Modulation of asialoglycoprotein receptor expression in liver by the endocytic compartment

CARLOS ENRICH* and W. HOWARD EVANS†
*Departamento de Biología Celular y Anatomía Patológica, Facultad de Medicina, Universidad de Barcelona, Av. Diagonal s/n, 08028-Barcelona, Spain and †Laboratory of Protein Structure, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

Receptors on animal cell surfaces regulate the uptake of a large variety of ligands, including peptide hormones, growth factors, adrenergic agonists and antagonists, metabolic carriers, e.g. transferrin, drugs as well as toxins [1]. Many viruses also enter cells by exploiting receptor-mediated endocytic pathways. It is assumed that endocytic processes, involving membrane recycling, degradation, and additionally in epithelial cells, transcytosis, control the expression of cell surface receptors [2]. The endocytic compartment is crucially involved in the regulation of intracellular membrane traffic. However, the possibility can be entertained that this complex organelle is implicated not only in 'inward sorting' of ligands and receptors, but also in the 'outward sorting' of receptors and other membrane proteins in concert with other organelles involved in regulating membrane traffic, e.g. the trans-Golgi apparatus.

In liver, receptors, to which circulating asialoglycoproteins and lipoproteins bind, illustrate its central role in controlling blood metabolites. The asialoglycoprotein receptor controls the uptake and degradation of modified plasma glycoproteins. Since its discovery in the early 1970s, the asialoglycoprotein receptor or hepatic lectin has been extensively studied and it has become a paradigm for the study of receptor-mediated endocytosis by the liver in vivo in isolated and perfused livers, in primarily cultured hepatocytes and more recently in the human hepatoma cell line Hep G2 [3-6].

The asialoglycoprotein receptor in hepatocytes has been found in two different major locations: at the cell surface (in the sinusoidal and lateral plasma membranes) and intracellularly, mainly in endocytic structures, and the Golgi apparatus [7]. The intracellular pool of receptors is substantially larger than the one found at the cell surface and this raises questions regarding the functional significance of intracellular reservoirs of receptors.

Antibodies to hepatic endosomes

Antibodies were prepared to the integral membrane proteins of hepatic endocytic membranes and these were shown by two-dimensional polyacrylamide-gel electrophoresis to recognize approximately ten antigens, with the asialoglycoprotein receptor being the immunodominant antigen. The antibodies recognized two isoforms of the asialoglycoprotein receptor that were shown to differ in their biochemical properties and in their topographical location in the hepatocyte. The two receptor isoform populations were shown to be located primarily in lateral plasma membranes and in endocytic structures and these differed in their sialic acid content, with the receptor in the latter membranes being depleted in sialic acid (C. Enrich & W. H. Evans, unpublished work [9]).

Until recently, a clear definition of the hepatic endocytic compartment in terms of its location has not been established, this in part being due to the lack of markers for this intracellular complex organelle [10]. Using these antibodies, we were able to immuno localize the endocytic structures in rat liver at the light and electron microscopy levels. Immunocytochemistry demonstrated that the endocytic isoforms of the receptor were located primarily in the vicinity of the cytoplasm abutting the bile canaliculus and the lateral plasma membranes of hepatocytes [9].

The endocytic compartment and hepatocyte surface polarity

Fig. 1 shows the various intracellular routes involving the endocytic structures in the polarized hepatocyte. The endocytic compartment is represented as the main intracellular store of asialoglycoprotein receptors. This endocytic intracellular pool of receptors differed from the cell surface pool by its content of sialic acid. The creation of two populations of receptors differing in their topographical location and sialic content may involve at least two mechanisms. In the first, the higher molecular mass isoform (48 kDa) is dispatched to the plasma membrane, but those receptors that are involved in recycling into the endocytic compartment lose sialic acid owing to the presence in endosomes of a neuraminidase activity which accounts for desialylation to the 43 kDa isoform. Indeed, conversion of the 48 to 43 kDa isoforms can be effected using isolated membranes by exposure to neuraminidases. A second possibility is that the

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Golgi apparatus dispatches the 48 kDa isoform primarily to the plasma membrane and the 43 kDa isoform to the endocytic compartment.

What modulates or controls this sorting of receptors in the endosomes and their subsequent expression at the cell surface? Ligand-induced modulation of this hepatic receptor, i.e. the classic down-regulation, in which the asialoglycoprotein receptor undergoes an accelerated rate of degradation as a result of the increased and persistent exposure to ligand, is one possibility [11]. However, other explanations can be advanced. A further possibility that explains the location of the asialoglycoprotein receptor at the lateral domain is that it fulfills a role in the adhesion of one hepatocyte to another [12-15]. The fact that during rapid growth, as exemplified in liver regeneration after partial hepatectomy, there is a decrease in the isoform found in the sinusoidal plasma membrane [16], suggests that modulation of the asialoglycoprotein receptor may be required for cellular recognition as a prerequisite for proliferative responses. Thus, the endocytic compartment appears to function as a receptor reservoir that, depending on the physiological, experimental or pathological situation, may sort specific receptors towards the sinusoidal plasma membrane or towards the lateral plasma membrane where the receptor may fulfill very different cellular functions.

This research was supported by the Comision Asesora de Investigacion Cientifico y Tecnica (PM88-0044).


GTP and its non-hydrolysable analogues stimulate polyphosphoinositide hydrolysis in plasma membranes of rat hepatocytes

JAVIER IBARRONDO, AIDA MARINO, JULIO FONT, MIGUEL TRUEBA and JOSE M. MACARULLA

Department of Biochemistry and Molecular Biology, Faculty of Sciences, Basque Country University, P.O. Box 644, 48080-Bilbao, Spain

Hormonally, specific phosphoinositidase (phospholipase C; PLC) activation leads to hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to give two intracellular messengers: inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) [1]. Non-hydrolysable analogues of GTP mimic the effect of calcium-mobilizing agents in permeabilized cells and stimulate PIP2 breakdown in plasma membranes from rat liver and other cells [2]. These findings indicate that a guanine nucleotide-regulatory protein(s), often termed Gp, with a GTPase activity, is involved in the functional coupling of several receptors to PLC; however, the molecular mechanisms involved in the PLC activation by guanine nucleotides and calcium have not been yet elucidated. Various reports have shown hormone or hormone-mimic guanine nucleotide effects on phosphoinositide hydrolysis in liver plasma membranes, but the significance of these observations remains questionable, since most deal with phosphatidylinositol (PI) hydrolysis, whereas hormonal effects on inositol phosphates are primarily due to PIP2 breakdown. Recently, two types of PLC have been described: a rat liver plasma membrane-bound PLC that was entirely calcium-dependent, stimulated by GTP and able to break down PIP2 and PIP, but not PI [3]; in addition, in platelets and calf brain, a soluble PLC, which shares similar properties to those reported for the membrane-bound enzyme. Moreover, it has been proposed that more than one G-protein could be involved in regulating the hydrolysis of PI, PIP and PIP2 in Swiss 3T3 cells [5]. Finally, the existence of, at least, two G-proteins (stimulatory and inhibitory), which are involved on PLC modulation in pituitary cells and platelets, has recently been proposed [6].

Here, we have examined the ability of calcium, GTP and non-hydrolysable guanine nucleotide analogues: guanylyl-5'-yl imidodiphosphate (GppNHp) and guanosine-5'-O-(3-thiotriphosphate) (GTPyS), to stimulate the PLC activity from myo-[3H]inositol-labelled plasma membranes of rat hepatocytes. Rat liver cells were incubated with 6 μM (3.5 mCi/μmol) of myo-[3H]inositol during 120 min at 37°C. After washing, a partially purified plasma membrane fraction was obtained as previously described [2]. Routinely, PLC activity was determined in a reaction mixture that contained 10 mM Tris/HCl, pH 8, 0.25 mM EDTA, 10−4 M-ATP, 10 mM LiCl, 5 mM MgCl2, 1 μM CaCl2 (between 10−4 and 10−7 m free calcium) and 300–600 μg of protein from freshly labelled plasma membranes; different proteinase inhibitors were also included in a final volume of 300 μl. The different inositol phosphates were determined by anion-exchange chromatography as described [2]. The free calcium concentration was determined by a calcium-sensitive fluorescent dye.