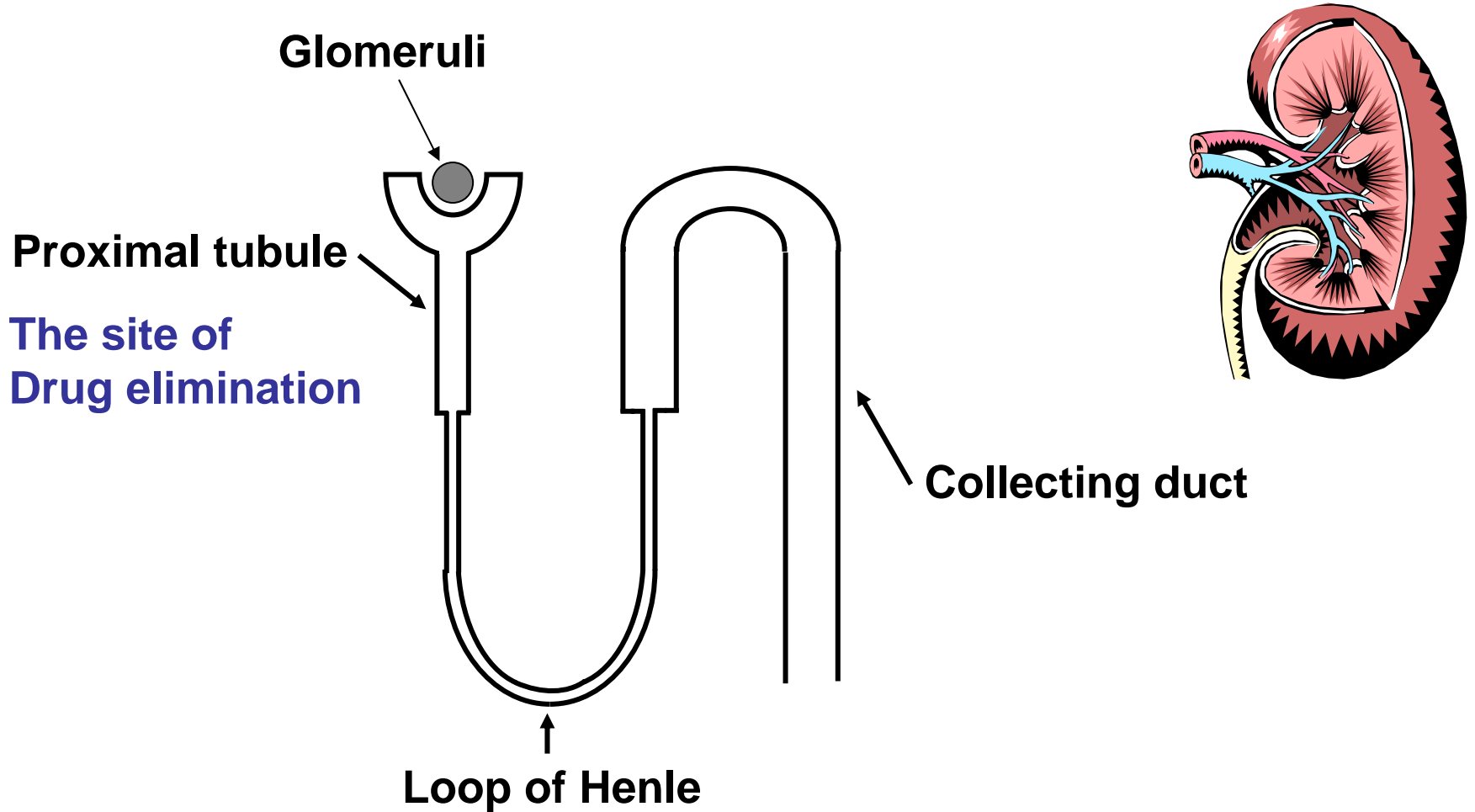


Regulation of Organic Anion Transporters and the Clinical Implication

OAT Family

| <u>Transporter type</u> | <u>Species</u> | <u>Tissue distribution</u> |
|-------------------------|---|----------------------------|
| OAT1 | Rat Mouse Human Flounder C.elegans Rabbit Pig | Kidney, Brain |
| OAT2 | Rat Human Mouse | Liver, Kidney |
| OAT3 | Rat Human Mouse | Kidney, Brain |
| OAT4 | Human | Kidney, Placenta |
| OAT5-7 | | |

Structure of Kidney Nephron



Anionic Substances Interacting with or Transported by OAT

Endogenous organic anions

| | |
|-------------------------------------|---|
| Cyclic nucleotides | cAMP, cGMP |
| Dicarboxylates | a-ketoglutarate, glutarate, succinate |
| Others | urate, folate, octanoate |
| Neurotransmitter metabolites | 4-hydroxy-3-methoxymandelic acid, 3,4-dihydroxyphenylacetic acid, |

Exogenous organic anions - drugs

| | |
|-------------------------|---|
| Antibiotics | penicillin G, carbenicillin, amoxicillin, piperacillin, cloxacillin, nafcillin, cephaloridine, cefadroxil |
| Anti-viral drugs | azidothymidine, acyclovir, amantadine |
| NSAIDs | salicylate, acetylsalicylate, indomethacin, antipyrine, benzydamine, paracetamol, diclofenac |
| Diuretics | furosemide, bumetanide, ethacrynic acid, acetazolamide, benthiazide |
| ACE inhibitors | captopril, enalapril, imidapril, delapril, benazapril, quinapril, ramipril |
| ATII antagonists | telmisartan, candesartan, valsartan, losartan |
| Anti-neoplastics | methotrexate, chlorambucil, 6-MP, thioguanine, dacarbazine, azathioprine, aclarubicin, |
| Anti-epileptics | valproate |

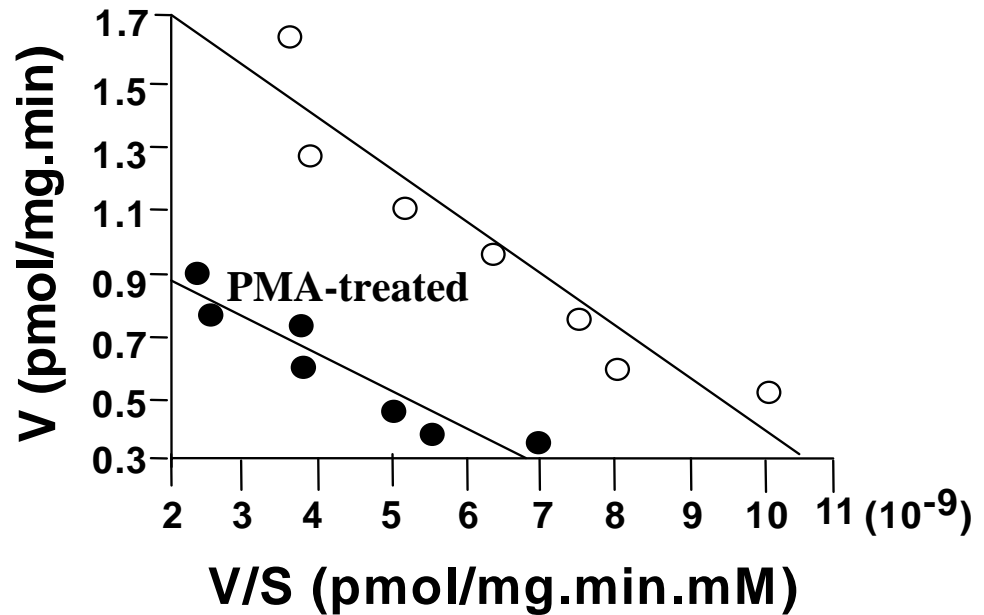
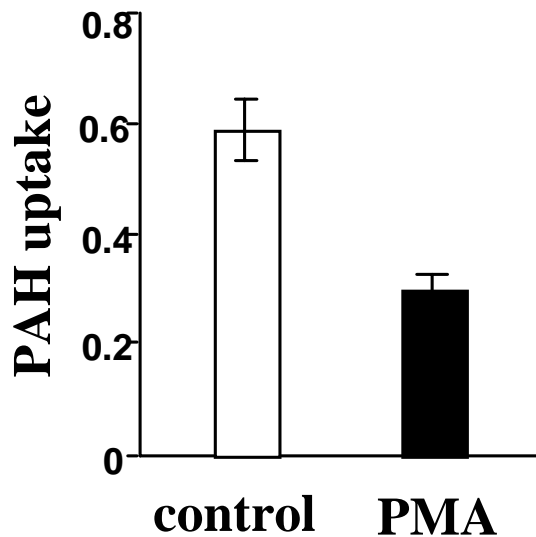
Exogenous organic anions - environmental chemicals

| | |
|-------------------------------|--|
| Mycotoxins | ochratoxin A, ochratoxin B, citreoviridin, citrinin, zearalenol, fumonisin B1, |
| Conjugated substances | |
| Sulphate conjugates | oestrone-S, p-nitrophenyl-S, 4-methylumbelliferyl-S, minoxidil-S, a-naphtyl-S, |
| Cysteine conjugates | S-benzyl-cys, CTFC, DCVC, N-acetyl-S-farnesyl-cys |
| Glucuronide conjugates | b-oestradiol 17-G, p-nitrophenyl-G, 4-methylumbelliferyl-G, |
| Glycine conjugates | PAH, o-hydroxyhippurate |

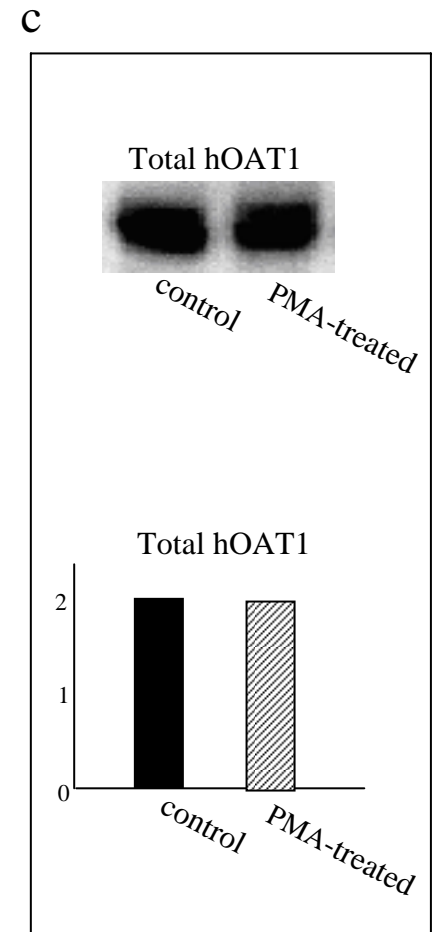
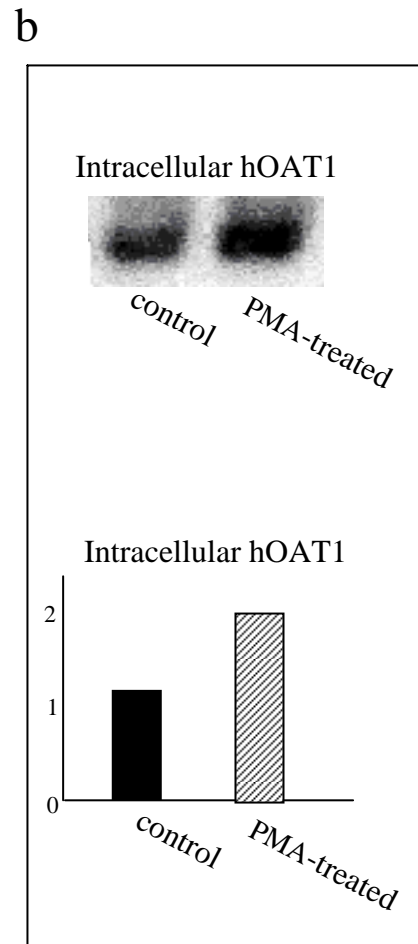
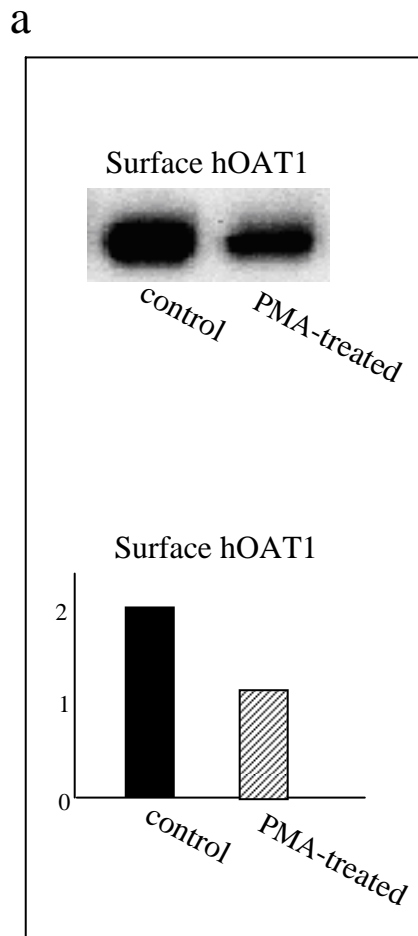
Post-translational Modification of OATs

- 1. Glycosylation**
- 2. Phosphorylation**
- 3. Disulfide Bonds**
- 4. Oligomerization**
 - Homo-oligomerization**
 - Hetero-oligomerization**

Effect of Protein Kinase C Activator PMA on OAT1 Activity



Activation of PKC Results in a Redistribution of OAT1 from Cell Surface to Intracellular Compartments



Biotinylation-based Strategy for Measuring OAT1 Internalization

Biotinylation of hOAT1-expressing cells with cell-impermeable biotinylation reagent sulfo-NHS-SS-biotin was performed under trafficking-impermissive condition (4°C).



The labeled cells were then rewarmed back to trafficking-permissive condition (37°C) to allow internalization to occur.

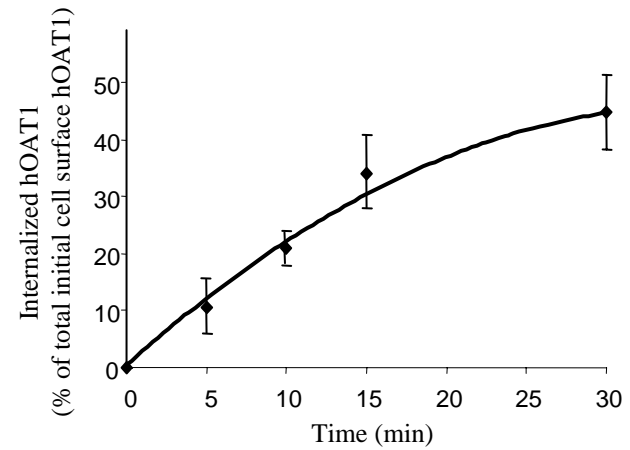
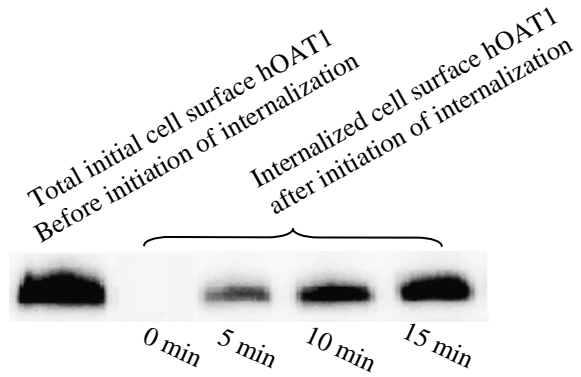


At the indicated time points after initiation of internalization, biotin from biotinylated proteins remaining on the surface was removed by treatment with MesNa, a nonpermeant reducing agent that cleaves disulfide bond and liberates biotin from biotinylated proteins at the cell surface.



The amount of biotinylated proteins resistant (inaccessible) to MesNa treatment was defined as "the amount of protein internalized".

OAT1 Undergoes Constitutive Internalization



Biotinylation-based Strategy for Measuring OAT1 Recycling

Biotinylation of OAT1-expressing cells with cell-impermeable biotinylation reagent sulfo-NHS-SS-biotin was performed under trafficking-impermissive condition (4°C).

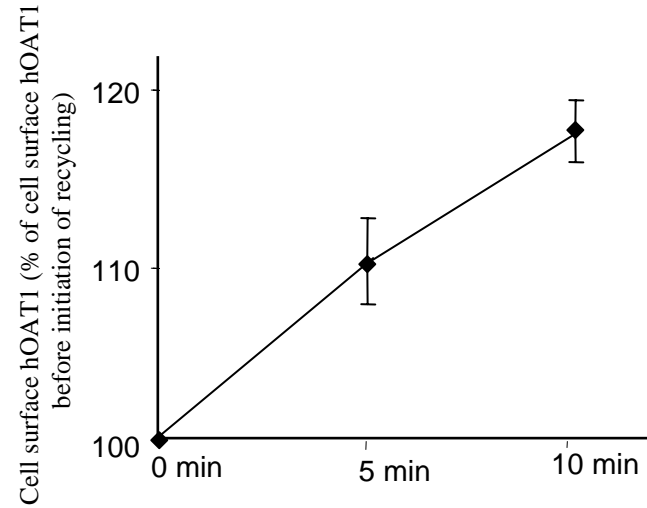
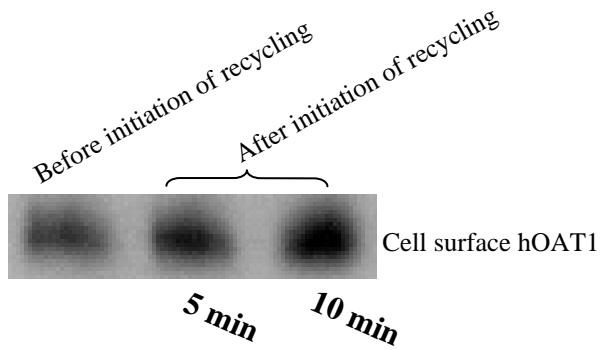


One set of the labeled cells were then rewarmed back to trafficking-permissive condition (37°C) to allow biotinylation to continue.

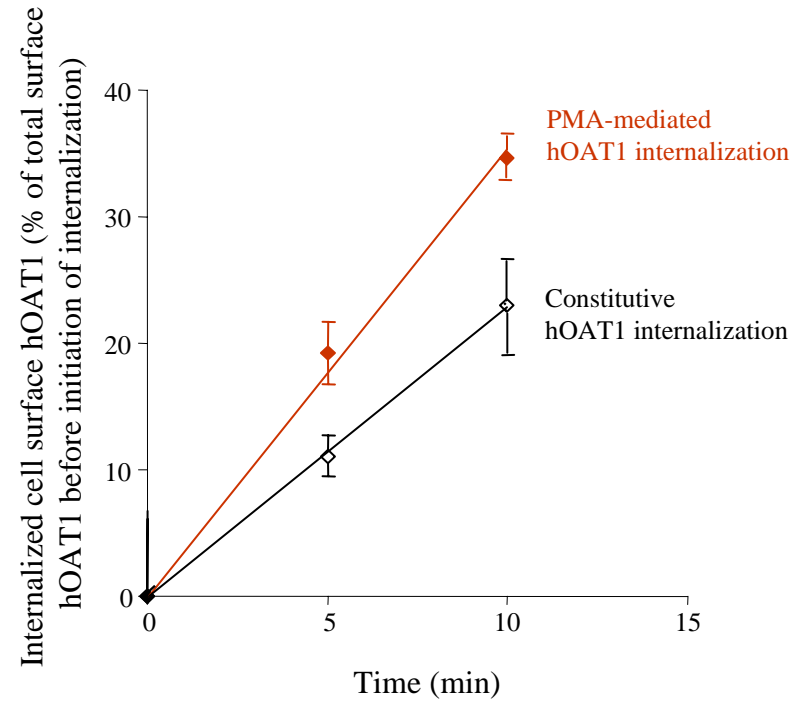
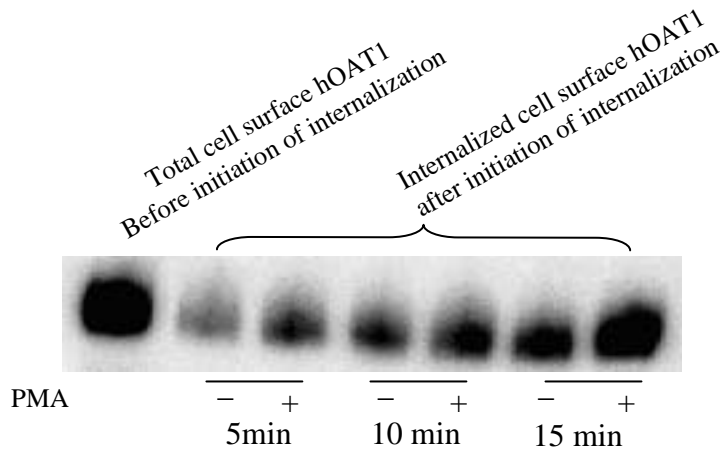


The difference between the amount of OAT1 biotinylated at 37°C and 4°C is defined as the amount of OAT1 recycled.

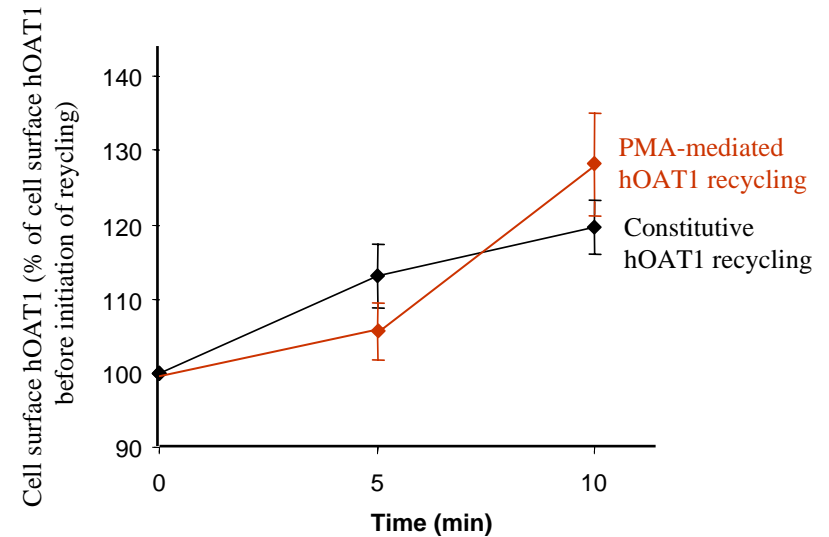
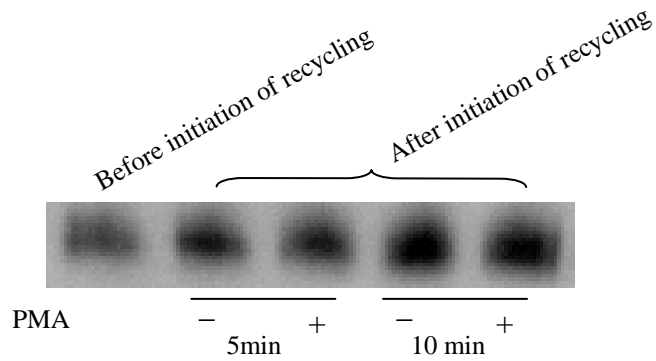
OAT1 Undergoes Constitutive Recycling



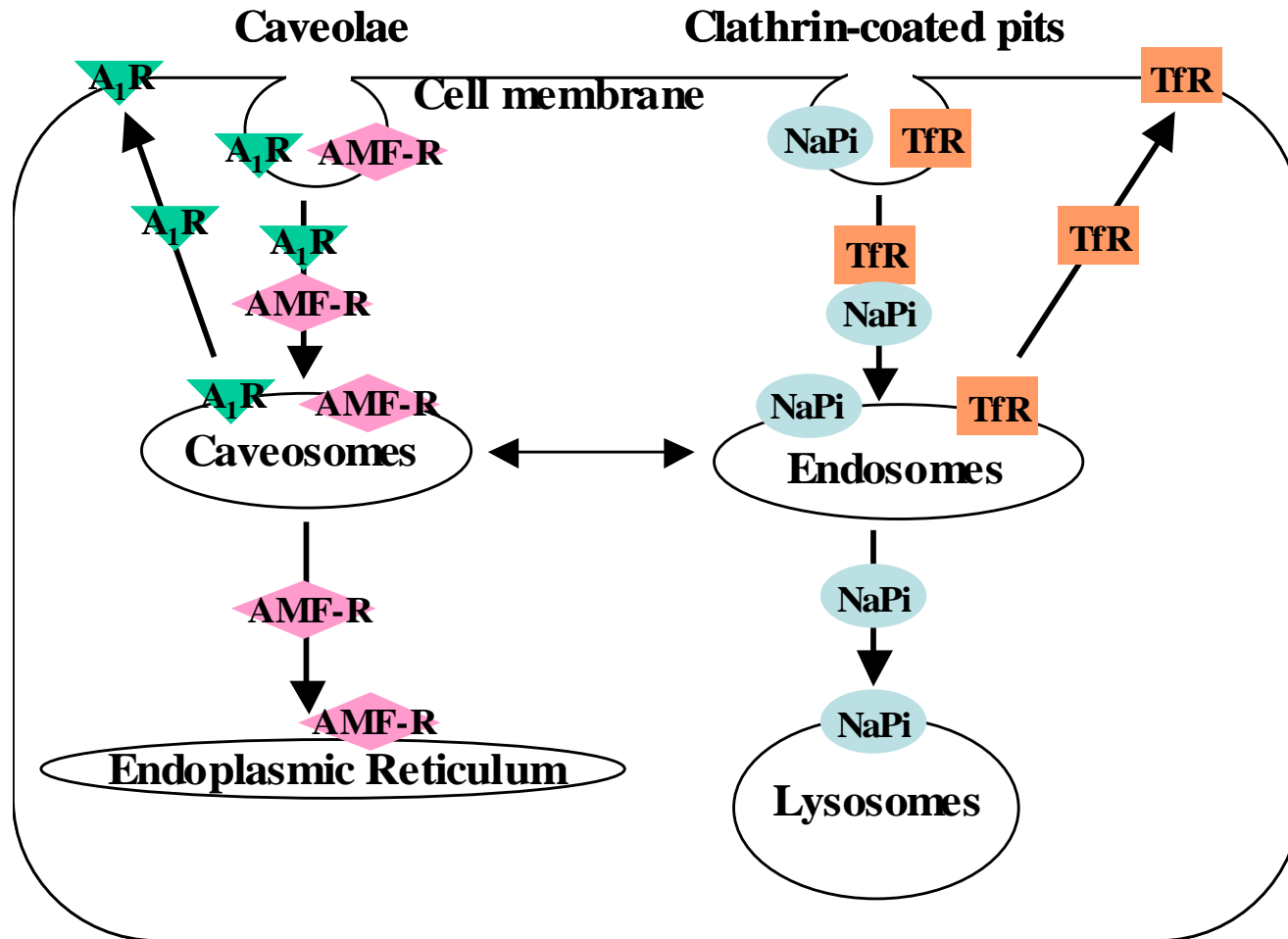
Activation of PKC Accelerates OAT1 Internalization



Activation of PKC Has No Effect on OAT1 Recycling

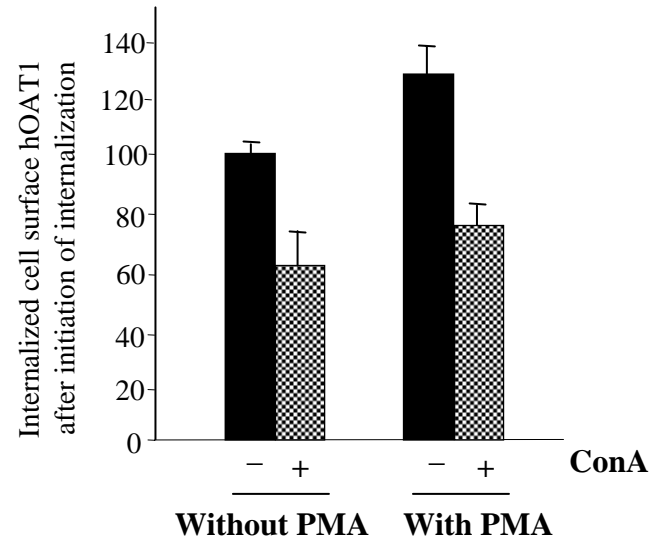
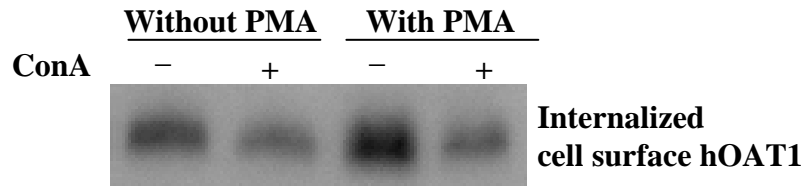


Trafficking Pathways of Membrane Proteins

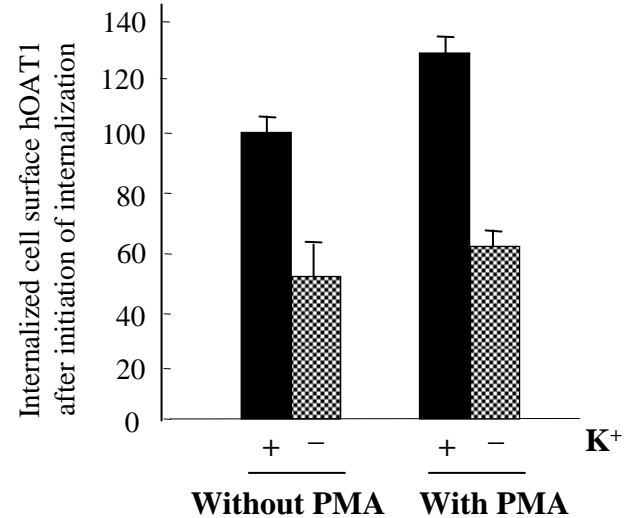
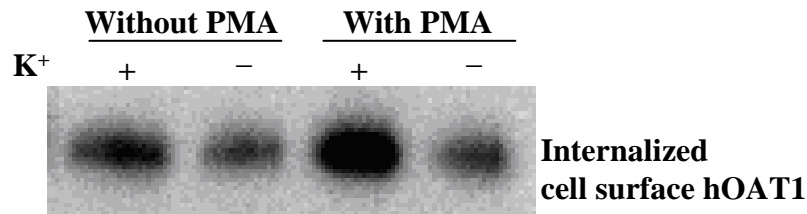


A₁R: A₁ adenosine receptor, TfR: transferrin receptor, NaPi: Na/phosphate cotransporter, AMF-R: autocrine motility factor receptor.

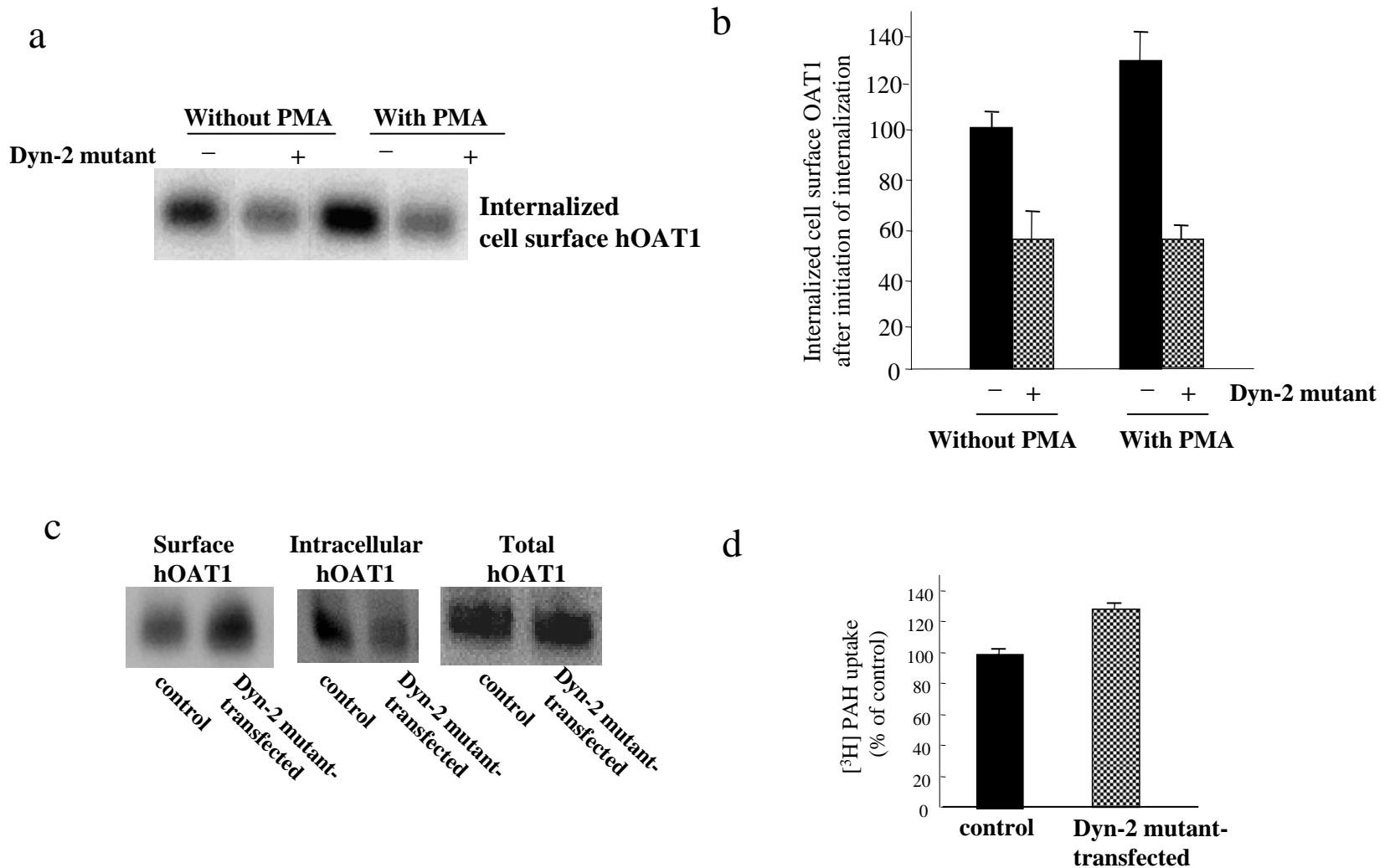
Concanavalin A (ConA) Blocked Constitutive and PKC-modulated OAT1 Internalization



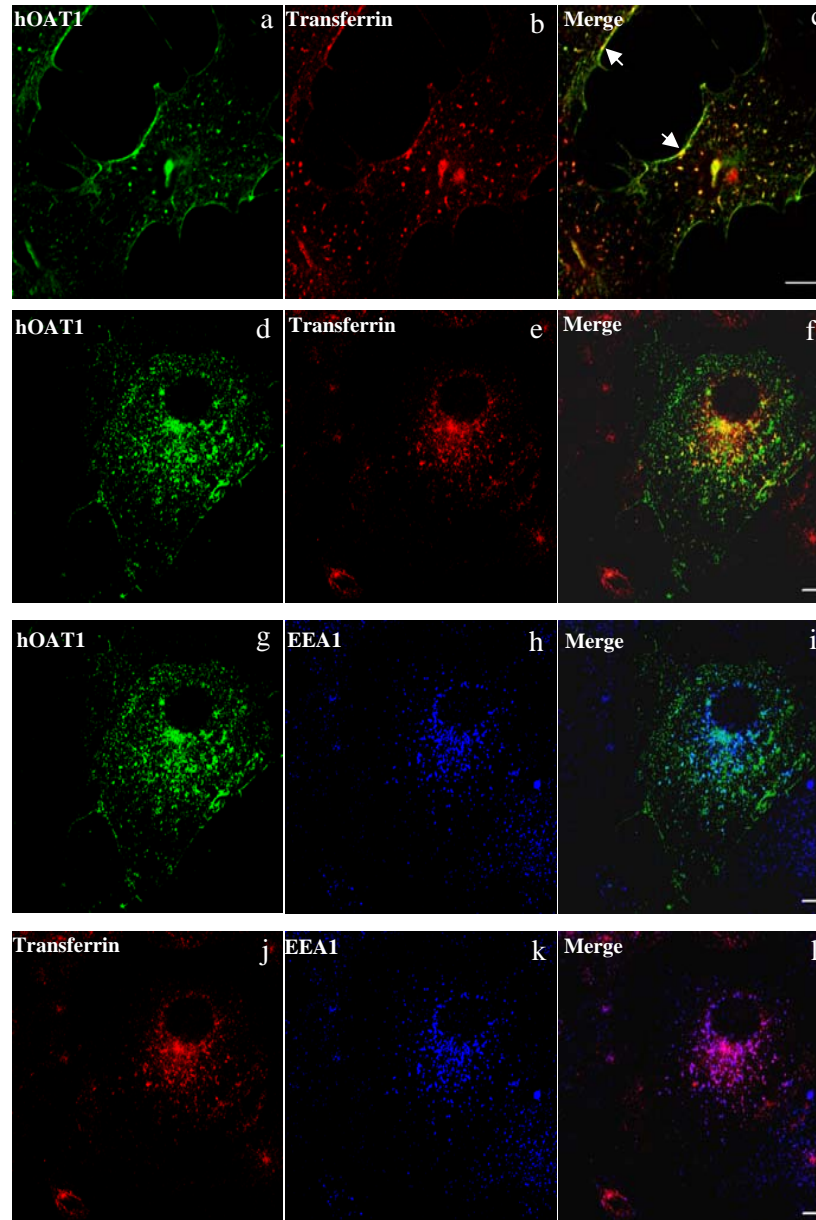
K⁺ Depletion Blocked Constitutive and PKC-Modulated OAT1 Internalization



Dominant Negative Mutant of Dynamin 2 Blocked Constitutive and PKC-modulated OAT1 Internalization



Immuno-localization of OAT1 and Transferrin



Conclusion

- (i) OAT1 constitutively traffics between cell membrane and recycling endosomes**
- (ii) PKC activation down regulates OAT1 activity by altering already existent OAT1 trafficking kinetics: accelerating OAT1 internalization without significantly affecting OAT1 recycling**
- (iii) OAT1 internalization occurs partly through a dynamin- and clathrin-dependent pathway**

Post-translational Modification of Drug Transporters

- 1. Glycosylation**
- 2. Phosphorylation**
- 3. Disulfide Bonds**
- 4. Oligomerization**

Post-translational Modification of OATs

1. Glycosylation
 2. Phosphorylation
 3. Disulfide Bonds
 4. Oligomerization
- Membrane Trafficking

Why would cells expend energy to constantly cycle transporters?

Perhaps the best explanation is that the transporter in a dynamic rather than a static state is more primed for the input to initiate trafficking, therefore is capable of providing quick and efficient fine-tuning in body response to environmental changes.

Pathophysiological Relevance of OAT1 Trafficking

In rats with bilateral ureteral obstruction (BUO), elimination of drugs was impaired partly due to a redistribution of OAT1 from cell surface to intracellular compartment (Villar, et al. 2005, *Kidney Int.* 68, 2704-13) . BUO is a serious and common disorder, which is associated with increased intraluminal pressure in the ureter and renal tubules that may cause renal parenchymal damage through a series direct and indirect effects, and is an important cause of acute renal failure.

We hypothesize that abnormal membrane trafficking of OAT1 may contribute to the impaired drug elimination in BUO.

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