Molecular cloning and characterisation of the mouse ‘system IMINO’ transporter

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The SLC6 family consists of transporters for amino acids, neurotransmitters and osmolytes. These transporters play an important role in the removal of neurotransmitters in brain tissue and in amino acid transport in epithelial cells. The SLC6 family also contains a number of orphan transporters. Recently we identified a new member of the SLC6 family (B^0AT1 or SLC6A19), which is closely related to the orphan transporters and transports neutral amino acids. We hypothesized that other orphan transporters may be amino acid transporters as well.

To test this hypothesis we studied the mouse slc6a20 gene. The mouse has two homologues that correspond to single the human SLC6A20 gene, which are known as XT3 and XT3s1. RT-PCR analysis revealed expression of XT3s1 in the brain, kidney, small intestine, thymus, spleen and lung, while expression of XT3 was restricted to kidney and lung. Subsequently, we isolated full-length cDNA clones of XT3s1 and XT3 from brain and kidney, respectively. In situ hybridisation showed strong expression of XT3/XT3s1 in the proximal tubules of kidney cortex, in intestinal villi and in the brain.

Expression of mouse XT3s1, but not XT3, in Xenopus laevis oocytes induced Na^+- and Cl^--dependent transport of proline, hydroxyproline, glycinebetaine, MeAIB and pipecolic acid. Activation analysis suggests a $1Na^+/1Cl^-$/proline cotransport, which would be electroneutral. However, uptake experiments under voltage-clamp conditions suggest translocation of 1 charge per proline molecule. This apparent discrepancy can be explained by the very high affinity of the chloride binding site - chloride transport is likely to occur by way of an exchange process and thus will not affect the electrogenicity of the transporter.

The substrate specificity and mechanism of transport by XT3s1 fits well with the properties of the classical ‘system IMINO’, one of the major proline resorption systems of the intestine and kidney (Stevens & Wright, 1985). Together, the expression pattern and functional characteristics of SLC6A20 suggest a possible involvement in the inherited aminoaciduria iminoglycinuria.

Stevens, B.R. & Wright, E.M. (1985) *Journal of Membrane Biology* **87**, 27-34.