MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF AN ORGANIC ANION TRANSPORTING POLYPEPTIDE (Oatp) FROM THE LITTLE SKATE, *RAJA ERINACEA*

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Organic anion transport is a fundamental property of hepatocytes throughout the vertebrate kingdom. To date, 8 organic anion transporting polypeptides (OATPs) have been cloned from human tissues, and 4 from rat tissues. Most of these genes encode for proteins of ~80kDa and are predicted to have 12 membrane-spanning domains. Three of the human and three of the rat OATPs have been characterized at the functional level, and have been shown to mediate the cellular uptake of a variety of organic solutes, many of which are drugs and xenobiotics. Previous functional studies in the small skate (*Raja erinacea*) and dogfish shark (*Squalus acanthius*) indicate that livers of these primitive marine vertebrates are also capable of transporting organic anions such as sulfobromophthalein (BSP), bilirubin, lucifer yellow and taurocholate from the portal circulation into hepatocytes by saturable, carrier-mediated transport systems on the hepatic sinusoidal plasma membrane. To study the evolutionary development of the OATP family, we have now cloned and functionally characterized an evolutionarily primitive Oatp from the liver of the small skate.

Three consensus regions (6-8 amino acids) were identified from alignments of the protein sequences of members of the OATP/Oatp family and two forward and two reverse degenerate primers were synthesized. Reverse transcription and touch down PCR were able to amplify a band with predicted size from skate liver total RNA. DNA sequencing confirmed that this gene fragment encoded for an Oatp. A skate liver cDNA library was then screened using this DNA fragment as probe. A strong positive hybridization signal was obtained for ~5,000 clones from one million plaques. Thirty positive plaques were analyzed in a second round of screening and most contained a 2.3 kb insert. Functional characterization of skate Oatp transport activity was carried out in *Xenopus laevis* oocytes injected with 5 ng of skate Oatp cRNA.

The full-length cDNA for this skate Oatp is predicted to encode a protein of 689 amino acids. Computer modeling predicts 12 transmembrane domains, 4 *N*-glycosylation sites and 5 protein kinase C phosphorylation sites. Sequence comparisons with other OATPs indicate that skate Oatp is 50.4% identical with OATP-F from human brain, an OATP that has not been functionally characterized, 43.4% and 41.2% identical with human Oatp2 and rat rlst1, which are liver specific OATPs, and 42.6% identical with human OATP1. A phylogenic analysis displays the evolutionary relationship with other members of the OATP family (Fig. 1). Northern analysis of multiple skate tissues (Fig. 2) indicates that skate Oatp is expressed predominantly in liver tissue, although weak signals were obtained in skate brain and testes. A peptide antibody identified an 85 kDa band from skate liver that is enriched in skate liver plasma membrane vesicles.

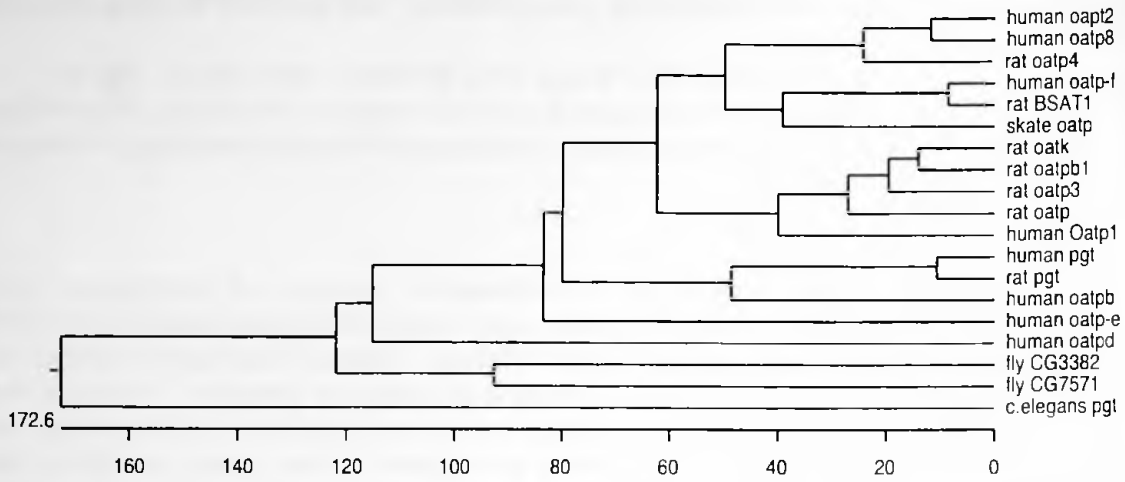

Figure 1. Phylogenic analysis of skate Oatps.

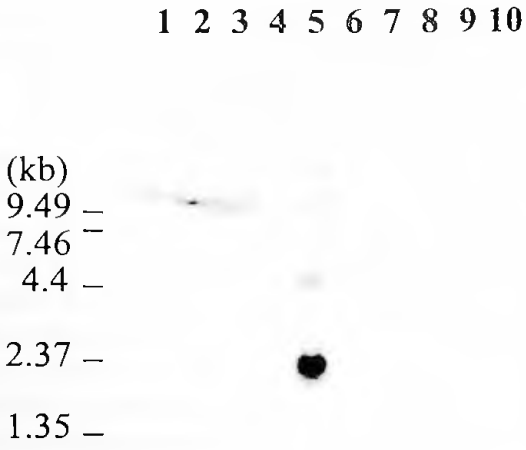

Figure 2. Northern analysis of skate Oatp.
15 ug of total RNA from skate tissue were loaded on each lane. Lane 1, Brain; lane 2, Heart; lane 3, Intestine; lane 4, Kidney; lane 5, Liver; lane 6, Pancreas; lane 7, Reetal gland; lane 8, Spleen; lane 9, Stomach; lane 10, Testes.

When the cRNA for skate Oatp was injected into *Xenopus laevis* oocytes, the oocytes demonstrated enhanced uptake of a broad range of organic solutes, including estrone sulfate, estradiol glucuronide, taurocholate, prostaglandin E_2 (PGE_2), and leukotriene C_4 (LTC_4), but not digoxin or tetraethyl ammonium. Skate Oatp is therefore a multispecific transporter, and most likely mediates cellular uptake of both endogenous and exogenous compounds. This substrate profile overlaps with, but is distinct from that of the characterized human OATP transporters. Uptake of [^{3}H]estrone sulfate in skate Oatp-expressing oocytes was unaffected when sodium was

replaced with equimolar concentrations of lithium or choline, indicating that transport is independent of the sodium gradient, in agreement with the mammalian OATPs. Uptake of estrone sulfate and taurocholate was saturable, with apparent Michaelis constants (K_m) of 61±11 and 85±7 μM, respectively. This K_m value for taurocholate compares favorably with that measured in cultured skate hepatocytes, 43±20 μM (Fricker *et al.*, Am. J. Physiol. 253:G816-G822, 1987).

Skate Oatp-mediated uptake of 50 nM [^{3}H]estrone sulfate was inhibited by the bile acids taurocholate, taurolithocolate, and sulfated taurolithocholate, as well as the major skate bile salt scymnol sulfate (at 200 μM). Transport was also inhibited by sulfobromophthalein (BSP) and bilirubin ditaurate, but not by ouabain. Interestingly, digoxin (500 μM) inhibited estrone sulfate uptake by about 70%, despite the fact that digoxin itself was not transported by skate Oatp-expressing oocytes.

These studies have identified and characterized an evolutionarily primitive form of an hepatic organic anion transporter that belongs to the OATP family of proteins. This transporter has a broad substrate preference, indicating an important role in the hepatic clearance of foreign chemicals. A comparison of the nucleotide and predicted amino acid sequence with the mammalian OATPs provides insight into conserved amino acid regions, and into the structure and function of this family of xenobiotic transporters.

We plan to examine the cellular and subcellular distribution of this protein in skate tissues and to further examine its substrate specificity. We also plan to search for additional members of the skate Oatp family of proteins, and establish their evolutionary relationships with mammalian proteins.

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