

Insights into regulatory mechanisms of organic cation transporters

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Abstract. Transport of organic cations (OC) is an important pathway for recycling and secretion of endogenous and exogenous OC. Numerous OC transporters have been molecularly identified, their regulation is just starting to be elucidated. Regulation of OC is subtype-, tissue- and species-dependent and involves phosphorylation, which alters substrate-affinity or membrane expression. (www.actabiomedica.it)

Key words: OCT, protein kinases, drug transport, site directed mutation

Introduction

Transport of organic cations (OCs) is mediated by polyspecific transporters (1). We present data on the regulation of rat and human OC transporters (OCTs) (2). OCTs have 11-12 transmembrane domains, a big extracellular loop with glycosylation sites and intracellular loops. The intracellular loops of all OCTs possess putative phosphorylation sites, which suggests that these residues play an important role in the regulation of OCs.

Material and methods

Experiments were performed with human embryonic kidney (HEK293) cells stably expressing rOCT1 or hOCT2. Uptake of the fluorescent substrates (ASP or amiloride) was measured with dynamic fluorescence microscopy (3-7). FACSscan flow cytometry together with an extracellularly oriented mouse monoclonal antibody was used to evaluate membrane trafficking. rOCT1 PKC phosphorylation sites were point mutated to evaluate PKC regulation.

Outcomes

PKC activation increased ASP uptake in WT-rOCT1, whereas it had no effect in rOCT1 mutated at the PKC-phosphorylation sites. Activation of PKA stimulated ASP-uptake in WT but only in some of the PKC-mutants. Inhibition of the p56lck tyrosine kinase reduced ASP-uptake in all rOCT1 forms. TEA, TPA and quinine inhibited ASP-uptake in WT-rOCT1. EC50 values obtained with mutated rOCT1 were significantly decreased. Activation of PKC decreased the EC50 values for TEA, TPA, and quinine in WT-rOCT1. Mutations of the potential PKC phosphorylation sites abolished these effects. Apparent substrate affinities were not affected by p56lck tyrosine kinase regulation. Using an extracellularly oriented monoclonal antibody the membrane-associated fluorescence in WT and 5x-PKC mutant was not altered after PKC activation. Stimulation of PKC increased rOCT1 phosphorylation in WT, but not in PKC-mutants.

Amiloride and ASP are fluorescent substrates of hOCT2-mediated. Uptake of both was inhibited by TEA, cimetidine, and TPA with similar EC50 values.

Amiloride and ASP-uptake was reduced by inhibition of Ca²⁺/calmodulin, stimulation of PKC and of PKA and increased by inhibition of PI3K. Inhibition of Ca²⁺/calmodulin resulted in a significant decrease of V_{max} and a reduction of the membrane-associated hOCT2 as determined by FACScan flow cytometry.

Conclusions

OCTs represent an important excretion and detoxification pathway for many endogenous and potentially harmful exogenous substances. For these reasons, regulation of their activity has a great physiological and pathophysiological significance. All OCTs possess several potential kinase-phosphorylation sites in the intracellular loops of the molecule. Different isoforms are differently regulated. For rOCT1 we demonstrate that the presence of all five potential PKC-phosphorylation sites in the intracellular domain of rOCT1 is essential for its regulation by PKC. These data suggested that the large intracellular loop of rOCT1 is part of the binding pocket or closely interacts with it and that under basal conditions rOCT1 is partially endogenously phosphorylated. Phosphorylation of different residues influences the binding site for various substrates differently and occurs at the S286, S292, T296, S328, and T550 residues. As PKA-mediated regulation of rOCT1 was still active in some but not in all PKC-mutants it is likely that modifications in one or more putative PKC sites prevents phosphorylation of another site by sterical alteration of the molecule. The p56lck tyrosine kinase mediated regulation is not affected by PKC site mutations. The regulatory effect of p56lck tyrosine kinase is not associated with affinity changes of the transporter.

hOCT2 transports the fluorescent substrates amiloride and ASP specifically, ASP with a higher af-

finity than amiloride. They apparently have partially overlapping binding sites in the binding pockets of hOCT2. Both underlie the same qualitative regulation and the inhibition of the Ca²⁺/CaM-complex causes a change of V_{max} via hOCT2-trafficking.

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