

Regulation of Slc26a6

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The vast majority of kidney stones are composed of calcium oxalate, and minor changes in urinary oxalate affect the stone risk. Knockout mice studies have indicated that intestinal oxalate secretion, mediated by anion exchanger Slc26a6, plays a major constitutive role in limiting net absorption of ingested oxalate, thereby preventing hyperoxaluria and calcium oxalate urolithiasis. This indicates that defects in the function or regulation of this key transporter are potential molecular mechanisms predisposing to calcium oxalate stones in humans. We previously reported that PKC- δ activation negatively regulates Slc26a6 activity expressed in *Xenopus* oocytes by reducing its surface expression. The physiological significance of our findings in *Xenopus* oocytes is underscored by the observation that PKC- δ activation also inhibits endogenous Slc26a6 activity in mouse duodenal tissue.

In view of the significance of Slc26a6-mediated intestinal oxalate secretion in the prevention of hyperoxaluria and calcium oxalate stones, we became interested in identifying the physiologic agonists acting upstream of PKC- δ . To identify such agonists, we used the human intestinal cell line T84, which endogenously expresses SLC26A6. We measured DIDS-sensitive [¹⁴C]oxalate uptake in the presence of an outward Cl gradient as an assay of Cl-oxalate exchange activity, the predominant transport mode described for SLC26A6. The cholinergic agonist carbachol is known to modulate intestinal ion transport through signaling pathways including PKC activation. We, therefore, examined whether carbachol affects Cl-oxalate exchange activity in T84 cells. We found that carbachol significantly inhibited oxalate transport by T84 cells, an effect blocked by the relatively selective PKC- δ inhibitor rottlerin. Under the same conditions, carbachol also led to significant translocation of PKC- δ from the cytosol to the membrane of T84 cells, thus providing further evidence that PKC- δ is the involved PKC isoform. We used pharmacological inhibitors to demonstrate that carbachol inhibition of oxalate transport resulted from activation of the M₃ muscarinic receptor and phospholipase C. We also found that carbachol caused significant stimulation of c-Src phosphorylation, and that inhibition of oxalate transport by both carbachol and PMA was significantly attenuated by the Src family kinase inhibitor PP2.

Taken together, these results indicate that carbachol inhibits oxalate transport by T84 cells through signaling pathways, including the M₃ muscarinic receptor, phospholipase C, PKC- δ , and c-Src kinase. These findings suggest that intestinal oxalate secretion is subject to cholinergic regulation, and future studies will be directed at testing this hypothesis in native tissue.
