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The distribution of mannose-6-phosphate receptors changes from newborns to adults in rat liver

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ABSTRACT

The co-existence of two types of mannose-6-phosphate receptors (CD-MPR and CI-MPR) in most cell types is still not well explained. Some evidence suggests that the CI-MPR could be actively involved in the regulation of growth factors in the early stages of mammalian organ development. In this study, it was demonstrated that both receptors are distributed in a non-overlapping fashion in rat liver, and that the distribution of CI-MPR changes over a percoll gradient between newborn and adult animals. By using marker proteins it was observed that in newborns the CI-MPR is located both in intracellular fractions and in fractions that coincide with a plasma membrane marker, whereas in adults it is only detected in intracellular fractions. It was also noted that *N*-acetyl-β-D-glucosaminidase distribution is closer to CI-MPR than to CD-MPR and that acid phosphatase did not match with any receptor. This evidence may also suggest that both receptors have different functions, mainly at early stages in the development of organs.

1. Introduction

Mannose-6-phosphate receptors (MPRs) mediate the selective transport of mannose-6-phosphate bearing glycoproteins from the *trans*-Golgi network to lysosomes [1–3]. Two types of MPRs have been described so far, the cation-dependent (CD-MPR) and the cation-independent MPR (CI-MPR) [4,5]. The two MPRs co-exist in most of mammalian cell types, however, the relevance of such co-existence has not been conclusively explained. In the phenotype of I-cell, excessive amounts of lysosomal enzymes are secreted by the fibroblasts, which induces an intracellular deficiency of acid hydrolases, confirming the importance of MPRs in the normal distribution of lysosomal enzymes [6]. Several attempts have been made to further explain the role of each MPR and found that expression of both receptors is spatially and temporally regulated during embryogenesis in mammalian and nonmammalian species [7,8]. The expression of CI-MPR has also been studied in several mammalian tissues during perinatal development, and it has been found that this receptor is highly expressed in the early stages of development and decreases gradually until adulthood [9–13]. Because the CI-MPR can also interact with multiple non lysosomal ligands, which are involved in embryogenesis and differentiation (e.g., insulin-like growth factor II, IGF-II and retinoic acid), it has been postulated that the CI-MPR can play an additional role in regulating cell proliferation at early stages of development. Recent findings suggest that the CI-MPR could be mostly be occupied in regulating the function of growth factors during early development, while at these ages the CD-MPR could be involved in the selective transport of lysosomal enzymes [14]. Thus, at the time that growth factors decrease, the CI-MPR could participate along with the CD-MPR in the transport and the proper distribution of lysosomal enzymes. If so, the CI-MPR could be relocated from the plasma membrane towards intracellular distribution during development. In this work, by using isopycnic gradients, it was compared the distribution of CI-MPR and CD-MPR in rat liver at two extreme ages, newborns and adults, and was observed changes in the distribution of both MPRs.

Abbreviations: AP, acid phosphatase; CD-MPR, cation-dependent mannose-6-phosphate receptor; CI-MPR, cation-independent mannose-6-phosphate receptor; MPR, mannose-6-phosphate receptor; NAG, *N*-acetyl-β-D-glucosaminidase.

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2. Material and methods

2.1. Reagents

The rabbit antirat CI-MPR was gifted from Dr. Nancy Dahms (Wisconsin University, USA). The polyclonal anti-CD-MPR raised in rabbit was generously given by Dr. Annette Hille-Rehfeld (Stuttgart, Germany). Na+–K+ ATPase and GM130 monoclonal antibodies were purchased from Sigma (St. Louis, MO, USA).The biotin-conjugated antirabbit IgG, horseradish peroxidase-conjugated avidin and 4-methylumbelliferyl substrates for *N*-acetyl-β-D-glucosaminidase (NAG) and acid phosphatase (AP) were also purchased from Sigma. Chemiluminescent reagents were purchased from Pierce Biotechnology Inc. (Rockford, Ill., USA). Percoll was purchased from ICN Biomedicals (Irvine, CA, USA).

2.2. Biological material processing

Newborns and 90-day-old rats (Sprague–Dawley) were sacrificed following the recommendations of the Guide for the Care and Use of Laboratory Animals as adopted by the US National Institutes of Health. In addition, the protocol used has the approval of the Committee for Use and Management of Animals of National University of Cuyo (Argentina). The livers of newborns (∗) or adult rats were removed and the tissues were homogenized in 1:5 (w/v) ice-cold 10 mM Tris acetate buffer (pH 7.2), containing 0.25 sucrose, 0.1% EDTA, 0.02% sodium azide, 5 mM glycerophosphate and 1 mM PMSF, according to Jofré et al. (2009). After low speed centrifugation (800*g*), the postnuclear supernatants (2 mg protein) were adjusted to 1.15 ml with homogenization buffer and were mixed with 4.6 ml of 15% Percoll, according to Stöckli and Rohrer [15]. The resulting mixture (12% Percoll) was loaded on the top of 0.25 ml cushion of 2.5 M sucrose and the tubes were centrifuged at 28,000*g* for 45 min at 4 °C in a L7-80 (Beckman) Ultracentrifuge (Ti 70.1 rotor). Fractions (12) of 0.5 ml were collected from the bottom of the gradient and each was adjusted to 6 ml with PBS. After centrifugation at 80,000g for 30 min at 4 °C, the pellets were resuspended in PBS and prepared for immunoblot. (∗)The organs of newborns were processed in pools of 500 mg tissue.

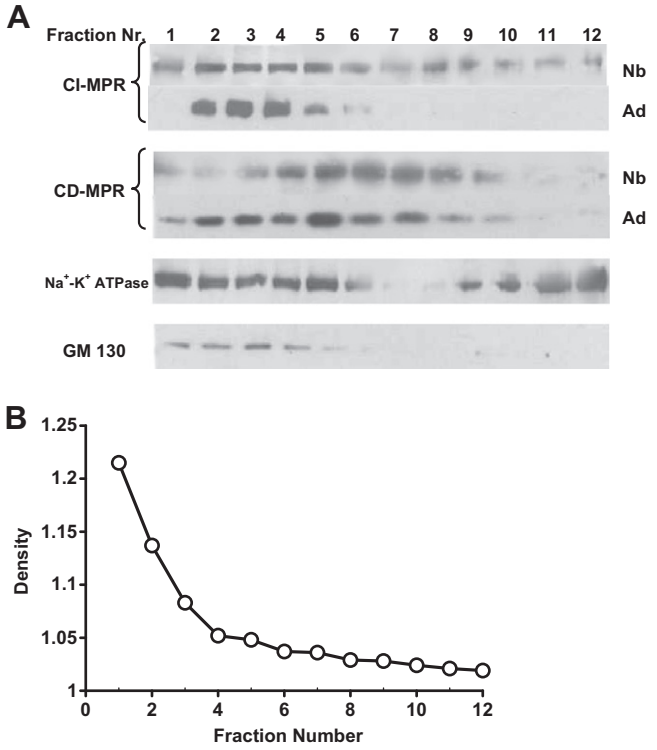

Fig. 1. Distribution of MPRs (CD-MPR and CI-MPR) in liver of newborn and adult rats along an isopicnic gradient. (A) The figure is representative of two independent experiments. To compare, the distribution of Na–K ATPase and GM130 was evaluated as markers of plasmalemma and Golgi cisternae respectively. (B) Densities of the gradient estimated through the ratio weight/volume.

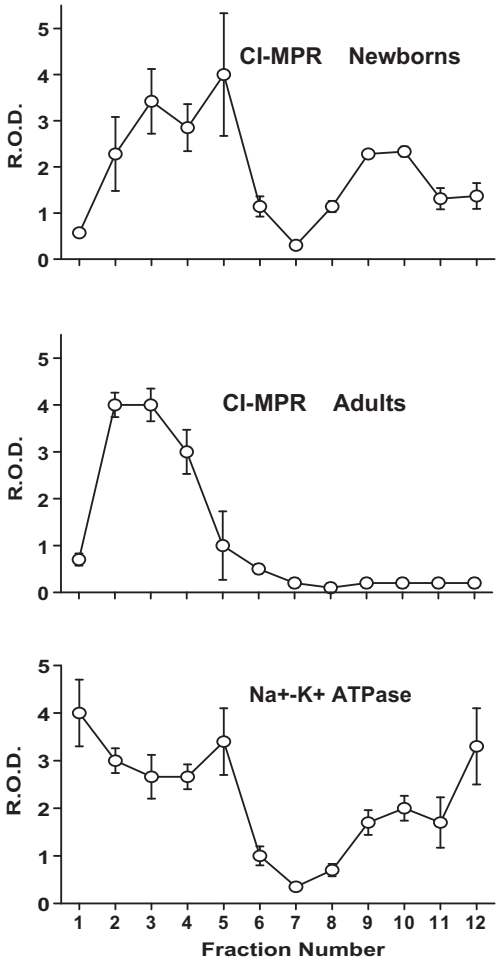

Fig. 2. Quantitation of the bands from immunoblots for CI-MPR in adults and newborns and the Na–K ATPase. Values represent the means of relative optical density (ROD) ± SD from two experiments.

2.3. Electroforesis and Western Blot

All procedures were carried out following the method of Jofré et al. [16]. Briefly, 50 μl of each fraction from the gradient were loaded onto SDS–PAGE for the detection of CD-MPR and CI-MPR (gels of 10% and 7.5% acrylamide, respectively). The proteins were electrotransferred to nitrocellulose membranes (pore size 0.2 μm; Schleicher and Schuell, USA), and the MPRs were detected with the corresponding antibody [12]. Na+–K+ ATPase and GM130 monoclonal antibodies were used at 1:300 and 1:500 dilutions, respectively. The specific proteins were revealed by a chemiluminescent method following the manufacturers' recommendations, and the bands were visualized on Kodax X-Omat films and quantified by densitometric scanning, and subsequent analysis using the Scion Image programme.

2.4. Other procedures

The activity of NAG and AP was measured fluorometrically on each fraction of the gradient using the corresponding