ISOLATION OF RAT LIVER CELLS CONTAINING CONCANAVALIN-A RECEPTOR SITES

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1. Introduction

There is experimental evidence suggesting that not all liver cells are performing similar functions at a given time. Albumin synthesis takes place in 10–30% of the hepatocytes [1,2]; also, the rate of glycogen synthesis varies within different liver lobes [3]. The present communication describes the selective isolation of a population of liver cells containing lectin receptor sites at the plasma membrane. This selected cell population reveals a higher degree of glycogenesis than the cells that fail to bind lectin.

2. Material and methods

2.1. *Isolation of rat liver cells*

Male albino rats weighing 200–250 g were deprived of food but not of water for 24 hr. The liver was removed under nembutal anesthesia (50 mg/kg of weight) and perfused during 20 min with a Miller perfusion apparatus using 50 ml of Hank's solution containing 0.02% chlostridial collagenase type I (Sigma Chemical Co.), 3.0% bovine serum albumin fraction v (Nutritional Biochemical Corporation) and 0.005% desoxyribonuclease I (Sigma Chemical Co.). The cells were dispersed as described by Ingebretsen and Wagle [4] and suspended in fresh Hank's solution. All the experiments were performed under a constant atmosphere of 95% O_2, 5% CO_2.

2.2. *Binding of Lectin*

A commercial nylon net with a 30 cm^2 mesh was thoroughly washed with distilled water prior to its use. Discs of approximately 2.8 cm in diameter containing 0.2–0.25 μmoles of leucine equivalents [5] were incubated at room temperature with 1 ml of 0.1 M phosphate buffer pH 6.6, 1 ml of 25% glutaraldehyde solution and 1 mg of Concanavalin-A grade IV (Sigma Chemical Co.). After 30 min the discs were washed several times with 0.2 N NaCl, until no more free Con-A* was released as determined by the ability of Con-A to agglutinate pathogenic strains of *Entamoeba histolytica.* If no agglutination occurs, less than 5 μg per ml of Con-A are present in the solution [6].

2.3. *Glycogen synthesis*

One ml aliquots (containing 6–10 × 10^6) of isolated liver cells were incubated for 2 hr at 37°C in 5 ml of Hank's solution containing 1% bovine serum albumin, 0.5 mM lactic acid, 0.125 μmoles of a standard mixture of 20 amino acids (Beckman Instruments, Inc.) and 1 μCi of uniformly labelled ^{14}C-glucose specific radioactivity 14.5 μCi/μmole; (Amersham Searle Co.). The reaction was ended after centrifugation of the cells at 2500 rpm for 15 min. Glycogen was extracted with 30% KOH [7]. Sufficient cold carrier glycogen was added in order to precipitate radioactive glycogen with 3 vol of cold ethanol. The precipitated glycogen was redissolved in water and precipitated again with ethanol.

* *Abbreviations used:* Con-A, Concanavalin A; Nylon-Con-A-disc, Concanavalin A covalently bound to nylon discs.

This procedure was repeated twice in order to remove free radioactive glucose. The final precipitate was dissolved in 2 ml of water and 0.5 ml aliquots were mixed with 15 ml of Bray's solution [8] and counted in a Beckman LS-150 liquid scintillation counter. The counting efficieny was 89%.

2.4. *Isolation of liver cells containing Con-A receptor sites*

Aliquots containing 6–10 X 10^6 cells in 2 ml of Hank's solution were incubated at 25°C for various periods ranging from 5–30 min with a nylon disc containing 37 μg of covalently bound Con-A, with gentle shaking (80 cycles/min). Preliminary experiments revealed that after 5 min of incubation there was only minimal increase in the number of bound cells; therefore, in all further experiments the incubation time was 5 min. The discs were removed, washed 3 times with 5 ml of fresh Hank's solution, and then incubated for 2 hr under the conditions described above for glycogen synthesis. Duplicate discs were used for DNA determination [9]. The cells that failed to bind to nylon were processed in a similar manner.

3. Results and discussion

3.1. *Isolation of rat liver cells*

The yield of liver cells obtained was similar to that reported by Ingebretsen and Wagle [4]. Over 90% of the cells were viable after isolation as judged by the exclusion of trypan blue, and remained viable for several hours.

The isolated liver cells incorporated radioactive amino acids into albumin and transferrin and also incorporated radioactive uridine into RNA. Examination of cells under the electron microscope showed preservation of their ultrastructure. The plasma membrane appeared intact in most cells examined.

3.2. *Binding of lectin*

Binding of Con-A to nylon discs was optimal at pH 6.6. The amount of lectin bound [10] varied from one experiment to another. For the experiments reported in this communication, nylon discs contained 37–70 μg of Con-A per disc, using bovine serum albumin as standard. A single set of nylon-Con-A discs was used for each experiment.

Table 1
Effect of Concanavalin-A upon glucose incorporation into glycogen by isolated liver cells*

	dpm/μg of DNA**
Control	16.8
50 μg of Con-A	56.7
100 μg of Con-A	50.5
300 μg of Con-A	13.2
400 μg of Con-A	13.6

* The results are average of duplicate samples.
** 1 X 10^6 cells contain 7.8 μg of DNA.

3.3. *Glycogen synthesis*

All the preparations of isolated liver cells incorporated radioactive glucose into glycogen. The incorporation varied from 15.3 dpm/μg of DNA to 28.7 dpm/μg of DNA. The average obtained in five different experiments was 23.0 ± 6.5 dpm/μg of DNA. Addition of Con-A to isolated liver cells stimulated the incorporation of radioactive glucose into glycogen (see table 1). The stimulation of glucose incorporation varied from 3.2–8.3-fold. The stimulatory effect on glycogen synthesis was obtained both with 50 or 100 μg of Con-A. Larger amounts of Con-A were inhibitory (see table 1). This insulin-like response induced by Con-A has been previously reported [11–13], and indicated that isolated liver cells possess Con-A receptor sites.

3.4. *Binding of isolated liver cells to nylon-Con-A discs*

The number of liver cells that bind to nylon-Con-A discs varied from one experiment to another, from 6.5–9.0%. In order to determine the efficiency of cell binding, a second nylon-Con-A disc was incubated with the same cell suspension, 5 min after removing the first nylon-Con-A disc. It was seen that no further cellular binding occured. Nylon discs containing only glutaraldehyde or covalently bound bovine serum albumin did not bind liver cells. Preincubation of nylon-Con-A discs with α-methyl-mannopyranoside prevented cell binding. These results suggest that cell binding is specific and that only those cells

Table 2
Effect of phospholipase-C digestion upon glucose incorporation into glycogen by isolated liver cells bound to nylon discs containing Concanavalin-A*

	Control	Nylon-Con-A-discs -Phospholipase		Nylon-Con-A-discs +Phospholipase**	
	dpm/μg of DNA†	dpm/μg of DNA†	Fold increase	dpm/μg of DNA†	Fold increase
Exp. 1	25.1	285.6	9.7	338.7	11.5
Exp. 2	15.3	1273.2	50.6	1548.7	61.6
Exp. 3	16.8	2582.0	191.2	††	††

* The results are average of duplicate samples

** $6-10 \times 10^6$ cells were incubated 5 min at room temperature in 2 ml of Hank's solution containing 40 μg of phospholipase-C type I (Sigma Chemical Co.). Nylon-Con-A discs were immersed in the cell suspension and incubation was continued for 5 more min. The rest of the experiment was performed as described for glycogen synthesis.

† 1×10^6 cells contain 7.8 μg of DNA.

†† Not determined.

containing Con-A receptor sites bind to the first nylon-Con-A disc.

Incorporation of radioactive glucose into glycogen was greatly enhanced in the cells bound to nylon-Con-A discs. As can be seen in table 2, incorporation of radioactive glucose increased from as low as 9.7-fold up to 191.2-fold. The incorporation of radioactive glucose into free cells, however, remained the same as in the controls, even in the presence of 50 μg of Con-A. This suggests that the Con-A-induced stimulation of glycogenesis in isolated liver cells can be accounted for by the increased activity of a limited population (∼10%) of cells possessing Con-A receptor sites.

It has been shown by Cuatrecasas [11] and Cuatrecasas et al. [14] that digestion of purified plasma membranes with phospholipase expose new receptor sites for insulin. This result however, does not establish whether the new receptors are in the same plasma membranes with the initially exposed receptors or in other plasma membranes. In order to show if phospholipase digestion has the same effect on the isolated liver cells, aliquots of liver cells were digested with phospholipase-C prior to incubation with nylon-Con-A discs. The percentage of bound cells was identical in the treated as in the control cells. The incorporation of radioactive glucose into glycogen, however, was 20% greater in the phospholipase-C digested cells as compared to the controls (see table 2). This result suggests that if new receptors are exposed, they must be present in the same cells as the original receptors; this is supported by the constancy in the number of cells bound to Con-A discs whether digested or not with phospholipase-C.

This study establishes that there is a discrete population of liver cells possessing specific Con-A receptor sites responsive to lectin binding with an increase in glycogenesis. Whether the small amount of cells binding to nylon-Con-A discs represent the actual number of cells containing lectin receptors in the intact liver is under investigation.

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