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Humanization of Rat Oatp2b1 to Identify the Estrone-3-Sulfate Binding Sites

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The organic anion transporting polypeptide 2B1 (OATP2B1) is a membrane transporter that mediates the cellular uptake of various endogenous compounds and drugs. OATP2B1 is widely expressed in tissues, including the placenta, the liver, and the small intestine. Recently it was reported that OATP2B1 possesses multiple binding sites and it was shown that the transport of E1S is mediated by two binding sites with different affinity. In a previous study, the transport of E1S by OATP2B1 was compared to the rat orthologue, revealing that Oatp2b1 probably has no low-affinity site for E1S.
The aim of this study was to identify which molecular identity in OATP2B1 is responsible for high- and low-affinity site. Since TM9 and TM10 seemed to be relevant for the binding and the transport of substrates, they were successfully humanized in the rat gene performing site-directed mutagenesis. Transport experiments with [3H]-E1S showed no differences in the transport activity of the TM9 and TM10 mutants compared to the rat wild-type. Expression of the proteins in HeLa cells was confirmed by Western blot analyses and immunofluorescence. However, low uptake rates of the rat and its mutants could be explained by the intracellular localization of the proteins, which probably were not enriched in the membrane. Therefore, it is necessary to improve and/or change the transfection method, in order to be able to perform transport experiments with properly expressed proteins and make comparable evaluations of the transport activities. A future project could involve a rat mutant containing the human ECL5, in order to investigate its role in the transport of substrates.

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