Study of hepatic binding protein activity in jejuno-ileal by-passed rat hepatocytes

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The kinetic constants of internalization of asialoorosomucoid were determined for normal and jejuno-ileal by-passed rat hepatocytes. In by-passed rats the maximum velocity of asialoorosomucoid internalization is decreased 3-fold, without any modification of apparant constant of internalization. Moreover, the rate constant of internalization was the same in the two groups of rats. These data suggest that the process of asialoorosomucoid internalization is not altered in by-passed hepatocytes and that the decrease of maximal velocity is only due to a decrease of total uptake receptor number.

Liver damage *Asialoorosomucoid* *Jejuno-ileal by-pass* *Isolated hepatocyte* *Hepatic binding protein*

1. INTRODUCTION

The endocytosis of asialoglycoproteins by normal hepatic parenchymal cells has been widely investigated by biochemical approaches as well as with histological methods (reviews [1,2]). The first stages of this receptor-mediated endocytosis (ligand binding, ligand internalization, ligand degradation in lysosomes) are now well described. In contrast, little information is available on the uptake of asialoglycoproteins by hepatocytes under pathological states [3–5]. Recently, we have provided evidence that in chronic liver damage the binding of asialoorosomucoid (ASOR) to isolated rat hepatocytes was dramatically altered, with a concomitant decrease in its total uptake [6]. This study was undertaken to measure the kinetic constants of internalization and the rate of internalization of surface-bound ASOR. Hepatocytes were isolated from rats with jejuno-ileal bypass, that leads to a liver injury similar to human cirrhosis, as shown by histological observations.

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2. MATERIALS AND METHODS

2.1. *Rat treatment and cell preparation*

Ten male Wistar rats (275–300 g) had an intestinal bypass type II from Cosne [7], i.e., the same length of ileum and jejunum was kept (approx. 5 cm). The anesthetic conditions were as follows: first preanesthesia with ether, then anesthesia with thiopenthal in the peritoneal cavity (60 mg/kg). Six control rats underwent sham operation.

Eleven weeks after operation, hepatocytes were isolated by perfusion with collagenase (Worthington) as described by Berry and Friend [8]. Final cell pellets were suspended in Krebs buffer, pH 7.4, and maintained with continuous gassing with O_2/CO_2 (95:5). In both control and bypassed rats, 80–90% viability was obtained as judged either by 0.05% trypan blue exclusion or the release of lactate deshydrogenase.

2.2. *Experiments*

Orosomucoid (a gift from Dr Wickerhauser, Bethesda, USA) was desialylated and radiolabeled as in [3].

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Experiments in duplicate were carried out in flat-bottomed tubes by incubating 1×10^6 cells at 37°C with different concentrations of [^{3}H]asialoorosomucoid at different times. The cells were kept in a gyratory shaking bath (60 rpm).

2.2.1. Determination of total uptake of asialoorosomucoid

Total uptake was measured only at 20 min with asialoorosomucoid (40–500 ng/ml). The reaction was stopped by adding 3 ml ice-cold Krebs buffer. The cells were washed 4 times and counted.

2.2.2. Determination of internalized asialoorosomucoid

Internalization was measured with [^{3}H]asialoorosomucoid (40–500 ng/ml) at different times, the reaction being stopped by adding 3 ml ice-cold Krebs buffer containing 20 mM EDTA (sodium salt). The cell pellet was resuspended in the same buffer and incubated for 15 min at 4°C. Then the cells were washed 3 times in the same buffer. This incubation released specific surface-bound [^{3}H]-asialoorosomucoid into the medium without any change in cell viability. The cell pellet was counted.

2.2.3. Determination of non-specific asialoorosomucoid

The determination of non-specific asialoorosomucoid taken up or internalized was evaluated by appropriate incubation as described above, but in the presence of 20 mM EDTA (sodium salt).

The cells were washed in a Krebs buffer containing 20 mM sodium salt and then counted.

The non-specific ^{3}H fixed by cells was subtracted from the corresponding values obtained either for total uptake or internalization.

3. RESULTS AND DISCUSSION

The kinetics of internalization of ASOR was studied at various times for different initial concentrations of ASOR. The initial velocities were calculated and then plotted as a function of the ASOR molar concentration (fig.1) and according to Eadie-Hofstee (fig.2). No significant modification of the apparent internalization constant was observed between control ($3.5 \pm 0.5 \times 10^{-9}$ M) and bypassed ($2.2 \pm 0.6 \times 10^{-9}$ M) rat hepatocytes. In contrast, the maximal velocity of

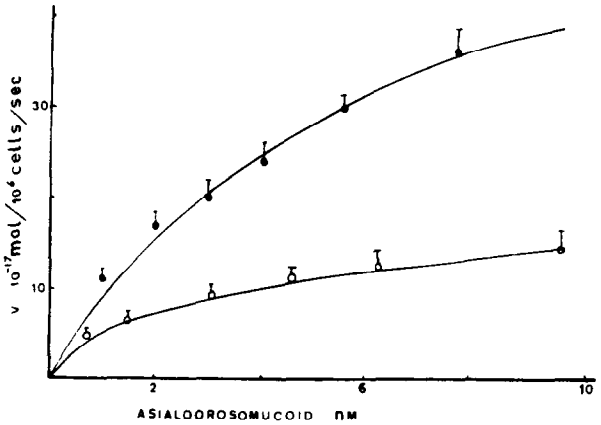

Fig.1. Initial velocity of asialoorosomucoid internalization as a function of asialoorosomucoid molar concentration. (●—●) Normal rat, (○—○) jejuno-ileal bypassed rat.

internalization was decreased about 3-fold in hepatocytes of bypassed rats ($16 \pm 4 \times 10^{-17}$ M/10^6 cells per s) as compared to control rats ($53 \pm 15 \times 10^{-17}$ M/10^6 cells per s).

The amount of surface-bound ASOR was then estimated at different concentrations of ASOR by incubating the cells at 37°C for 5 min, the time needed to obtain a steady state in free and occupied receptors. The specific surface-bound ASOR was measured as described in section 2. The control and bypassed rat hepatocytes bound a maximum of $0.24 \pm 0.025 \times 10^{-12}$ M and $0.07 \pm 0.01 \times 10^{-12}$ M ASOR/10^6 cells, respectively. From the values measured for the maximal velocity and the maximal amount of surface-bound ASOR, the rate constant for internalization of ASOR could be evaluated. We found the same value for both control and bypassed rats ($220 \pm 15 \times 10^{-5}$ and $216 \pm 25 \times 10^{-5}$ s^{-1}, respectively), close to that reported by Weigel [9], Tolleshaug [10] and Bridges et al. [11] for normal rat hepatocytes. Under our experimental conditions, the time needed to internalize an amount of ASOR quasi-equivalent to the total receptor number was about 8 min for both control and bypassed rats.

These data show that the decreased total uptake of ASOR previously observed with bypassed rat hepatocytes [6] was not due to an alteration of the rate constant of internalization but could be attributed to a decrease of cell surface receptors.

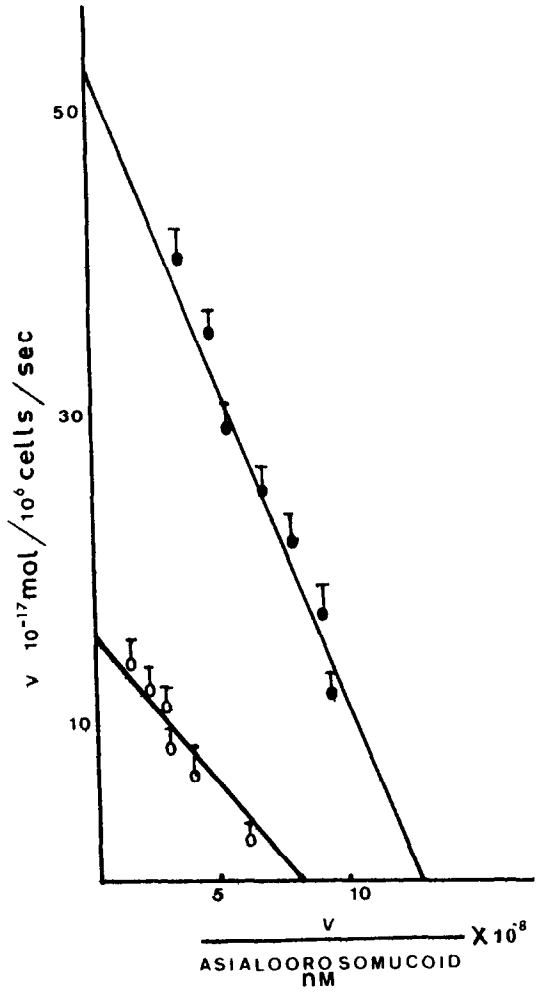

Fig.2. Initial velocity of asialoorosomucoid internalization as a function of the initial velocity/asialoorosomucoid molar concentration ratio according to Eadie-Hofstee. (●—●) Normal rat, (○—○) jejuno-ileal bypassed rat.

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