Vol. 210, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 5, 1995 Pages 138-144

DIFFERENTIAL EXPRESSION OF THE ASYALOGLYCOPROTEIN RECEPTOR IN DISCRETE BRAIN AREAS, IN KIDNEY AND THYROID

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Received March 21, 1995

The asyaloglycoprotein receptor is a dimer formed by two polypeptide chains abundantly expressed in the liver (RHL-1 and RHL-2). Using specific primers for the two polypeptide chains we measured, by semiquantitative reverse PCR (RT-PCR), the corresponding mRNAs in different rat tissues. We found that both RHL-1 and RHL-2 mRNAs are expressed in the liver, kidney, brain and thyroid. Under the same conditions we did not detect any specific mRNA in the spleen. In the brain these sequences are expressed along a posterior-anterior gradient. Cerebellum and brainstem display the highest expression of the brain RHL-1 and RHL-2 mRNAs. Tissues and regional distribution of this receptor suggest that other body districts besides liver may participate in the clearance of serum glycoproteins.

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Abbreviations: RHL= Rat hepatic lectin; RT-PCR= Reverse Transcriptase Polymerase Chain Reaction; CRD= Carbohydrate Recognition Domain; GAPDH= Glyceraldehyde-3-phosphate dehydrogenase; mRNA= messenger RNA.

0006-291X/95 $5.00

Asyaloglycoprotein receptors are present on the surface of liver cells. They specifically bind, internalize and dispose desyalated serum glycoproteins (1-4). The specific carbohydrate recognition domain (CRD) recognizes terminal galactose or N-acetyl-glucosamine, which are exposed on the surface of desyalated glycoproteins. The CRD motif is highly conserved in glycoprotein-binding proteins and in the asyaloglycoprotein receptors from different species (5). There are different forms of asyaloglycoprotein receptor: homodimeric or heterodimeric (RHL-1/2) (6, 7, 8). Only the heterodimers are able to internalize and deliver the ligands to lysosomes (9). The simultaneous expression of RHL-1 and RHL-2 is a prerequisite for the recycling of the receptor between lysosomes and plasma membrane (10). The unbalanced expression of the two components apparently results in the formation of receptors anchored to the plasma membrane (11). RHL-1 and RHL-2 have been traditionally considered to be expressed only in the liver. Recent data suggest that they are expressed elsewhere: peritoneal macrophages (12), Sertoli cells, epididymal sperm (13), late-stage spermatids (14) and neonatal enterocytes (15).

We report here that the specific mRNA of RHL-1 and RHL-2 are cohordinately expressed in brain, kidney and thyroid. Furthermore, their expression is restricted to selected brain areas (brainstem and cerebellum), suggesting that districts other than liver partecipate to the clearance of glycoproteins.

MATERIALS AND METHODS

Materials: Guanidinium thiocyanate from FLUKA; TAQ polymerase and PCR Buffer from POLYMED; dNTPs Lithium salt from BOHERINGER MANNHEIM; reverse transcriptase and nick translation kit from GIBCO BRL; random hexamers from PHARMACIA; [α-^{32}P]-dGTP from AMERSHAM; DNase and RNasin from PROMEGA; other chemicals from SIGMA.

RNA preparation: Total RNA was extracted from tissues by acid guanidinium thiocyanate-phenol-chloroform method (16). Total RNA was

digested with DNase at 37°C for 15 minutes to eliminate genomic contamination.

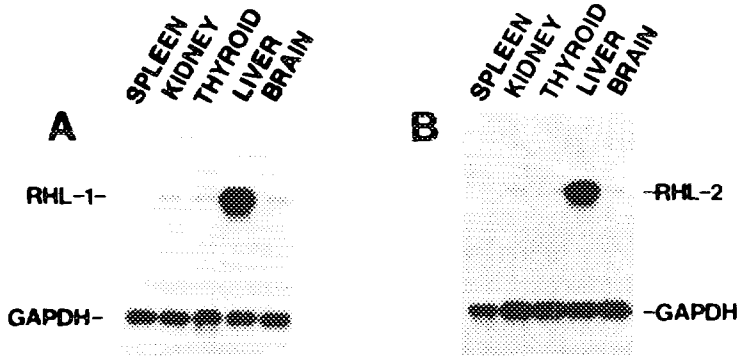
cDNA preparation: Single strand cDNA synthesis was performed according to the manufacturer instructions on 2 μg of total RNA in 20 μl of reaction buffer containing 15 pmol of random hexamers and 200 U of M-MLV RNase H reverse transcriptase (SUPERSCRIPT GIBCO BRL) at 42°C for 1 hour.

PCR amplification: 1/20 of cDNA reaction was amplified in 75 μl of PCR Buffer containing 15 pmol of each oligonucleotide primer:
RHL-1 forward: 5'-CGAAGCTTGAGCTGCCAGATGGCC-3'; 5' nucleotide 367 (17).
RHL-1 reverse: 5'-GGAACGGGTTGAGACAGAGTGT-3'; 5' nucleotide 814 (17).
RHL-2 forward: 5'-TCCTGAGCAACGGCACAGAATGC-3'; 5' nucleotide 659 (9).
RHL-2 reverse: 5'-AAGTGTTCGGGTCGCCAAGTGGA-3'; 5' nucleotide 1056 (9).
GAPDH forward:5'-TTCACCACCATGGAGAAGGCT-3'; 5' nucleotide 369 (18).
GAPDH reverse: 5'-ACAGCCTTGGCAGCACCAGT-3'; 5' nucleotide 715 (18).
1 unit of TAQ DNA polymerase was added after the first denaturation step (5 minutes at 97°C). Samples were then subjected to 20 cycles consisting of 1 min. at 95°C, 1 min. at 60°C, 1 min. at 72°C. The last extension was carried out for 10 min.. The GAPDH primers were added after the first 5 cycles.
The amplified DNA was electrophoresized on 1.5% agarose gel, blotted and hybridized in 0.5 M Na_2HPO_4, 7% SDS, 1 mM EDTA pH 8 buffer solution at 65°C for 16-18 hours, with specific probes: RHL-1, RHL-2 and GAPDH obtained by PCR from rat liver cDNA. The specific amplified fragments were subjected to DNA sequence analysis.

RESULTS AND DISCUSSION

Specific primers corresponding to the carbohydrate recognition motifs of the extracellular domains of rat liver asyaloglycoprotein receptor 1 and 2 (RHL)s were synthesized and used to amplify reverse transcribed RNA from different rat tissues. The regions of the receptor chosen to amplify total RNA are higly conserved in evolution and contain an invariant tryptophan residue at position 157 of RHL-1 and position 174 of RHL-2 (9, 17) coding sequences.

Amplification of rat liver cDNA produced a 450 bp (RHL-1) and 400 bp (RHL-2) fragments, respectively. The 450 bp and 400 bp bands were confirmed to correspond to the RHL-1 and RHL-2 mRNAs respectively by DNA sequence analysis (data not shown). The mixture of amplified DNA was separated on agarose gel and hybridized with specific DNA probes (Fig.1). The specific hybridization signal was linearly dependent on the initial cDNA concentration. The measurements of RHL-1 and RHL-2 mRNA content were performed at least at two cDNA concentrations and were normalized to the GAPDH mRNA and to the liver RHL-1 and RHL-2 mRNA contents (Fig.1). The presence of introns in the amplified regions of RHL-1 and -2, and the absence of specific bands when reverse transcriptase was omitted indicated that we were measuring transcribed RNA.

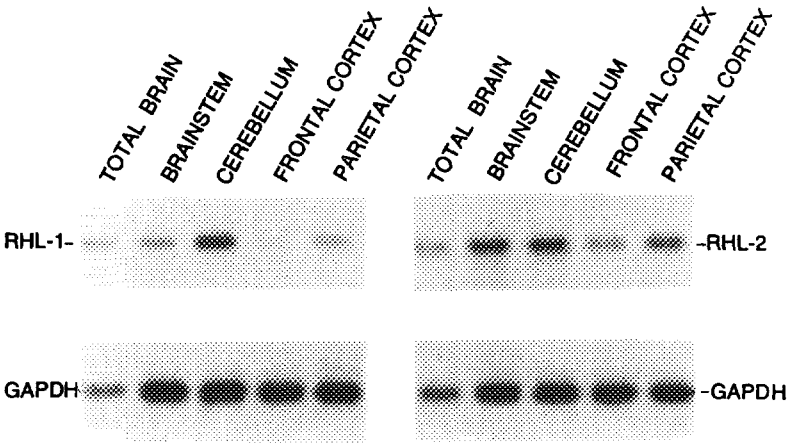


RHL-1	cDNA
Liver	100
Kidney	1.75
Brain	1.20
Spleen	—
Thyroid	1.15

RHL-2	cDNA
Liver	100
Kidney	3.40
Brain	3.90
Spleen	—
Thyroid	3.80

Fig. 1. **A)** Blot analysis of RT-PCR co-amplified products of RHL-1/GAPDH mRNAs from rat tissues. In the table is shown the RHL-1 mRNA level normalized to the liver RHL-1 mRNA content. **B)** Blot analysis of RT-PCR co-amplified products of RHL-2/GAPDH mRNAs from rat tissues. In the table is shown the RHL-2 mRNA level normalized to the liver RHL-2 mRNA content.

Figure 1 shows that, albeit at low levels, RHL-1 and RHL-2 sequences are transcribed in brain, thyroid and kidney, but not in spleen. The absence of specific RHL sequences in spleen indicates that the signal found was not due to "leaky" transcription of the RHL-1 and -2 genes present in every tissues. Moreover, the ratio between RHL-1 and RHL-2 expression appeared to be ca. 1:1 in the brain (Fig.2). This suggests that the receptor expressed in vivo in the brain and in the other tissues is a functional heterodimer RHL-1 and RHL-2. In the thyroid, for example, this receptor may be involved in the internalization and delivery to the lysosomes of thyroglobulin (the main glycosylated protein present in thyroid and precursor of thyroid hormones) from the follicular lumen.



RHL-1	cDNA
Cerebellum	100
Brainstem	40.3
Total brain	20.6
Frontal cortex	3.5
Parietal cortex	14.7

RHL-2	cDNA
Brainstem	100
Cerebellum	63.6
Total brain	25.3
Frontal cortex	31.8
Parietal cortex	51.3

Fig. 2. Blot analysis of RT-PCR co-amplified products of RHL-1 and RHL-2/GAPDH mRNAs from discrete brain areas. The tables show RHL-1 and RHL-2 mRNA levels normalized to the cerebellum RHL-1 and brainstem RHL-2 mRNA content, respectively.

The presence of asyaloglycoprotein receptor mRNAs in the brain prompted us to investigate in detail whether these sequences were expressed in a specific brain district or throughout the central nervous system. To this end, we isolated total RNA from several brain areas, i.e, cortex, cerebellum and brainstem, and measured the levels of RHL-1 and RHL-2 mRNA sequences. Figure 2 shows that cerebellum and brainstem expressed ca. 80% of the total RHL-1 and RHL-2 sequences present in the brain. Cortex RNA, derived from different areas (parietal and frontal) contained ca. 10% and 40% of total brain RHL1 and RHL2 mRNAs, respectively. Considering that brainstem contains mainly myelinated axons, we suppose that the RHL-1 and -2 mRNAs are distributed and localized mainly along the plasma membrane of the axons. We also tested primary cultures of neurons and glial cells derived from the different brain areas illustrated in Figure 2 for the presence of RHL-1 and RHL-2 sequences. We found that these RNAs are present in neurons and glial cells derived from cerebellum and are not detectable from cultures derived from cortex (data not shown).

The peculiar distribution in the brain of RHL-1 and RHL-2 mRNAs suggests a general role for these receptors in the central nervous system as plasma-tissue exchangers of biologically relevant molecules such as neurotrophins and hormones. It is peculiar that the other tissue expressing asyaloglycoprotein receptors is the testis (13, 14). The brain and the testis are both characterized by a tight barrier between the serum and the tissue: the haemato-testicular and the haemato-enkephalic barriers. It is likely that the presence of these receptors on the cell surface contacting the barrier may facilitate the selective removal and uptake of glycoproteins, including hormones and growth factors.

Even though we did not demonstrate directly the presence of the asyaloglycoprotein receptors on the surface of the cells, we believe that the sequences detected represent a true expression of these proteins above the background. This is also the first demonstration of their expression in discrete brain areas. We suggest that the demonstration of the cohordinate expression of RHL-1 and RHL-2 in thyroid , kidney and brain, albeit lower than in the liver, strongly argues for the presence of the functional asyaloglycoprotein

receptors in these tissues and suggests a more general function of these molecules than previously thought.

ACKNOWLEDGMENTS

We thank Dr. Gennaro Schettini for helpful discussion and for providing rat brain total RNA. We also thank Prof. Silvestro Formisano and Dr. Bruno Di Jeso for critical reading of the manuscript.This work was supported by AIRC (Associazione Italiana per la Ricerca sul Cancro) grants.

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