Characterization of a bile salt transport system in isolated hepatocytes from shorthorn sculpin, *Myoxocephalus scorpius*

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The Na^+-dependent bile salt transport system plays an important role in hepatic uptake of bile salts in humans and rodents, but it is uncertain whether such a system exists in marine species. Here we demonstrate that isolated shorthorn sculpin hepatocytes specifically transported ^{3}H-taurocholic acid in a time-, dose- and Na^+-dependent manner with a K_m=15 μM, a much higher affinity for bile salts than in sea lamprey, suggesting that a Na^+-taurocholate cotransporting polypeptide (NTCP, SLC10A1) is expressed in sculpin liver.

Sodium (Na^+)-dependent bile salt transport system encoded by the Na^+-taurocholate cotransporting polypeptide (NTCP/Ntcp, SLC10A1/Slc10a1) plays an important role in hepatic uptake of bile salts in humans and rodents[6]. In contrast, studies using isolated hepatocytes from the marine skate and rainbow trout (two species that represent elasmobranchs and teleosts, respectively) failed to show Na^+-dependent taurocholic acid (TCA) transport activity in these cells, indicating an Ntcp ortholog may not have evolved in these species[4, 5]. However, our previous studies in hepatocytes from sea lamprey, a more primitive vertebrate, have demonstrated Na^+-dependent uptake activity for TCA, although with low affinity (K_m = 115μM), indicating that a Ntcp ortholog evolved early in vertebrate evolution[1]. These observations indicate transport specificity of Ntcp ortholog for TCA in evolutionarily primitive species may vary among species. In this report we examined whether a Na^+-dependent TCA transport system could be identified in the liver of shorthorn sculpin (*Myoxocephalus scorpius*), a marine teleost**,** hereafter referred to as sculpin.

Sculpin were procured by MDIBL and maintained in 14˚C circulating seawater tanks with a 12-hour light-dark cycle. A ll animals were anesthetized in 0.1 g/L Tricaine and sacrificed by detaching the head from the spinal cord using a sharp knife before liver removal for collagenase perfusion. Hepatocyte preparation was carried out as we have previously described[5]. Briefly, a collagenase (type VIII, 0.05% in marine teleost Ringer) perfusion was carried out through the portal vein at 15˚C for about 25 minutes. After collagenase digestion, the liver was disrupted using forceps. Hepatocytes were obtained by low-speed centrifugation with viability averaging ~75%. A ^{3}H-TCA (1 μCi/ml, 10 μM) uptake assay was performed as previously reported. Total cellular protein concentration was used to normalize radioactivity. Non-specific binding activity to cell membranes was determined by subtracting radioactivity determined at 0˚C and 0 minute uptake as background. To assess the Na^+-dependence of the transport system, NaCl in marine teleost Ringer was replaced with equal molar amounts of choline chloride or LiCl.

TCA uptake activity occurred in a time- and dose-dependent manner at 15˚C in isolated sculpin hepatocytes with V_{max} and K_m at about 80 pmol/mg protein and 15 μM, respectively (Fig 1). ^{3}H-TCA uptake in these cells was almost completely competitively inhibited by excessive amount of the bile acid taurochenodeoxycholic acid (TCDCA, 200 μM), further suggesting there is a specific bile acid transporter in hepatocytes of this species. When NaCl in the Ringer buffer was substituted with choline chloride, the transport activities were significantly reduced by 50% after both 10 min and 30 min incubations at 15˚C (Fig 2), suggesting the presence of a Na^+-dependent bile salt transporter. Interestingly, when NaCl in the Ringer was replaced with LiCl the transport activities were significantly increased (Fig 2) by more than two-fold, in contrast to transport activity of mammalian NTCP/Ntcp.

Sculpin hepatocytes demonstrated TCA uptake activity in a Na^+- and dose -dependent manner, with a K_m = 15 μM that is comparable with human NTCP (K_m = 6 μM)[3]. This suggests an Ntcp ortholog is expressed in this marine teleost and functions in a similar fashion as its mammalian orthologs. As compared to a similar transport system in sea lamprey, sculpin Ntcp demonstrated a much higher affinity for TCA (15 μM *vs.* 115 μM). The lower K_m for TCA in sculpin hepatocytes coincides with the evolution of C24 bile acids in teleosts, further suggesting that sculpin Ntcp may be the functional determinant for hepatic bile acid uptake in this species.

Interestingly, sculpin hepatocytes demonstrated enhanced uptake of TCA when Na^+ was replaced with Li^+ in the Ringer solution. This finding indicates that there are structural differences between sculpin Ntcp and its mammalian orthologs because NTCP/Ntcp in humans and rodents lost transport activity when Na^+ was replaced with Li^+. Of note, this discrepancy was described occasionally for certain other transport systems in teleosts[2]. Future studies will be needed to sequence the sculpin *Ntcp* gene and characterize its transport activity *in vitro* in transfected cells.

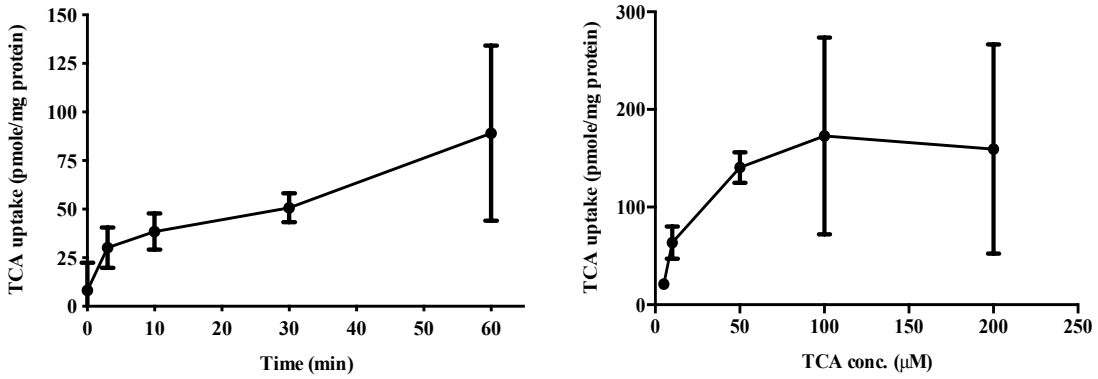

Figure 1. Isolated sculpin hepatocytes demonstrated TCA uptake activity in a time (left) and dose-dependent (right) manner at 15˚C. Michaelis-Menten analysis of TCA uptake at 15˚C for 15 minutes; n = 3-4.

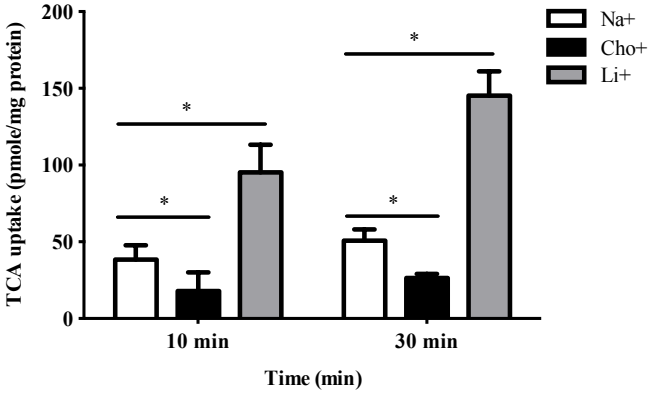

Figure 2. TCA uptake in sculpin hepatocytes is Na^+-dependent and Li^+-enhanced. $^*p < 0.05$; n = 3.

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