

OCTN1 Cellular Uptake Assay in CHO cells

The OCTN1 transporter (SLC22A4) is an integral membrane protein expressed in the kidney, skeletal muscle, placenta, prostate and heart. OCTN1 is localized in the luminal membrane of renal proximal tubules (**Figure 1**). OCTN1 is polyspecific and transports monovalent organic cations like tetraethylammonium (TEA), quinidine, verapamil. The transport is independent from an inwardly directed sodium gradient and membrane potential.

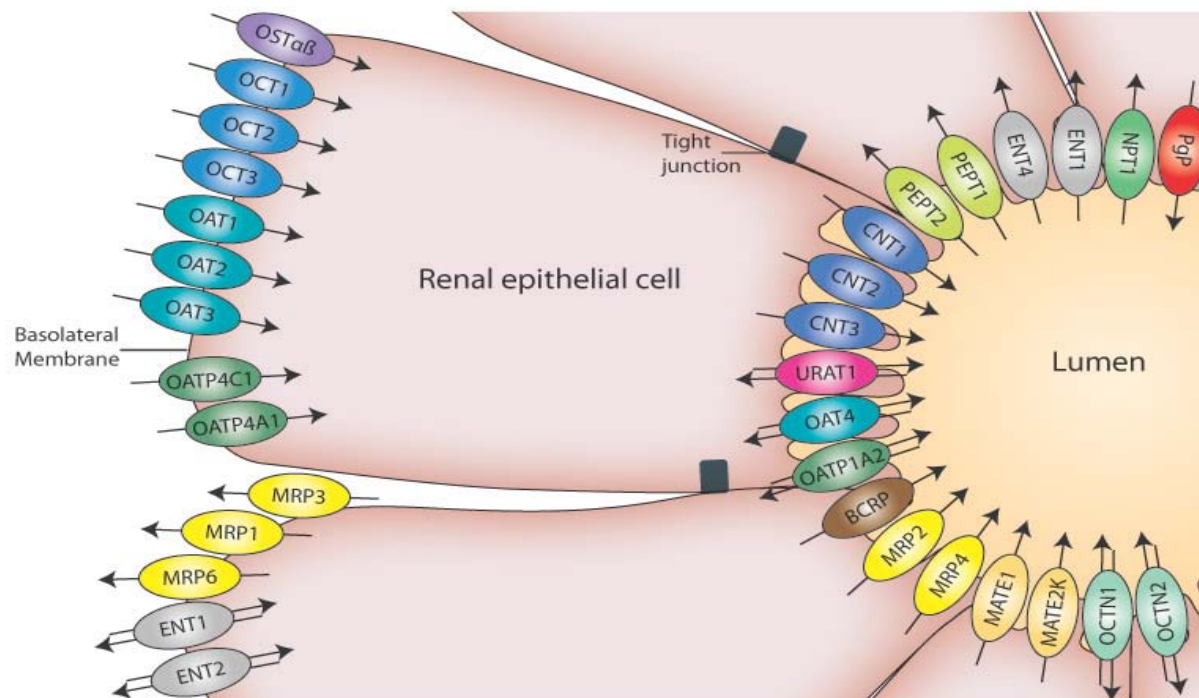


Figure 1 - Localization of transporters in human kidney.

A simple method for measuring OCTN1-mediated transport is the cellular uptake assay. SOLVO generated OCTN1-CHO cell lines stably expressing OCTN1, and developed an inhibition assay for the determination of the interaction of test drugs with this transporter.

The interaction is detected through modulation (inhibition) of the initial rate of ¹⁴C-tetraethylammonium (TEA) transport into OCTN1-CHO cells.

The OCTN1-mediated transport is saturable and fits well to the Michaelis-Menten kinetics (Fig. 2.). K_M was found to be $398 \pm 88 \mu\text{M}$ and V_{max} was $196 \pm 15 \text{ pmol/mg protein/min}$. Based on these data $3,6 \mu\text{M}$ was chosen for probe substrate (TEA) concentration.

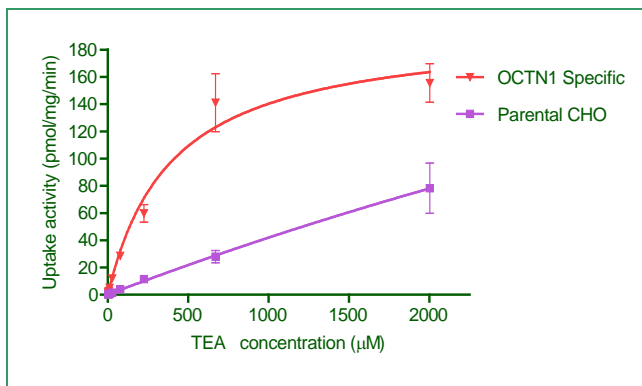


Figure 2 – Substrate saturation with TEA on OCTN1-transfected and parental CHO cells. Closed triangle symbols (\blacktriangle) show the transporter specific accumulation. Cells were incubated in the presence of different TEA concentrations for 60 minutes at 37°C .

Organic solvent dependence of OCTN1 transporter was also tested (Fig. 3.). OCTN1 was found to be sensitive to DMSO, the most frequently used solvent of test articles. Ethanol was selected as the solvent for the OCTN1 assays.

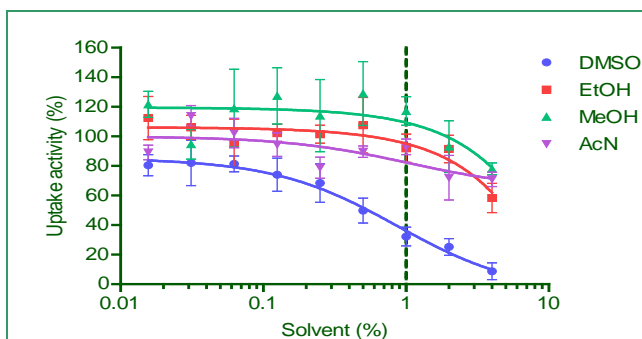


Figure 3 - OCTN1-mediated uptake of TEA in the presence of different organic solvents. The experiment was performed with $3,6 \mu\text{M}$ TEA, at 37°C .

Inhibition of TEA uptake in the presence of several compounds (verapamil, quinidine, nelfinavir, cimetidine, atenolol, metformin) and organic solvents (etanol, DMSO) was also tested (Fig. 4.). As expected, verapamil, nelfinavir and quinidine inhibited the

OCTN1-mediated TEA transport, as well as 1% V/V DMSO, whereas cimetidine, atenolol, metformin did not show significant inhibition.

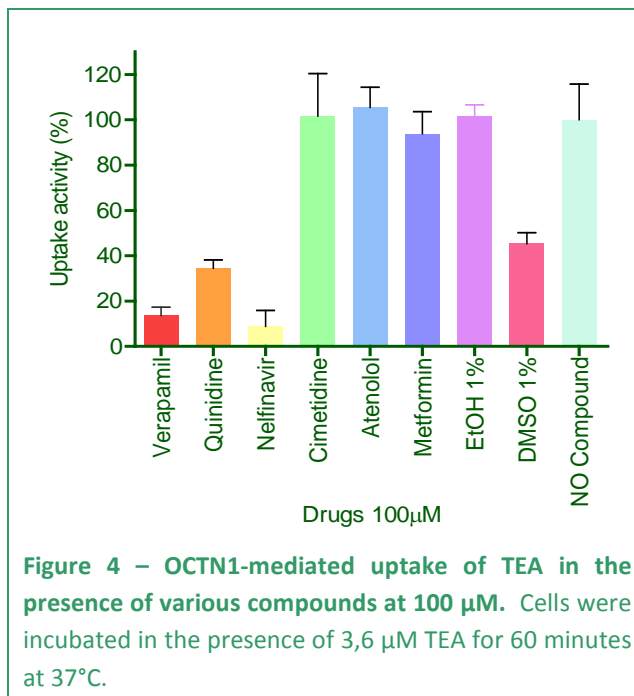


Figure 4 – OCTN1-mediated uptake of TEA in the presence of various compounds at $100 \mu\text{M}$. Cells were incubated in the presence of $3,6 \mu\text{M}$ TEA for 60 minutes at 37°C .

IC_{50} value was determined for verapamil (Fig. 5.). IC_{50} value $2,69 \pm 1,21 \mu\text{M}$. Verapamil was selected as a positive control of inhibition for OCTN1-CHO studies.

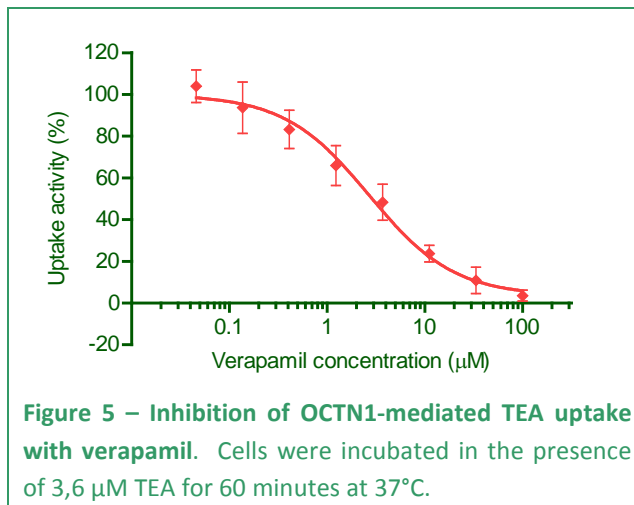


Figure 5 – Inhibition of OCTN1-mediated TEA uptake with verapamil. Cells were incubated in the presence of $3,6 \mu\text{M}$ TEA for 60 minutes at 37°C .

Optimized assay parameters ensured a signal-to-noise ratio higher than 3 with an acceptable standard deviation of about 10 %.