Characterization of a bile salt transport system in isolated hepatocytes from adult sea lamprey (*Petromyzon marinus*)

Shi-Ying Cai[1], Daniël A. Lionarons[1], and James L. Boyer[1,2]
[1] Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520
[2] Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672

The Na^+-taurocholate cotransporting polypeptide (NTCP, SLC10A1) is the major hepatic bile salt uptake transporter in humans and rodents, but not in fish such as marine skate and rainbow trout. Last summer, we found a Na+-dependent uptake system with low affinity for taurocholate in isolated hepatocytes from adult sea lamprey. Here we identified the ortholog gene of Slc10a1 in sea lamprey. Functional characterization indicates that lamprey Slc10a1 transports both petromyzonol sulfate (its endogenous bile salt) with high affinity, but taurocholate with low affinity. The multi-tissue expression pattern suggests that lamprey Slc10a1 can transport other substrates in addition to bile salt.

The Na^+-taurocholate cotransporting polypeptide (NTCP, SLC10A1) is a liver specific bile salt transporter in humans and rodents[4]. It is localized on the basolateral membrane of hepatocytes and facilitates the uptake of conjugated bile acids from blood, thereby maintaining concentrations of bile acids in enterohepatic circulation in these species. In addition to bile acids, NTCP/Ntcp from these species can also transport thyroid sulfates and steroid sulfates, such as estrone-3-sulfate and dehydroepiandrosterone 3-sulfate[2,5]. However, little is known about the function of this gene in non-mammalian vertebrates, although genome annotation indicated that Slc10a1 gene has been involved throughout vertebrate evolution. Our previous study indicated that there is a Na^+-dependent bile salt uptake system in the hepatocytes of sea lamprey (*Petromyzon marinus*), but with very low affinity for taurocholic acid (TCA)[1]. It was not known whether an Slc10a1 ortholog in lamprey is responsible for this function. In this report we identified this ortholog in lamprey and characterized its substrate specificity for TCA and petromyzonol sulfate (PZS), the major endogenous bile salt in sea lamprey.

Total RNA was isolated from upstream migratory sea lamprey caught in the Kenebunck River in southern Maine. BLAST search of lamprey genome was performed to identify NTCP/SLC10A1 ortholog candidate gene. Reverse-transcription PCR (RT-PCR) was used to clone a fragment of lamprey Slc10a1 (lpSlc10a1) followed by 5′ and 3′ RACE PCR to obtain the full-length cDNA. A luciferase-based NTCP-farnesoid X receptor α reporter (FXR/NR1H4) assay was utilized to detect lpSlc10a1 substrate specificity for bile salts, because conjugated bile salts require a specific transporter, *e.g.,* NTCP, to cross the cell membrane, and they are ligands for human FXR[3]. Thus, FXR will be transactivated, if lpSlc10a1 is able to transport a given bile salt into the transfected cells, *i.e.,* HEK293T cells.

Molecular cloning identified an ortholog of Slc10a1 in lamprey liver and intestine. Its full-length cDNA contains 2.1 kb and encodes 366 amino acids and shares ~ 45% sequence identity to human NTCP. Phylogenetic analysis indicated lpSlc10a1 is an ortholog of NTCP/SLC10A1 in lamprey. RT-PCR revealed that lpSlc10a1 mRNA was abundantly expressed in many tissues, including liver, kidney, intestine and brain, but not gill in adult lamprey. This is in striking contrast to its mammalian ortholog, which appears to be a liver specific gene. To test whether lpSlc10a1 could transport TCA and PZS, we cloned the full-length coding sequence into pcDNA3 vector. After co-transfecting FXR report constructs into HEK293T cells, the dual-luciferase assay indicated that lpSlc10a1 transported these bile salts. As shown in Figure 2, only high concentrations (25 μM) of TCA markedly increased report gene expression in lpSlc10a1 transfected cells, whereas 1 μM of TCA greatly induced report gene expression in human NTCP transfected cells. Interestingly, lpSlc10a1 demonstrated high sensitivity to PZS, as 1 μM of PZS substantially stimulated reporter gene expression.

In summary, we identified a NTCP/SLC10A1 ortholog in sea lamprey. Reporter assay demonstrated that lpSlc10a1 can effectively transport PZS, but has low affinity for TCA. This finding is consistent with our early observations[1]. Tissue distribution of lpSlc10a1 suggests this transporter may transport other substrates in addition to its endogenous bile salt. Further analysis and comparison of the sequence and substrate specificity of lpSlc10a1 and other members in this family will yield additional insight into the function of Slc10a1 in vertebrate evolution.

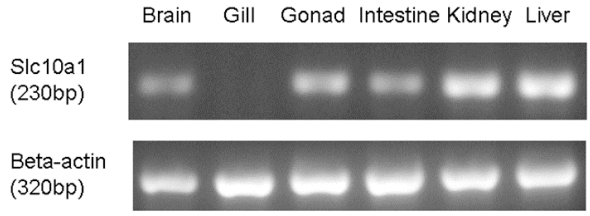

Figure 1. mRNA tissue distribution of lpSlc10a1 in adult lamprey determined by reverse-transcription PCR. Amplified Beta-actin fragment was used as positive controls for each tissue.

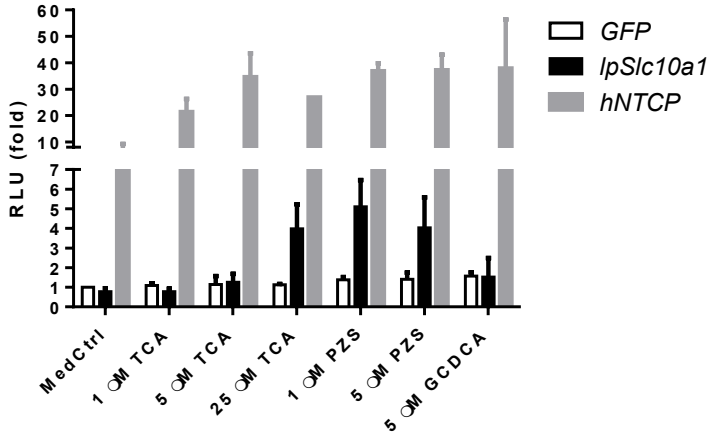

Figure 2. A dual-luciferase reporter assay demonstrated lpSlc10a1 transported TCA and PZS. HEK293T cells were transfected with FXR, RXR, hIBABP-Luc and Renilla Luc, as well as control vector with GFP, or lpSlc10a1, or human NTCP (hNTCP) vectors. Transfected cells were treated with indicated bile salts (μM = micromolar) for 24 hrs. Firefly luciferase activities were normalized to Renilla luciferase, and the fold change is relative to cells transfected with GFP expression vector by setting its medium treated value as 1. All values represent three independent experiments and are presented as means ± SD.

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