A new sodium-D-glucose cotransporter in *Squalus acanthias* intestine

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In the past few years we have obtained growing evidence that the spiny dogfish (*Squalus acanthias*) expresses different isoforms of sodium glucose cotransporters (SGLT) in kidney and intestine. From kidney we could clone a transporter that was clearly identified as SGLT2[1,2]. For intestine we had only indirect hints that were pointing to an SGLT1-type transporter, but the transporter itself remained unknown[3]. Now we can report the successful identification of a transporter with high homology to SGLT1.

We created degenerate primers from the sequence for the extramembraneous loop 13 that has a sequence very unique for SGLT1 and SGLT2 from skate and shark, respectively. Total RNA isolated from intestine of shark was reverse transcribed into cDNA which was subsequently used in PCR with the above mentioned degenerate primers to screen for the presence of SGLT. PCR gave a signal of the estimated size for SGLT1 of 340 bp in shark intestine. Sequencing of the DNA extracted from the respective band confirmed the first impression: the fragment from shark intestine showed high homology with SGLT1. According to the new sequence information we prepared new specific primers to be used in RACE-PCR (SMART-RACE kit, BD-Bioscience) to obtain the complete sequence. In a first attempt we obtained a fragment for the 3'-end. The 5'-end imposed several problems, but a combination of new primers and fresh RNA gave a fragment and eventually we could clone the full-length transporter.

Translation of the nucleotide sequence showed 72% identity to human SGLT1. Homology to the previously cloned SGLT1 from *Leucoraja* kidney and SGLT2 from *Squalus* kidney was 88% and 62%, respectively. Computer analysis of the amino acid sequence revealed more features of SGLT. Based on hydrophobicity, 14 transmembrane regions separating 13 loops were predicted. Typically, the largest loops are 6 and 8 (extracellular) and 13 (cytosolic). We found several conserved amino acids, that had previously been associated with functions as Na^+-, glucose- and phlorizin-binding. Furthermore there are several possible sites for N-glycosylation and phosphorylation. This new sequence information helped to identify some regions of the transporter that are highly conserved up to mammals and therefore most likely involved in the function of the protein. At other positions there are distinct differences between SGLT1 and SGLT2 that might explain functional differences.

Experiments to express the transporter in *Xenopus laevis* oocytes for functional characterization are in progress. Preliminary data support the classification of the intestine sequence as SGLT1. Compared to controls, cRNA-injected oocytes show uptake of D-glucose in the presence of Na^+. This uptake is inhibited by phlorizin and D-galactose and has a rather high affinity for glucose.

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