INTERACTION AND FUNCTIONAL REGULATION OF XENOBIOTIC TRANSPORTERS BY PDZ ADAPTOR PROTEINS

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Xenobiotic transporters have been proposed to be involved in membrane penetration of various therapeutic agents. As little information is available on the molecular mechanisms of functional regulation of the transporters, we attempted to clarify the protein-protein interactions of such transporters as a first step to identify the regulators. Yeast two-hybrid screening revealed interactions between the carboxyl terminus of various xenobiotic transporters (PEPT1, PEPT2, OCT3, OCTN1, OCTN2, OAT4, OATP-A, OATP-D and OATP-F) and PDZ (PSD95, Dlg and ZO1) domain-containing proteins. Specific interaction was confirmed in pull-down and immunoprecipitation studies. The deletion of the last four amino acids of the PEPT2, OCTN1 and OCTN2 C-termini abrogated such interaction. The interaction of PDZK1 with the carboxyl terminus of OCTN2 was also confirmed in a pull-down study using kidney brush-border membrane vesicles. Immunohistochemical analysis revealed colocalization of OCTN2 and the PDZ protein in renal brush-border membranes. Double transfection of OCTN2 with PDZK1 greatly stimulated the uptake by OCTN2 of its endogenous substrate carnitine, and this increase was consistent with a 6-fold increase in transport capacity. Such an increase was not observed for OCTN2 with deletion of the last four amino acids. Thus, the present findings are the first to identify a functional regulator of OCTN2, operating through direct interaction between multiple PDZ domains and the carboxyl terminus of OCTN2. The present findings also imply that various apical membrane transporters are localized within a protein network and are functionally regulated by PDZ adaptors.