MOLECULAR IDENTIFICATION AND FUNCTIONAL COMPLEMENTATION BETWEEN A NOVEL HUMAN AND MOUSE ORGANIC SOLUTE TRANSPORTER, AND A TRANSPORTER FROM THE LITTLE SKATE, *RAJA ERINACEA*, OSTα/OSTβ

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Cellular homeostasis requires the regulated entry and exit of a multitude of compounds across the plasma membrane. Cells must take up specific amounts of nutrients, metabolic precursors, inorganic ions, signaling molecules, and other macromolecules, while also exporting signaling molecules, hormones, electrolytes, metabolic waste products, and xenobiotics. Recent studies have described some of the genes involved in these transport processes; however, it is clear that many other genes and gene products remain to be identified and characterized.

Using a comparative approach, we recently identified a novel type of organic solute and steroid transporter in the liver of the evolutionarily ancient vertebrate, the little skate *Raja erinacea* (Wang et al., Proc. Natl. Acad. Sci. U.S.A. 98:9431-9436, 2001). In contrast to all other organic anion carriers, this skate transporter requires the co-expression of two distinct and novel gene products, *Ostα* and *Ostβ*. Substrates for this multispecific transporter include estrone sulfate, taurocholate, digoxin, and prostaglandin E_2. Interestingly, the overall predicted membrane topology of skate *Ostα/Ostβ* is similar to that of the heterodimeric sensory rhodopsins, suggesting that *Ostα/Ostβ* may have evolved from an ancestral rhodopsin-like molecule, but has acquired the ability to transport steroids and eicosanoids, compounds that also function as ligands for some G-protein-coupled receptors. Initially, *Ostα* and *Ostβ* orthologues were not identified in the human genome, or in any other sequenced genomes, indicating that these genes may be specific to marine elasmobranchs. However, sequences for hypothetical human and mouse proteins have recently been entered into the databases that exhibit 25-41% predicted amino acid sequence identity with skate *Ostα* and *Ostβ*. The present study tested whether these mammalian genes are expressed, whether they encode for orthologues of the skate gene products, and if so, whether they functionally complement each other's transport activity.

An RT-PCR-based strategy was employed to generate cDNAs for the predicted open reading frames (ORF) of putative human *OSTα* and *OSTβ* from human liver mRNA, and of putative mouse *Ostβ* from mouse liver mRNA. The human and mouse liver mRNA was purchased from Clontech. The cDNA for the putative mouse *Ostα* was purchased from the American Type Culture Collection. Sequence analysis of the cDNAs for the four genes indicated that mouse *mOstα* and *mOstβ* clones were identical to published GenBank sequences, whereas the human *OSTα* and *OSTβ* sequences varied by a single nucleotide in the reading frame of each gene when compared to the published GenBank sequences. Both differences in the human gene sequences result in amino acid substitutions. It is unclear whether the observed differences are due to polymorphisms or whether they result from mutations introduced during PCR. They are unlikely to be sequence artifacts as both occur in regions of strong sequence data. A search of the human genomic database (http://www.ncbi.nlm.hih.gov: 80/BLAST/) revealed that human *OSTα* is located on chromosome 3q29 and is coded by 9 exons, whereas human *OSTβ* is on chromosome 15q21 and is coded by 4 exons. Mouse *Ostα* is located on chromosome 16B2 and is coded by 9 exons, whereas mouse *Ostβ* is found on chromosome 9C and is coded by 4 exons.

To assess whether the human and mouse proteins function as organic solute transporters, uptake of [^{3}H]estrone sulfate was measured in *Xenopus laevis* oocytes injected with cRNA synthesized from the human, mouse, or skate genes. As expected, co-expression of skate *Ostα* and *Ostβ* was required to generate transport activity. When the putative human *OSTα* and mouse *Ostα*

were expressed individually in oocytes, no transport activity was detected; however, when these genes were co-expressed with skate *Ostβ*, a strong transport signal was obtained, indicating that human *OSTα* and mouse *Ostα* can functionally complement the corresponding skate gene. Likewise, human *OSTβ* and mouse *Ostβ* did not induce transport activity when expressed individually in oocytes, but generated a functional transporter when co-expressed with skate *Ostα*. Thus, the human and mouse proteins are not only functional orthologues of the skate proteins, but are able to complement each other across species. Moreover, co-expression of the two human or the two mouse genes generated a very strong transport signal, as did the human-mouse α/β pairs.

Oocytes injected with human *OSTα* and *OSTβ* cRNA (1 ng each) or with mouse *Ostα* and *Ostβ* cRNA (1 ng each) were able to transport taurocholate, estrone sulfate, digoxin, and prostaglandin E_2, but not estradiol glucuronide or *p*-aminohippurate, indicating that this transport system is multispecific and that it may participate in cellular transport of conjugated steroids and eicosanoids. This substrate profile is similar to that of skate Ostα/Ostβ (Wang et al., Proc. Natl. Acad. Sci. U.S.A. 98:9431-9436, 2001). The skate, mouse, and human transporters shared a number of other features as well. Transport was sodium-independent, saturable, and inhibited by bile salts, steroids, and other organic anions. Replacement of the NaCl in the oocyte incubation medium with either choline chloride or lithium chloride had no effect on estrone sulfate uptake, indicating that transport is not coupled to the sodium electrochemical gradient. Initial rates of estrone sulfate uptake into human OSTα/OSTβ or mouse Ostα/Ostβ-expressing oocytes were saturable, although the apparent Michaelis constants (Km) were relatively high (320 ± 30 μM and 290 ± 24 μM, respectively; n=3, ±SEM). The Km for estrone sulfate uptake by skate Ostα/Ostβ is lower, 85 ± 16 μM, although still relatively high (Wang et al., Proc. Natl. Acad. Sci. U.S.A. 98:9431-9436, 2001). Uptake of [^{3}H]estrone sulfate in hOSTα/hOSTβ and mOstα/mOstβ-expressing oocytes was inhibited by a variety of bile salts, steroids, and other organic anions. [^{3}H]Estrone sulfate uptake was strongly inhibited by sulfated steroids, including lithocholic acid sulfate and taurolithocholic acid sulfate. As previously reported for skate Ostα/Ostβ (Wang et al., Proc. Natl. Acad. Sci. U.S.A. 98:9431-9436, 2001), taurine-conjugated bile salts were more effective than the corresponding glycine-conjugated compounds, and the addition of a sulfate group further enhanced inhibitory potency. Estrone sulfate transport was also inhibited by spironolactone, digoxin, sulfobromophthalein, bilirubin ditaurate, probenecid, and indomethecin.

OSTα and *OSTβ* mRNA levels were measured in nineteen human tissues and were expressed relative to β-actin mRNA levels using quantitative real time PCR analysis. The results revealed that *OSTα* and *OSTβ* are widely expressed in human tissues. Tissues that had high levels of *OSTα* mRNA generally also had high levels of *OSTβ* mRNA, indicating co-expression of these genes. Relatively high levels of both mRNAs were found in testes, colon, liver, small intestine, kidney, ovary and adrenal gland, and lower levels were measured in heart, lung, brain, pituitary, thyroid gland, uterus, prostate, mammary gland, and fat. The mRNA for *OSTα* and *OSTβ* was below our limit of detection in skeletal muscle and peripheral blood leukocytes.

In summary, using a comparative approach the present study identified a novel mammalian organic solute transporter that is widely distributed and highly expressed in human tissues. This transporter is unique among mammalian organic anion transporters in that it requires the expression of two distinct gene products, a putative 7-TM domain membrane protein, OSTα, and a smaller, single-TM domain polypeptide, OSTβ. Interestingly, the human and mouse proteins were able to complement each other, as well as those from the skate, indicating a high degree of conservation throughout evolution. Although the physiological functions of this transporter are not known, its broad tissue expression and its ability to transport steroids and PGE_2 suggest an important role in cellular functions. (Supported by ES03828, ES01247, ES06484, DK25636, DK48823, and ES07026).