LIGAND-INDUCED CHANGES AT THE HEPATOCYTE SINUSOIDAL PLASMA MEMBRANE

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1. Introduction

The plasma membrane of the hepatocyte is a highly polarised structure both in terms of function and composition [1]. The blood-facing sinusoid region is itself highly complex, actively participating simultaneously in both exocytotic and endocytotic processes. We are exploring the functional heterogeneity of the hepatocyte sinusoidal plasma membranes, encouraged by the finding using subcellular fractionation techniques that part of the plasma membrane alkaline phosphodiesterase is located to membrane of different density to that containing 5′-nucleotidase [2].

Utilising perfusion of rat liver with radiolabelled ligands together with single-step sucrose density gradient centrifugation of whole liver homogenates [2], we can determine rapidly and quantitatively, the subcellular localization of membrane–ligand complexes. Here we show that glucagon and antibodies to alkaline phosphodiesterase (a plasma membrane ectoenzyme [3]) bind to membrane of differing density, and are then internalised at different rates to another membrane location. The properties of this latter membrane indicate that it does not derive from the Golgi-apparatus, endoplasmic reticulum or lysosomes. Furthermore, within the timescale of the experiment, no evidence for degradation of glucagon was obtained.

2. Materials and methods

2.1. *Subcellular fractionation and enzyme assays*

Subcellular fractionation of whole rat liver homogenates and enzyme assays were carried out as in [2]. In some experiments, digitonin (Sigma, St Louis, MO) was included in the homogenization medium to selectively increase the equilibrium density of cholesterol-containing membrane [4].

2.2. *^{125}I-Labelled glucagon*

Glucagon (Sigma) was iodinated with chloramine T and purified by chromatography on QAE–Sephadex by the method in [5].

2.3. *^{125}I-Labelled anti-(alkaline phosphodiesterase)*

Alkaline phosphodiesterase (EC 3.1.4.1.) was purified from mouse liver as in [6]. Rabbit anti-(mouse alkaline phosphodiesterase) was prepared as in [7] and a γ-globulin fraction iodinated using the iodogen technique [8].

2.4. *Perfusions*

Livers of nembutal anaesthetised male rats (Sprague-Dawley) were perfused in situ using the method in [9]. A flow rate of 15 ml/min was maintained throughout the perfusion and the perfusate kept at 37°C unless otherwise stated.

3. Results

Perfusion of rat liver with a pulse of ^{125}I-labelled anti-(alkaline phosphodiesterase) followed by a wash with Krebs Ringer alone for different times showed that the ligand bound initially to membrane of density 1.13 g/cm^3 (fig.1A), coincident with the low density peak of alkaline phosphodiesterase and galactosyltransferase activities. After a chase period of 15 min (fig.1B), the distribution of label mirrored that of alkaline phosphodiesterase. When the perfusion was done at 4°C, conditions that allow binding of ligand but not internalization [10], fractionation showed the label to be bound to membrane of density 1.15 g/cm^3, intermediate in position with respect to

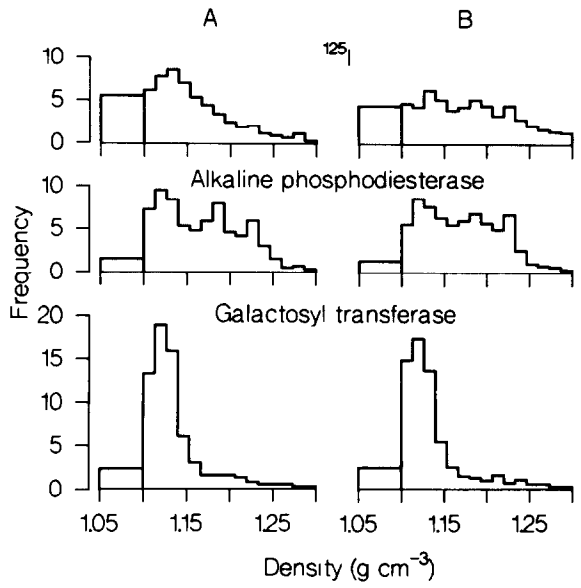

Fig.1. Fractionation of rat liver following perfusion with ^{125}I-labelled anti(alkaline phosphodiesterase). Rat liver was perfused in situ as in section 2, with 75 ml Krebs-Ringer bicarbonate containing ^{125}I-labelled anti-(alkaline phosphodiesterase) (7.5×10^2 cps/ml) for 2.5 min. The liver was then perfused with Krebs Ringer alone for 5 min (A) or 15 (B). At the end of the perfusion the liver was rapidly removed, 2 g peri-lobular tissue homogenized and subjected to analytical sucrose density gradient centrifugation. The resulting fractions were assayed for ^{125}I content and for various organelle marker enzymes. The results are expressed as frequency-density histograms [15].

the lower density peaks of alkaline phosphodiesterase.

Perfusion with ^{125}I-labelled glucagon using the same procedures as above, showed that glucagon bound initially (fig.2A) to membrane of density 1.18 g/cm^3, coincident with the peak of 5'-nucleotidase and the high density components of alkaline phosphodiesterase. With a chase period of 15 min (fig.2B) ~50% of the label was associated with the low density membrane.

The effect of homogenisation with digitonin on the distribution of label and marker enzymes, after perfusion with ^{125}I-labelled glucagon and a 15 min chase, is shown in fig.3. Both ^{125}I and alkaline phosphodiesterase were shifted to high density regions of the gradient, whereas the distribution of galactosyl transferase was only slightly affected.

The identity of the radiolabel in fractions containing the maximum radioactivity after perfusion with ^{125}I-labelled glucagon and washing with ligand free medium for both 5 and 15 min was investigated.

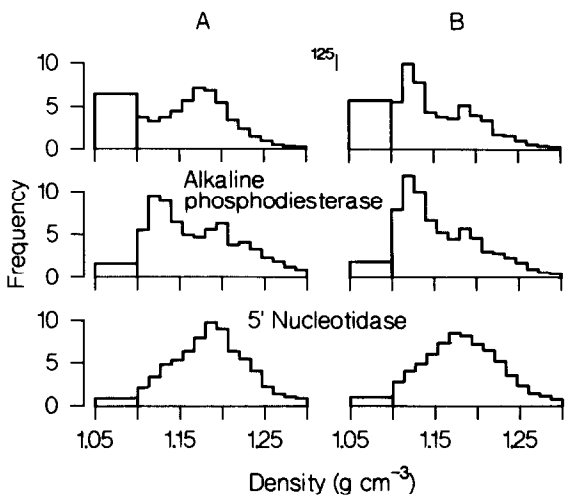

Fig.2. Fractionation of rat liver following perfusion with ^{125}I-labelled glucagon. Rat liver was perfused as before for 5 min with 75 ml Krebs-Ringer bicarbonate containing ^{125}I-labelled glucagon (1.1×10^3 cps/ml). The liver was then perfused with Krebs Ringer alone for 5 min (A) or 15 min (B). The liver was then fractionated and the fractions analysed as in fig.1.

The fractions were centrifuged at $10^5 \times g$ for 90 min. After this time 85–96% of the total radioactivity in the fraction was associated with the pellet. Resuspension of the pellets in 0.1 M HCl at 4°C for 30 min followed by centrifugation as before released 91–97% of the label into the supernate. The supernatants were neutralised with NaOH, lyophilised, and dissolved in 0.5 ml 20 mM Tris buffer (pH 8.0) containing 100 mM NaCl and 0.1% bovine serum albumin. The solutions were chromatographed, using the same buffer, on a G-25 column (55 × 1 cm) pre-calibrated with blue dextran, glucagon and NaI. In each case the elution profile of the radiolabel was identical to that of glucagon.

4. Discussion

The regional polarity of the hepatocyte is obvious from a functional viewpoint and recent studies have shown this to be reflected in the distribution of some membrane components at the apical (biliary) and baso-lateral (sinusoidal) faces [11]. In view of the extensive range of physiological processes occurring at the sinusoidal domain, we now search for biochemical correlates of the functional differentiation.

Perfusion of rat liver with iodinated antibodies to alkaline phosphodiesterase at 37°C appears to show

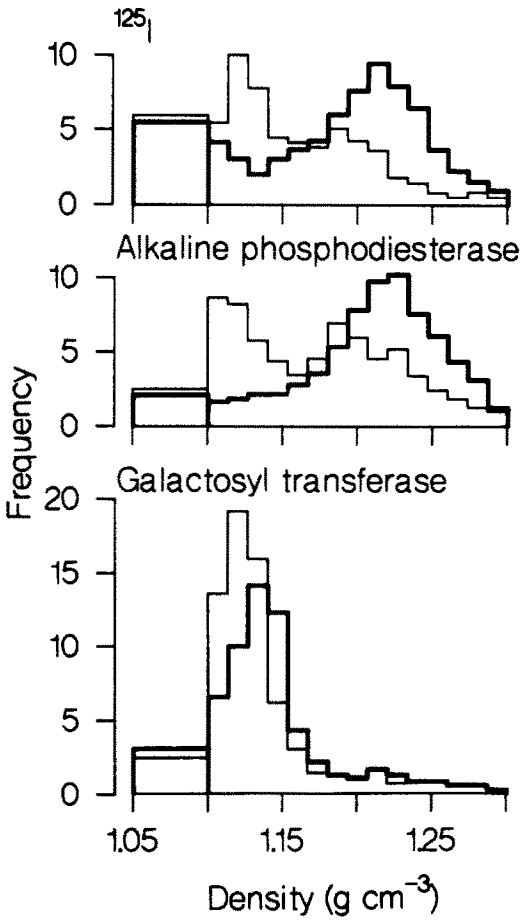

Fig.3. Fractionation in the presence of digitonin of rat liver perfused with ^{125}I-labelled glucagon. Rat liver was perfused as in fig.2B (5 min pulse; 15 min wash), but homogenized in medium containing 1 mg digitonin/ml. The homogenate was fractionated and the fractions analysed as before. The thick line indicates the distribution in the presence of digitonin; the thin line, the distribution in a control gradient.

that the antibody is associated with very low density membrane (1.12 g/cm^3) and is then translocated to higher density membrane. However perfusion at 4°C indicated that binding is initially to membrane of density 1.15 g/cm^3. Thus at 37°C, the antibody must undergo rapid (<4 min) migration to the very low density membrane. In the case of glucagon, binding is to membrane of density 1.18, followed by a much slower migration to the very low density membrane.

We interpret these results as representing the same process in both cases, but differing in their respective time scales, and that the shift to low density membrane represents interiorization of these ligands. The slower rate of translocation shown by glucagon may reflect events necessary for the activation of adenylate cyclase.

The very low density membrane components, by virtue of their position on the sucrose density gradients are clearly non-lysosomal, and furthermore in view of their perturbation by digitonin are clearly different to Golgi-type membranes. Since autoradiographic and preparative experiments have indicated that insulin is internalized to either lysosomal [13] or Golgi-type components [14], these results suggest that processing of receptor-bound glucagon is different to that for insulin. The nature of these ligand-sensitive membrane fragments remain to be determined.

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