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

Shi-Ying Cai[1], Alison Pierik[2] 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^+-dependent bile salt transport system plays an important role in hepatic uptake of bile salts in humans and rodents but not in fish such as marine skate and rainbow trout. Our previous studies indicate that adult sea lamprey liver is able to remove bile salts from plasma despite being cholestatic. Here we demonstrate that isolated adult lamprey hepatocytes specifically transported ^{3}H-taurocholic acid in a time-, temperature-, dose- and Na^+-dependent manner with K_m=115 μM, that is much higher than in skate and rainbow trout. This finding suggests that a functional albeit low affinity ortholog of Na^+-taurocholate cotransporting polypeptide (NTCP, SLC10A1) exists in lamprey liver.

The sodium (Na^+)-dependent bile salt transport system plays an important role in hepatic uptake of bile salts in humans and rodents. The Na^+-taurocholate cotransporting polypeptide (NTCP, SLC10A1) is responsible for this function in these species[3]. In contrast, studies using isolated hepatocytes from marine skate and rainbow trout (two species represent elasmobranchs and teleosts, respectively) did not show Na^+-dependent taurocholic acid (TCA) transport activity in these cells, indicating that an Ntcp ortholog may not have evolved in these species[1,2]. In this report we determined whether there is a Na^+-dependent TCA transport system in the liver of sea lamprey (*Petromyzon marinus,* here after lamprey, a species of agnatha)**.**

Upstream migratory sea lamprey were caught in the Kenebunck River in southern Maine and were maintained at MDIBL in 11˚C circulating fresh water tanks with a 12 hour light-dark cycle. Males and females were kept separated. All animals were anesthetized in 0.1 g/L Tricaine before liver removal for collagenase perfusion. Hepatocyte preparation was carried out as we previously described[2]. Briefly, collagenase (type VIII, 0.05% in lamprey Ringer) perfusion was carried out through the portal vein at 15°C for about 40 minutes. After collagenase digestion, the liver was disrupted using forceps. Hepatocytes were obtained by low-speed centrifugation with viability averaging ~75%. ^{3}H-TCA (1 μCi/ml, 1 μM) was incubated with aliquots of hepatocytes (~1 million cells) at 0˚C or 15˚C for the designated times. The cells were then quickly washed three times by centrifugation, and lyszed using 1N NaOH for 1 hour. After neutralization using equal volume of 1N HCl, aliquots of the lysate were subject to liquid scintillation detection. Protein concentration was used to normalize radioactivity. Non-specific binding activity was subtracted by setting 0˚C 0-minute uptake as background. To assess the Na^+-dependence of the transport system, NaCl in lamprey Ringer was replaced with equal molar of choline chloride or LiCl.

As shown in Figure 1A, the isolated hepatocytes had significantly higher uptake activity for ^{3}H-TCA at 15˚C than at 0˚C for all time points starting at 3 minutes of incubation. This activity increased in a linear range over the first 10 minutes of incubation at 15˚C and reached a plateau after 30 minutes. These observations indicate that there is a specific bile salt transport system in the hepatocytes of adult lamprey that takes up TCA in a temperature- and time-dependent manner. When NaCl in lamprey Ringer was substituted by choline chloride or LiCl, the transport activities were significantly reduced by more than 50% after both 10 min and 60 min incubation at 15˚C (Fig 1B), suggesting the presence of a Na^+-dependent bile salt transporter. To test whether other bile salts could compete with ^{3}H-TCA uptake in these hepatocytes, we added 50 μM of cold TCA or taurochenodeoxycholic acid or petromyzonol sulfate (the major endogenous bile salt in lamprey) in the uptake assay. As shown in Figure 1C, all three bile salts significantly decreased ^{3}H-TCA uptake by more than 50%, consistent with a specific bile salt transport system in these hepatocytes. These findings also suggest that taurochenodeoxycholic acid and petromyzonol sulfate could be substrates of this transport system. Finally, to determine the V_{max} and K_m for this TCA transport system, we assayed 10-min uptake of ^{3}H-TCA at substrate concentrations of 0.2 μM to 400 μM. Data from this experiment indicates that the V_{max} and K_m are 35 ± 7.8 pmol/mg protein/min and 115 ± 8.7 μM, respectively (Fig 1D).

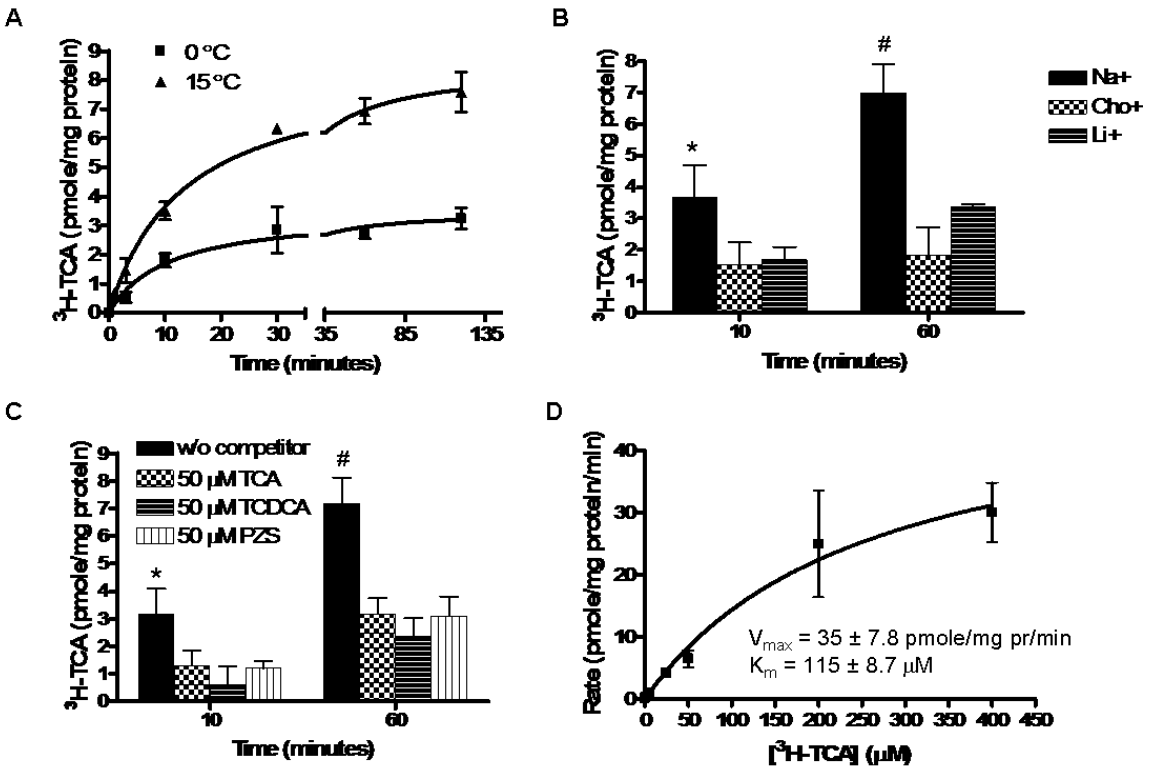

Figure 1. Characteristics of a TCA transport system in the isolated hepatocytes of adult lamprey. A, Temperature- and time-dependence (n = 3-5); B, this transport is also Na^+-dependent; C, competition experiment at 15°C using cold taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), and petromyzonol sulfate (PZS); D, Michaelis-Menten analysis of TCA uptake at 15°C for 10 minutes (n=3). $^*p < 0.05$, $^\#p < 0.01$, n = 3-4.

In summary, using a newly developed isolated lamprey cell culture system, we have demonstrated there is a Na^+-dependent bile salt transport system in the hepatocytes of the adult lamprey, with a very low affinity for TCA (K_m = 115 ± 8.7 μM). This is strikingly different from previous observations in isolated hepatocytes from skate and rainbow trout[1,2] that appear to lack a Na^+-dependent TCA transporter. As NTCP/SLC10A1 is the functional determinant for the hepatic Na-dependent bile salt transport in humans and rodents, we speculate a Slc10a1 ortholog may also carry out this function in liver from the adult lamprey. Indeed, genome annotation indicates there is a Slc10a1 gene in the lamprey. Reverse-transcription PCR also showed this gene is expressed in the liver of adult lamprey, although it is not a liver specific gene as in humans and rodents. Future studies will try to identify a full-length lamprey Slc10a1 and characterize its transport activity for TCA and other bile salts and steroid metabolites. Whether this low affinity uptake system is an adaptive response to cholestasis will need to be assessed by comparing its expression and function in the larval pre-metamorphosis form.

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1. **Råbergh CM, Ziegler K, Isomaa B, Lipsky MM, Eriksson JE.** Uptake of taurocholic acid and cholic acid in isolated hepatocytes from rainbow trout. *Am J Physiol*. 267:G380-G386, 1994.
2. **Smith DJ, Grossbard M, Gordon ER, Boyer JL.** Isolation and characterization of a polarized isolated hepatocyte preparation in the skate Raja erinacea. *J Exp Zool*. 241:291-296, 1987.
3. **Stieger B.** The role of the sodium–taurocholate cotransporting polypeptide (NTCP) and of the bile salt export pump (bSEP) in physiology and pathophysiology of bile formation. *Handb Exp Pharmacol*. 201:205-59, 2011.