

Possible approaches to reduce renal radioactivity retention after radiolabelled peptides

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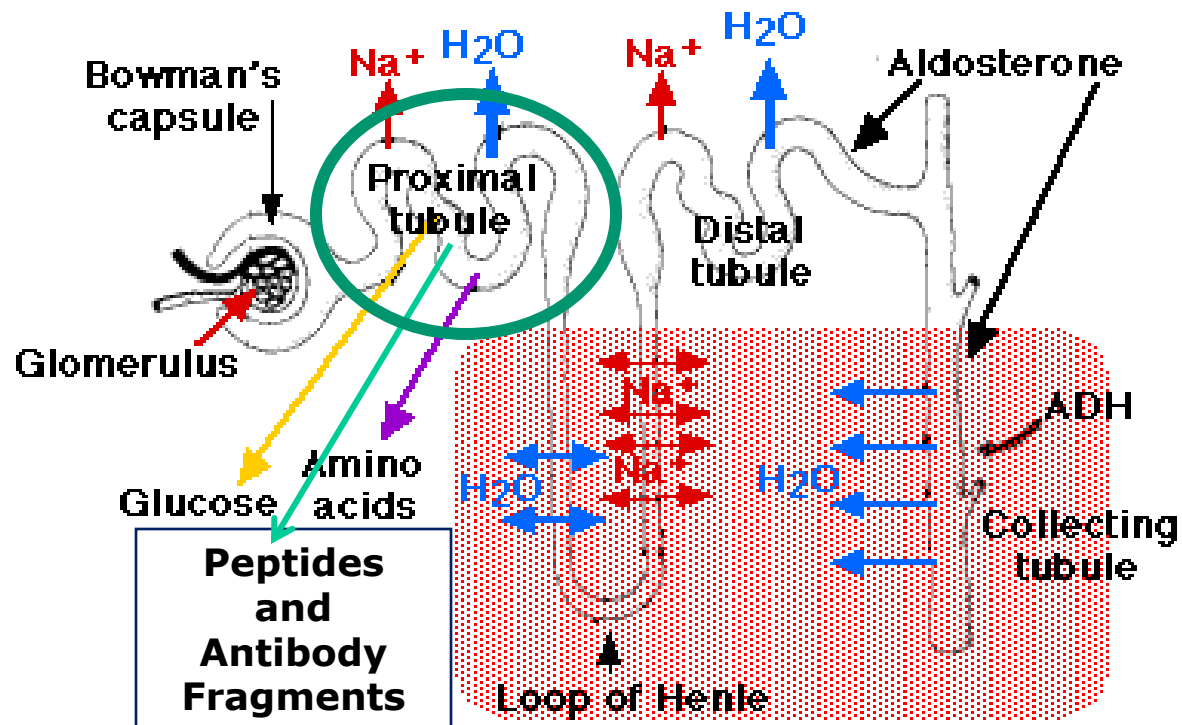
Strategy of our group

Study of transport mechanisms of new receptor-specific radiopharmaceuticals in preclinical experiments at different levels

- radiolabelling and stability studies
- biodistribution studies in experimental animals
- perfused rat kidney and liver
- cell cultures
- microautoradiography of organs of interest

Kidney transport mechanisms

<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/K/Kidney.html>



Kidney protection is one of the aims of our studies

The kidneys are the major dose-limiting organs in peptide receptor radionuclide therapy because of tubular reabsorption and retention of radioactivity.

Decrease of renal radioactivity uptake will allow for higher tumour radiation dose.

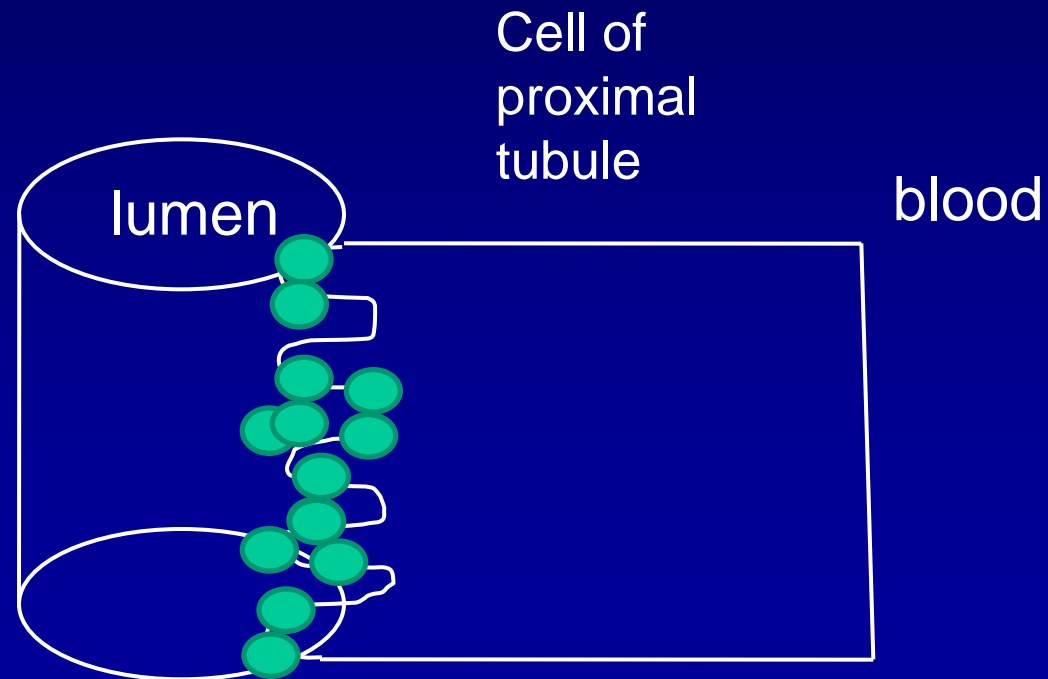
Possible strategies

1. infusion of amino acids mixtures (Lys + Arg) - competition at the binding site
2. administration of drugs employing the same transport system (megalin/cubilin transporter) in the renal reabsorption - competition in the transport mechanisms
3. increasing of the radioactivity supply to the somatostatin receptor positive organs by reducing glomerular filtration rate for example by captopril – increasing AUC in the central compartment and maintaining the radioactivity passing through the kidney
4. employment of a partial extra-renal elimination of the radiopeptide (hemoperfusion, hemodialysis)
5. reversible inhibition of receptor-mediated endocytosis in the proximal tubular cells of the kidney by inhibitors of HMG-CoA reductase – statins

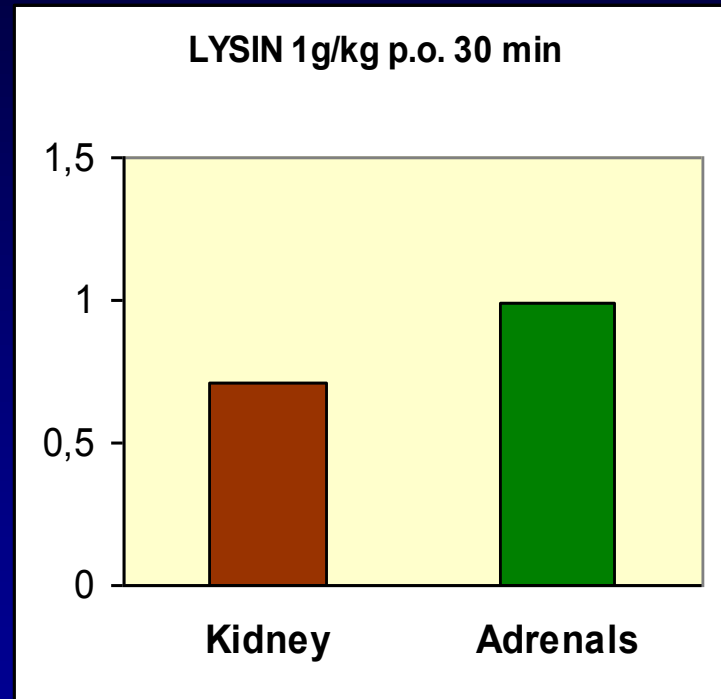
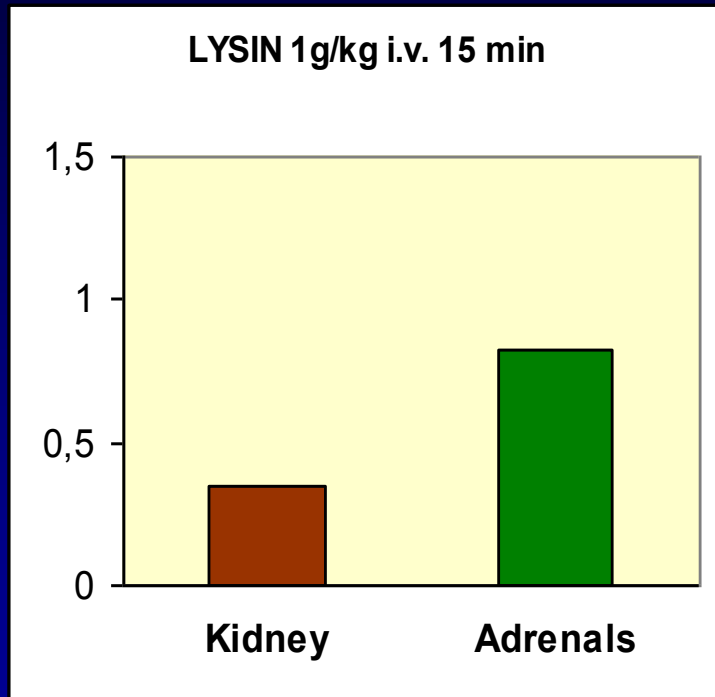
1. competition at the binding site

infusion of basic amino acids mixtures (Lys + Arg, polaminoacids)

Behr TM et al: Reduction of renal uptake of monoclonal antibody fragments by amino acids infusion . J Nucl Med (1996)



Inhibition of receptor-mediated endocytosis of ^{111}In -DOTA-tate by lysin in rats



High doses of aminoacids produce serious hyperkalaemia and severe vomiting

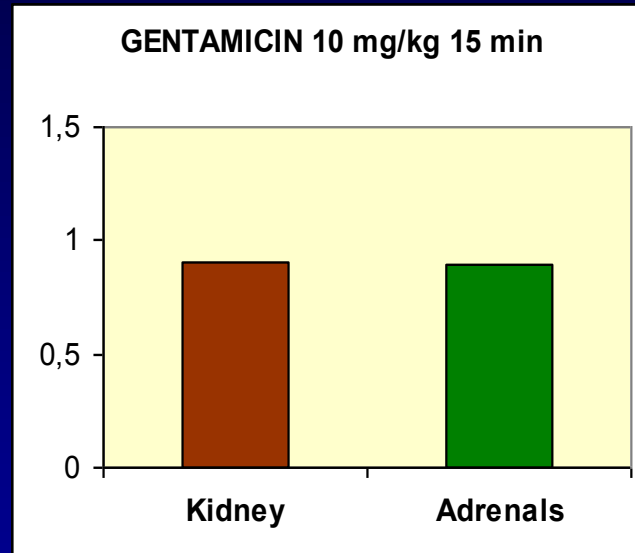
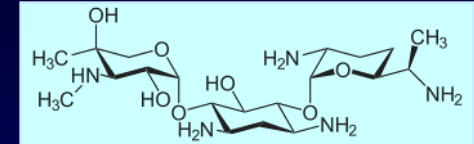
2. competition in the transport mechanisms:

administration of drugs employing the same transport system (megalin/cubilin transporter) in the renal reabsorption:

Suitable candidates

- clinically used drugs eliminated by the kidney in unchanged form
- are taken up in the proximal tubules by the system mediated by the same transporter
- and are able to compete with radiopeptides.

An attempt to inhibit receptor-mediated endocytosis of ^{111}In -DOTA-tate by gentamicin (aminoglycoside antibiotic)



Gentamicin is internalized by megalin transport system in renal proximal tubules

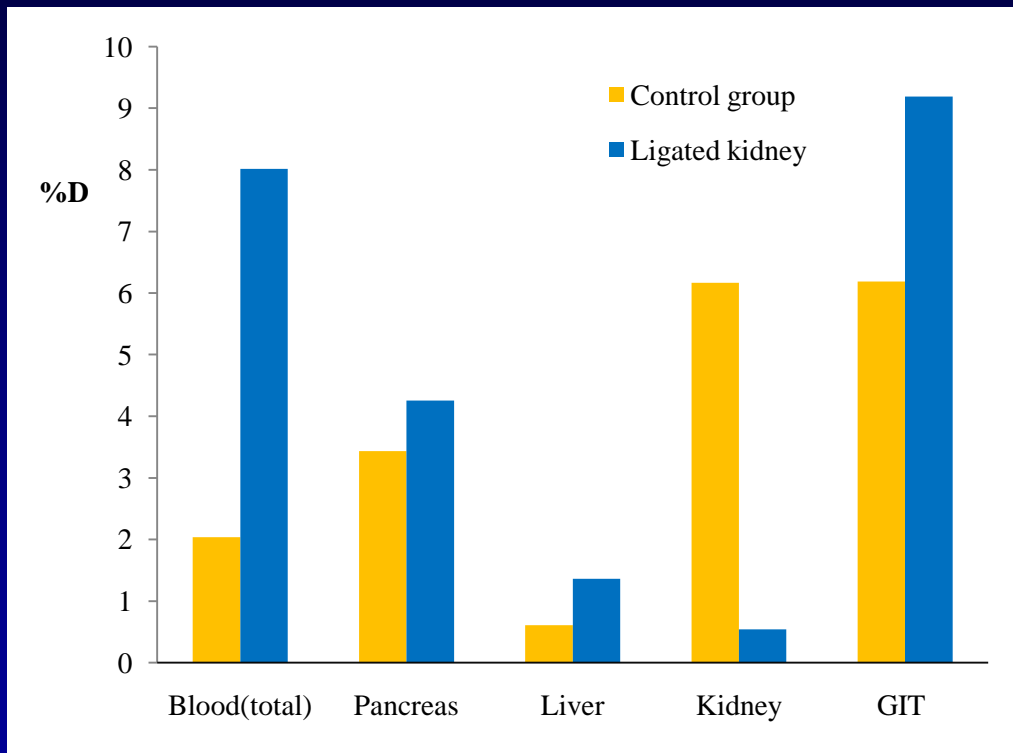
3. Increasing of the radioactivity supply to the somatostatin receptor positive organs by reducing glomerular filtration rate (captopril)

Renal excretion rate of radiopeptide \approx GFR \times f_u

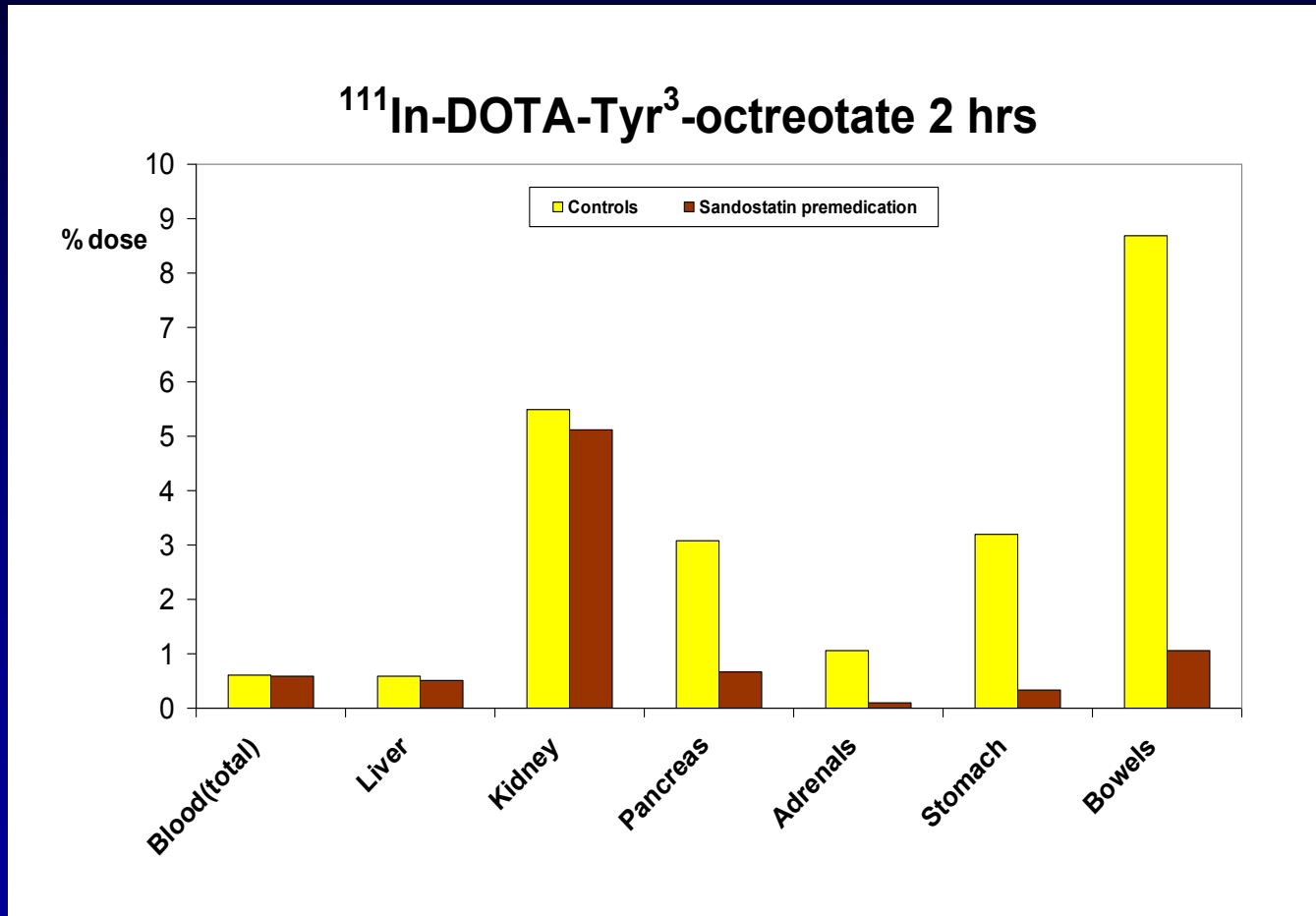
\downarrow GFR \rightarrow \uparrow AUC in plasma \rightarrow \uparrow supply of the peptide in the target

Radioactivity passing through the kidney is unchanged!

Influence of kidney ligation on distribution of ^{111}In -DOTA-tate



The effect of somatostatin receptor blockade by octreotide



4. employment of a partial extra-renal elimination of the radiopeptide (hemoperfusion and/or hemodialysis)

Peptide uptake in the tumor is very rapid (few minutes) its elimination rate is substantially slower (few hours).

If there is an external system making an efficient removal of the peptide from the blood possible, than an employment of this system shortly after the peptide administration should markedly decrease radioactivity uptake both in elimination organs and in organs of deep compartment.

At equilibrium, the proportion of the activity eliminated by the kidney Q_k and by external procedure Q_{ex} is given by:

$$P = Q_k / Q_{ex} = GFR \times f_u / BF \times E$$

5. reversible inhibition of receptor-mediated endocytosis in the proximal tubular cells of the kidney by inhibitors of HMG - statins

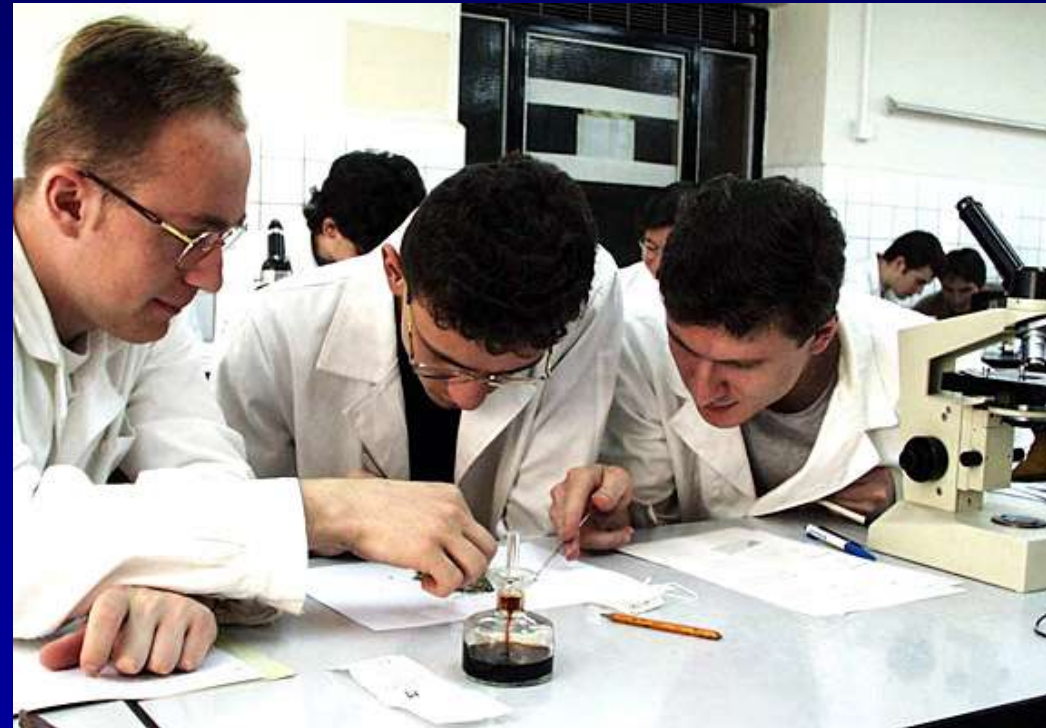
/3-hydroxy-3-methylglutaryl/-CoA reductase

(potent inhibitors of sterol biosynthesis)

Statins are usually used for therapeutic reduction of plasma cholesterol levels. In the absence of cellular toxicity, they inhibit protein uptake by the human proximal nephron via inhibition of HMG-CoA reductase and reduce prenylation of proteins involved in endocytosis.

Similar effects could be expected for radiopeptide endocytosis.

We call other members of COST
for collaboration in this area of
research!



Thank you for your attention!

GFR

Captopril inhibits the formation of angiotensin II (causing vasoconstriction of the efferent arterioles) and causes vasodilatation of that arterioles resulting in a lowering of filtration pressure in the kidney affected by renal artery stenosis.

Through this mechanism impairment of glomerular filtration accures while renal blood flow remains stable.

H.Yoe Oei, F.H.M. Derkx: renography in renovasculat hypertension,
Mallinckrodt: MAG 3 Todays routine

Statins are usually used for therapeutic reduction of plasma cholesterol levels.

In the phase III studies of a new statin, rosuvastatin, which included comparison with other statins and placebo, there was an observation of proteinuria in some subjects, most frequently in those taking rosuvastatin above the currently recommended dose range.

The proteinuria observed with rosuvastatin was generally transient, not associated with worsening renal function and mainly of tubular type, suggesting reduced reabsorption of normally filtered proteins.

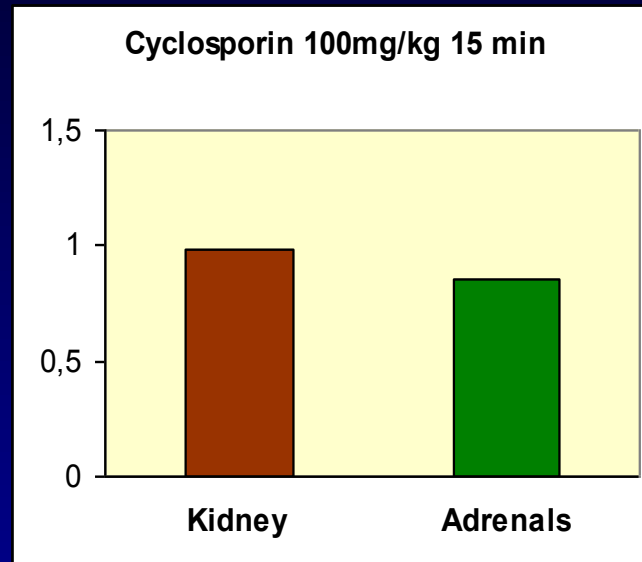
The observation in OK cells suggest that the mechanism for a reduced rate of protein reabsorption is linked to inhibition of HMG-CoA reductase in the proximal tubule cells which in turn leads to a depletion of the cellular GGPP pool (geranylgeranyl pyrophosphate) and thereby to reduced function of one or more GTP-binding proteins (proteins that bind to guanosine triphosphate), known to be involved in the process of endocytosis.

Anja Verhulst, Patric C. D'Haese, Marc E. de Broe: Inhibitors of HMG-CoA Reductase, Receptor –mediated Endocytosis in Human Kidney Proximal Tubular Cells, *J. Am Soc Nephrol* 15: 2249-2257 (2004)

Distribution of somatostatin analogues in rats 24 hours after dosing

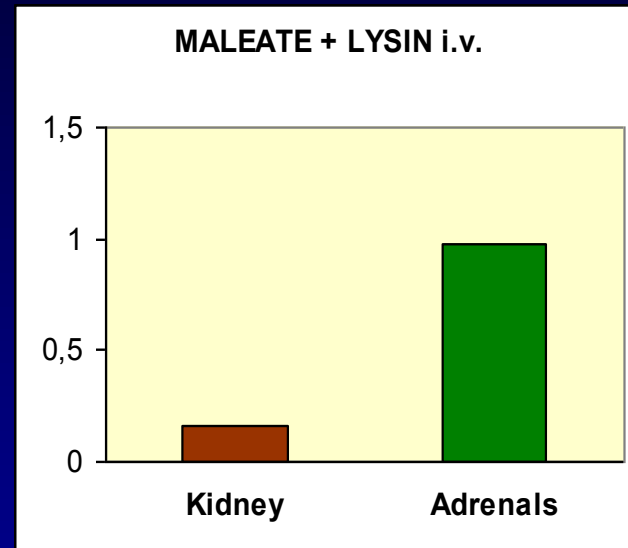
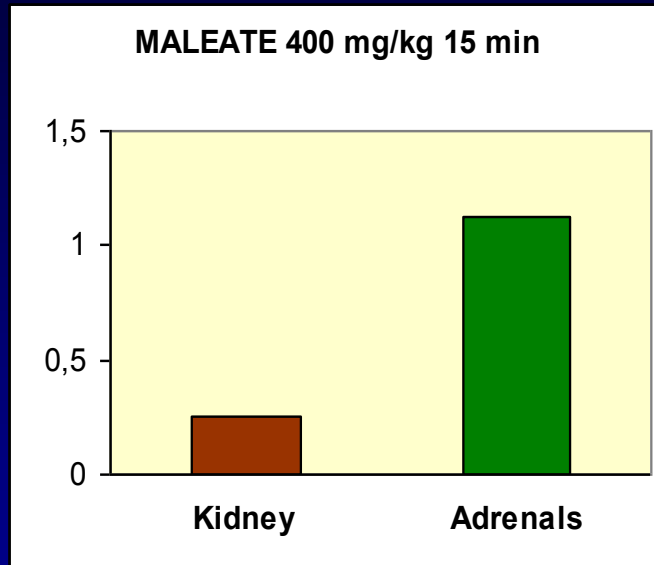
Peptide	Kidney		Adrenals	
In-DTPA-oc	3.92	0.62	0.10	0.02
In-DOTA-oc	4.35	0.52	0.18	0.02
In-DOTA-toc	5.12	0.64	0.20	0.02
In-DOTA-t-GA-tate	14.4	3.44	0.49	0.12
Y-DOTA-t-Ga-tate	4.20	0.98	0.32	0.09
In-DOTAGA-tate	11.1	2.06	0.76	0.13
Y-DOTAGA-tate	3.23	0.83	0.50	0.08
In-DOTA-tate	5.55	1.18	0.71	0.14
Y-DOTA-tate	2.07	0.43	0.92	0.14
Sm-DOTA-tate	4.39	0.90	0.81	0.07
I-DOTA-tate	0.40	0.13	0.008	0.002

Effect of cyclosporine to receptor-mediated endocytosis of ^{111}In -DOTA-tate



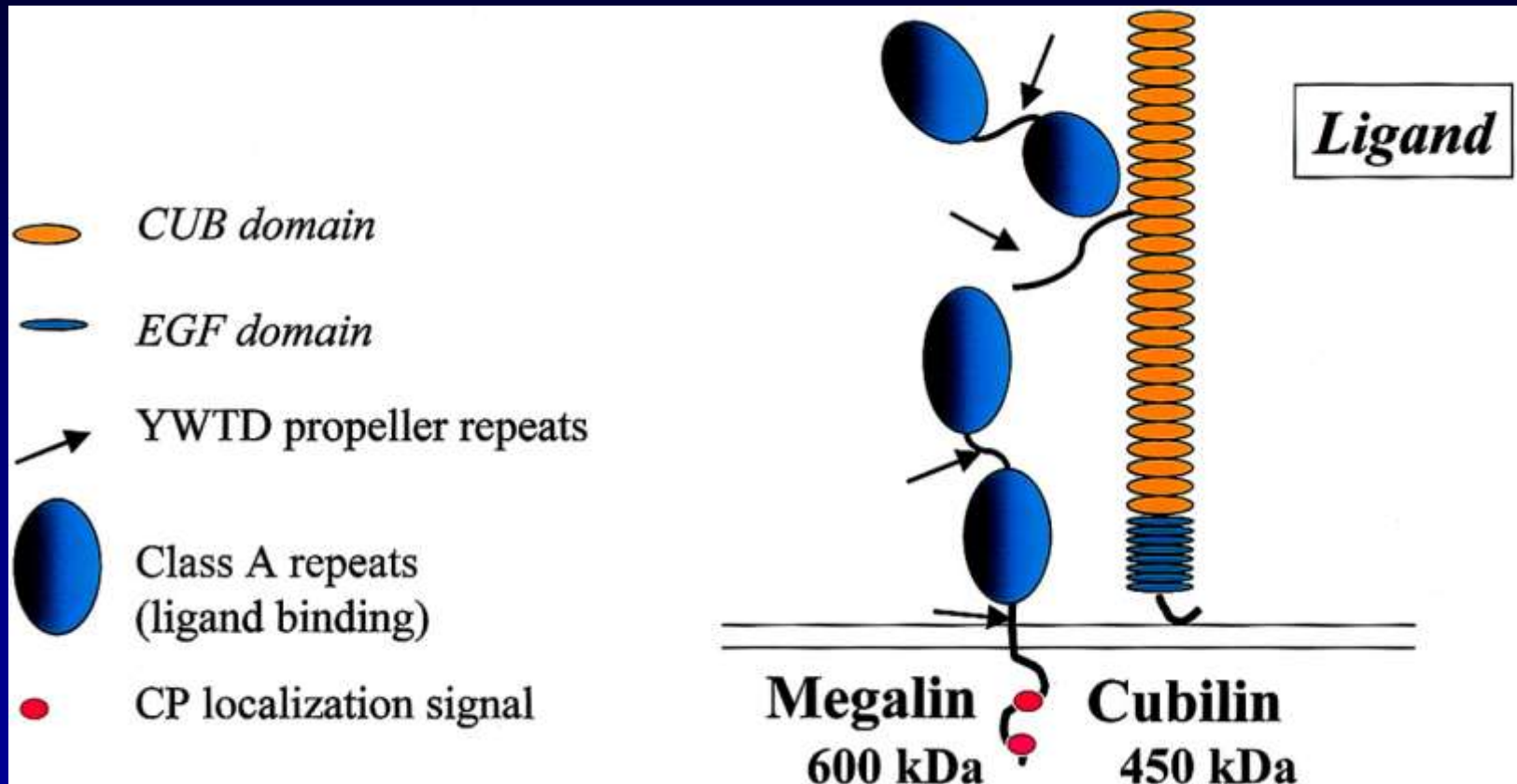
Cyclosporine A is an inhibitor of P-glycoprotein-mediated transport. P-glycoprotein was found to be located in the luminal membrane of the renal proximal tubular cells.

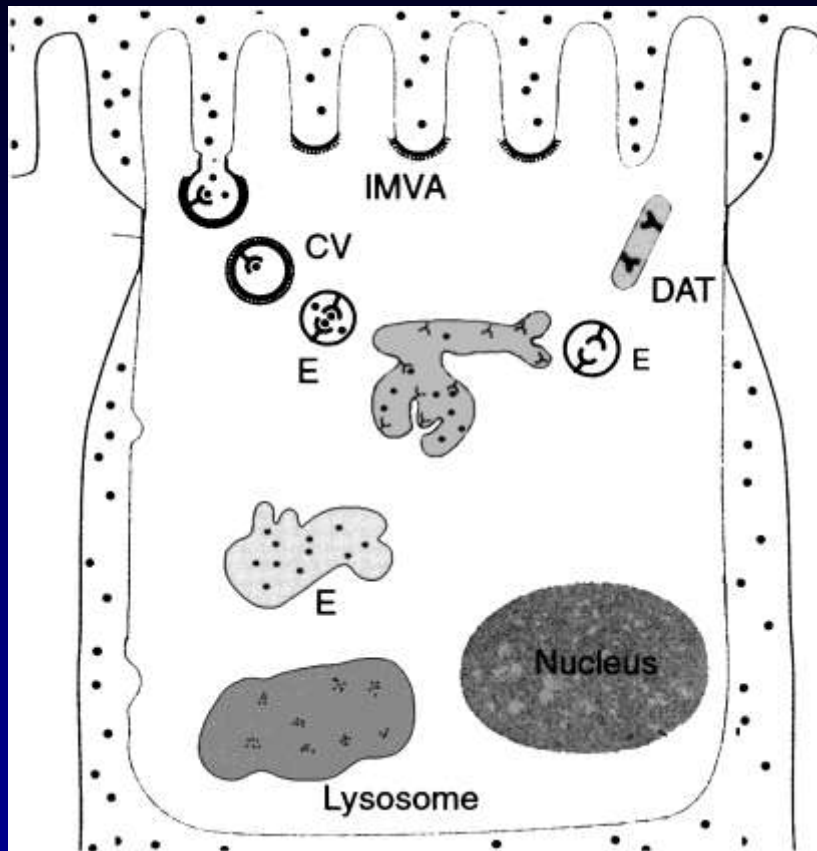
Inhibition of receptor-mediated endocytosis of ^{111}In -DOTA-tate by maleate and its combination with lysine



Maleate inhibits a variety of renal transport systems by inhibiting the citric acid cycle in tubular cells and reduces ATP supply

Schematic presentation of the two endocytic receptors, megalin and cubilin.





Schematic drawing illustrating the megalin/cubilin-mediated endocytic process in the renal proximal tubule.

Ligands are internalized through apical clathrin-coated pits in intermicrovillar areas into coated vesicles and subsequently to endosomes in which the ligands dissociate from the receptors. The ligands are transferred through endosomal compartments to lysosomes for degradation and further processing. The receptors are returned to the apical plasma membrane through dense apical tubules. While the proteins are degraded in lysosomes, vitamins and different trace elements are returned to the circulation by so far poorly defined pathways.

Pierre J. Verroust and Erik I. Christensen: *Nephrol Dial Transplant* (2002) 17: 1867-1871

Mechanisms of the peptide transport in renal tubular cells

Inui K.-I. et al.: Kidney Int. (2000) 58: 944:

„Filtered peptides are reabsorbed across brush-border membranes by at least two H^+ -coupled cotransporters PEPT1 and PEPT2. P-glycoprotein pumps various hydrophobic agents into the lumen.“

