a rapid change in membrane fluidity, as suggested by Nassar et al (15). It is only when the diabetic state is more prolonged and/or more severe that the decrease of total receptor number is significantly accentuated. This is probably related to a progressive deficiency of the intracellular protein synthesis. A similar deterioration was observed by Jefferson (16) in diabetic liver.

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