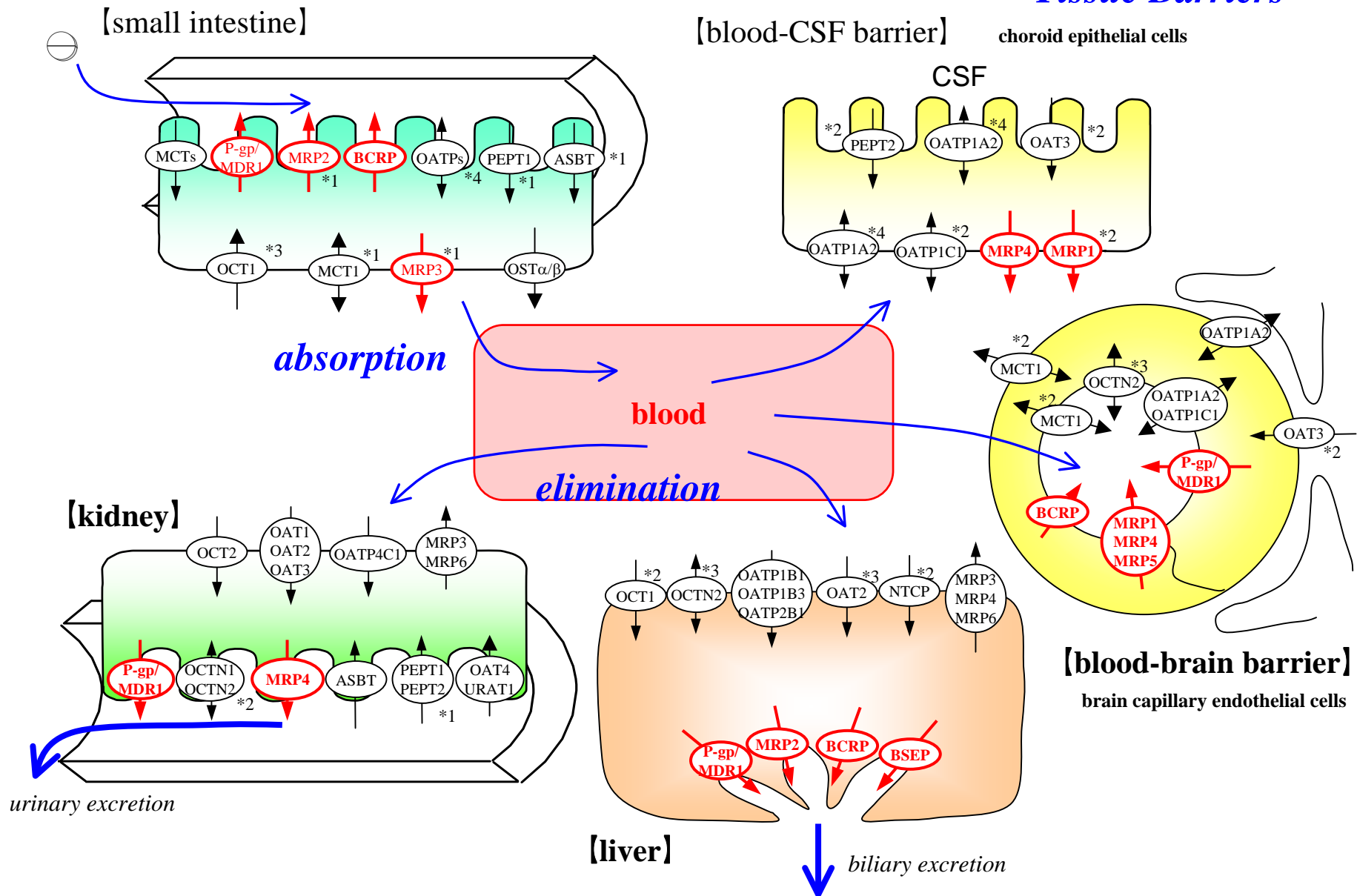


腎尿細管、血液脳関門において取り込みおよび排出に働くトランスポーターの分子実体と機能の比較論的解析

東京大学大学院薬学系研究科

楠原 洋之

Tissue Barriers



Transporters involved in the drug disposition

Organic anion transporter 3 (551 a.a., M.W. 60kDa)

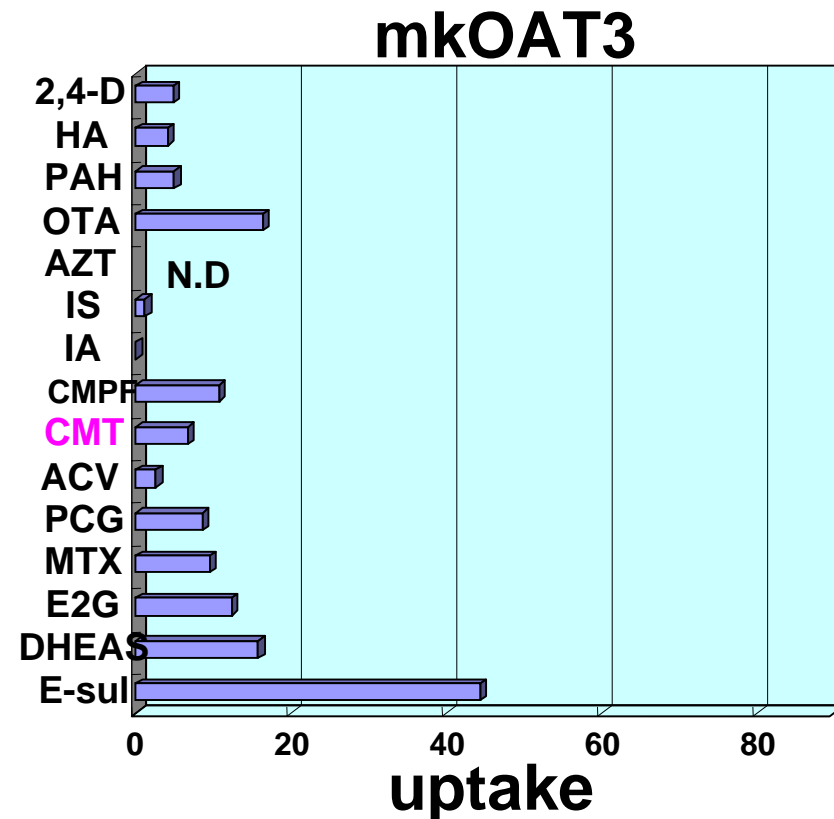
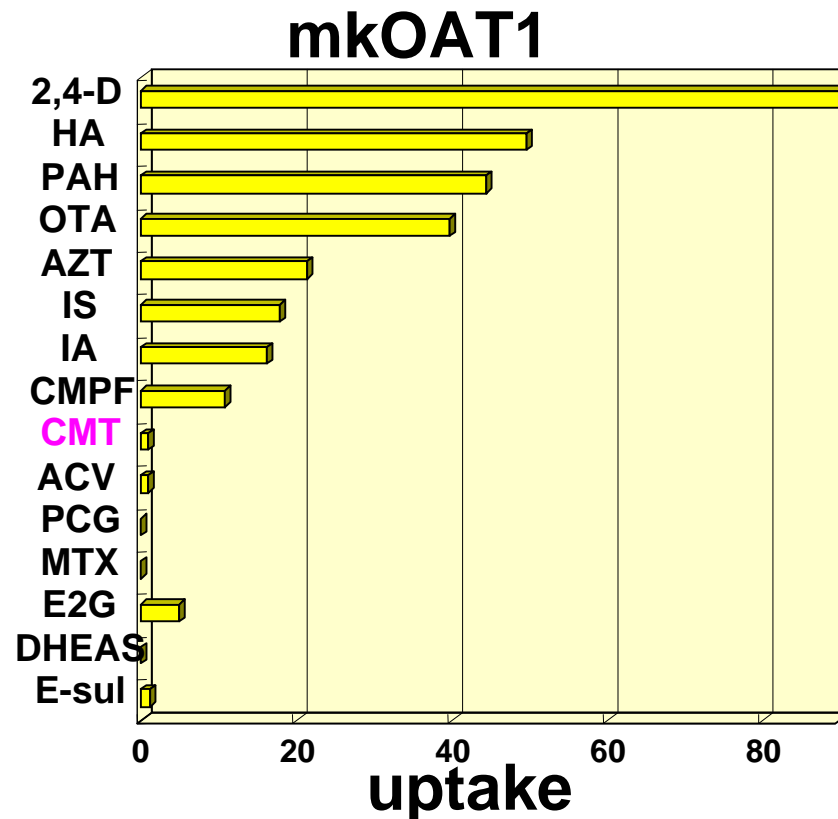
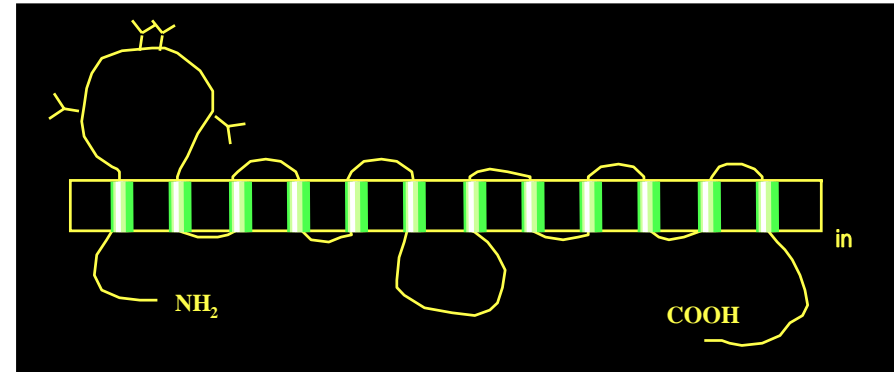
Tissue distribution:

kidney (basolateral membrane)

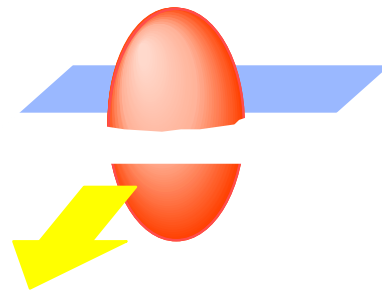
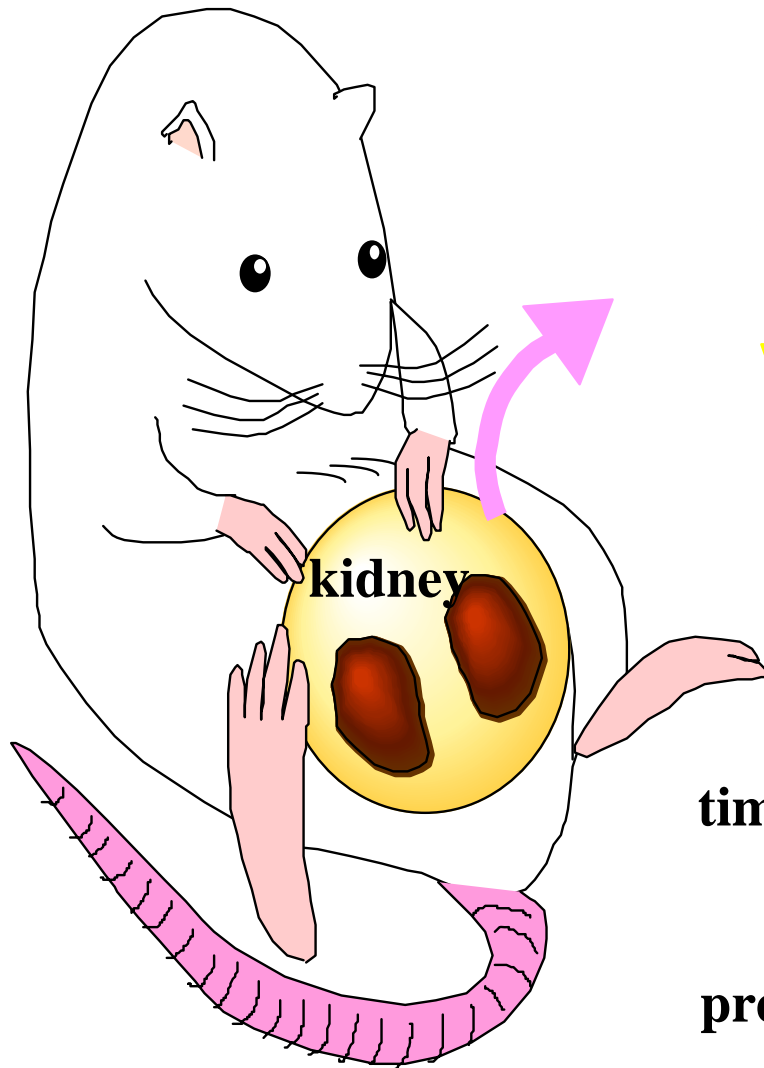
liver (sinusoidal membrane?)

brain capillary (abluminal membrane)

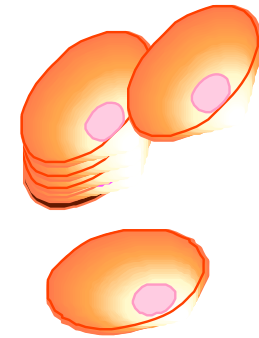
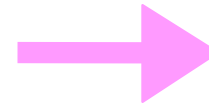
choroid plexus (brush border membrane)



Kidney slice

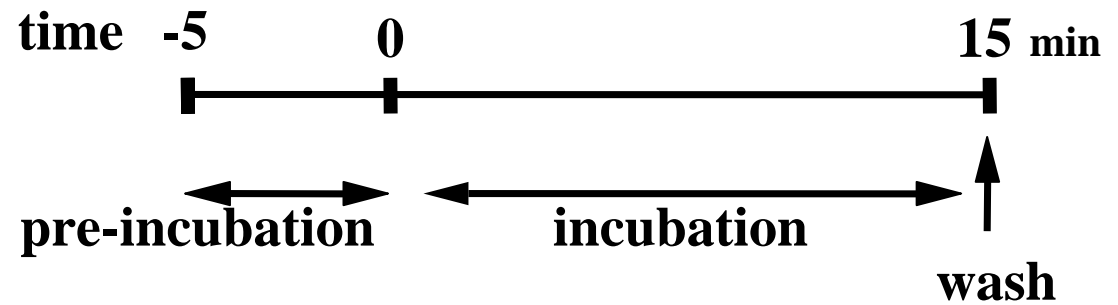


cutting

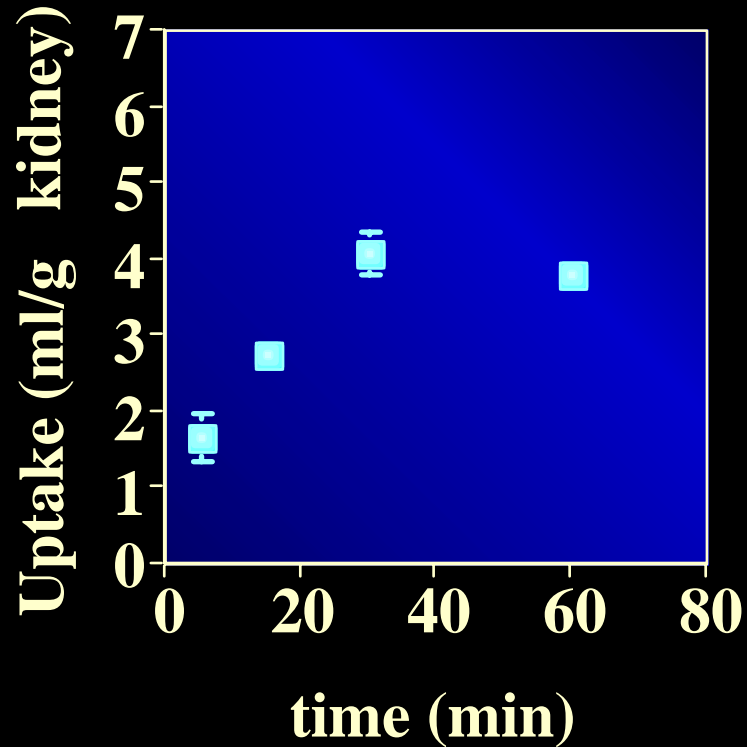


kidney slices
(300 μ m)

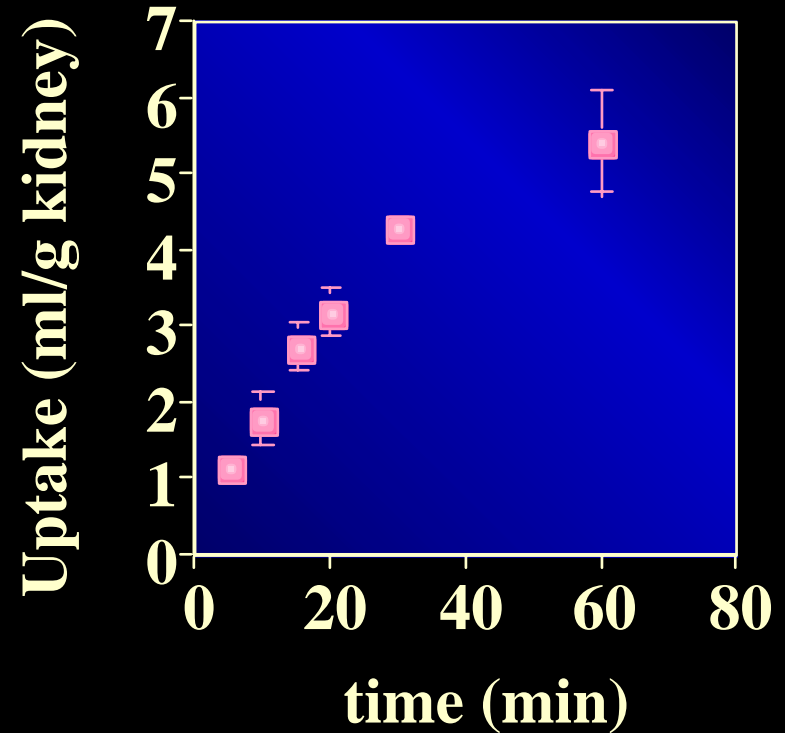
time table



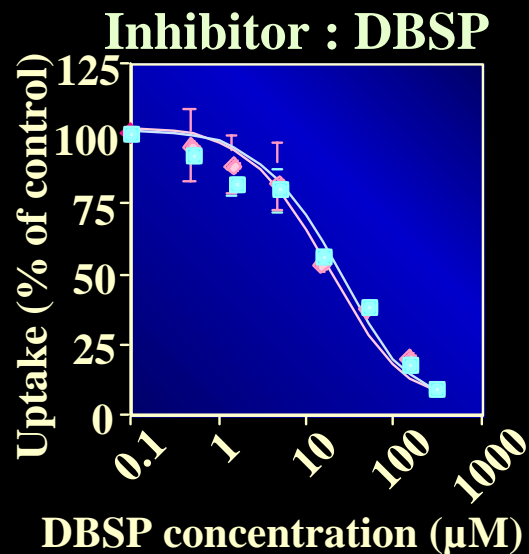
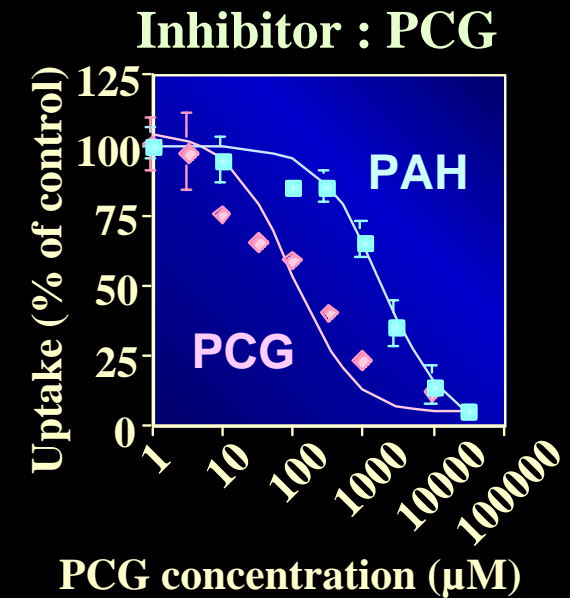
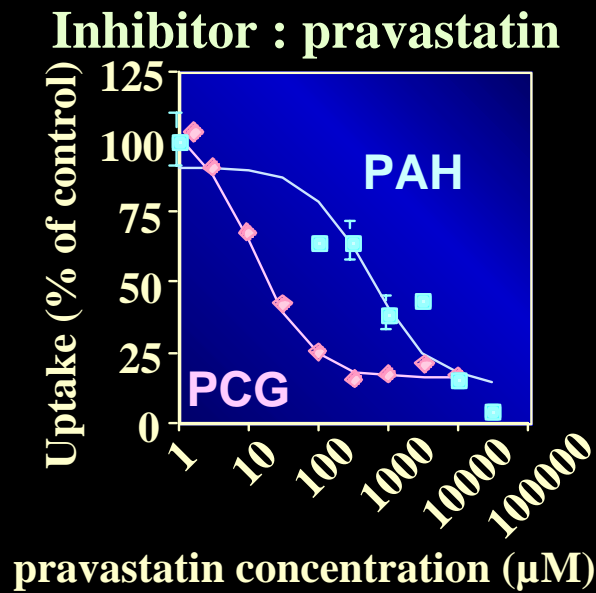
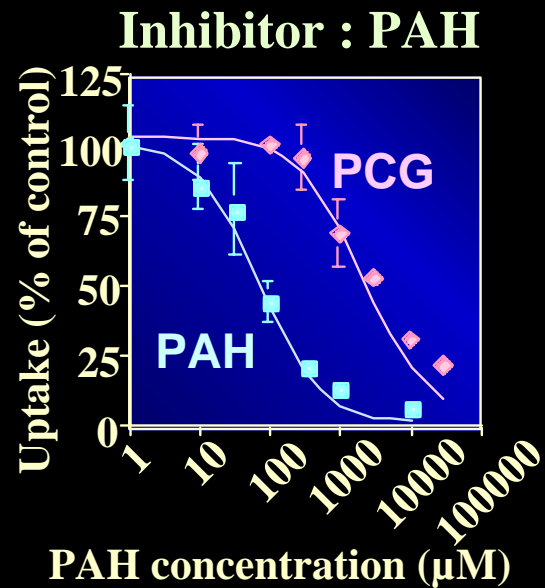
PAH uptake



Pravastatin uptake



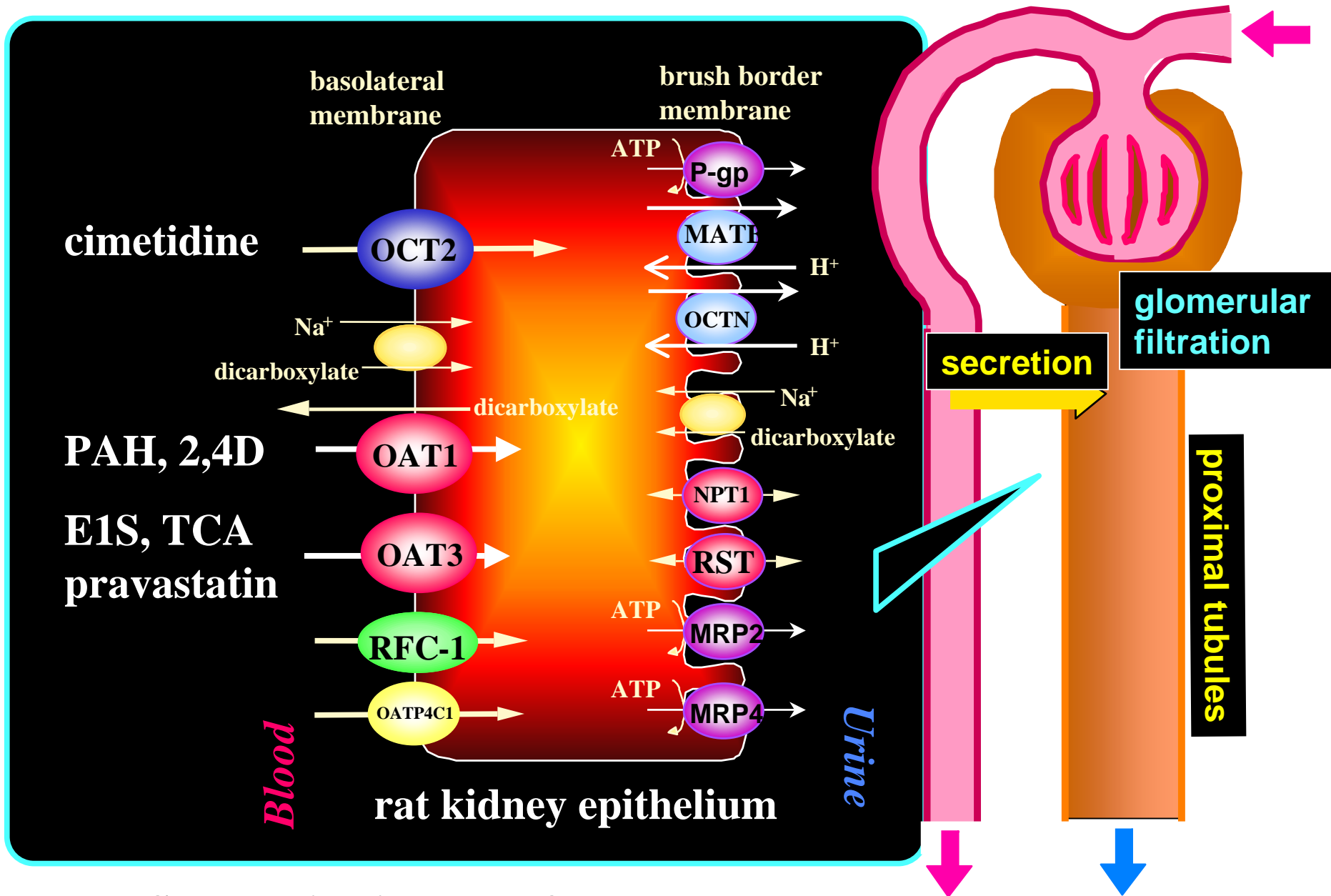
Time Profile of PAH and pravastatin uptake by kidney slices



K_m and K_i values of PAH and pravastatin uptake by kidney slices and gene expression systems.

	PAH uptake		pravastatin uptake	
	Oat1	Kidney slice	Oat3	Kidney slice
PAH	$59.9 \pm 5.0 \mu\text{M}$	$69.0 \pm 8.6 \mu\text{M}$	$1.35 \pm 0.51 \text{mM}$	$2.09 \pm 0.43 \text{mM}$
pravastatin	$1.15 \pm 0.46 \text{mM}$	$534.4 \pm 399.1 \mu\text{M}$	$13.4 \pm 2.3 \mu\text{M}$	$11.4 \pm 3.1 \mu\text{M}$
PCG	$633.1 \pm 249.5 \mu\text{M}$	$1.93 \pm 0.33 \text{mM}$	$52.8 \pm 9.0 \mu\text{M}$	$94.0 \pm 44.2 \mu\text{M}$
DBSP	$2.74 \pm 0.26 \mu\text{M}$	$22.2 \pm 5.8 \mu\text{M}$	$2.15 \pm 1.13 \mu\text{M}$	$17.0 \pm 4.4 \mu\text{M}$

Inhibitory effect of four inhibitors on PAH and pravastatin uptake by kidney slices

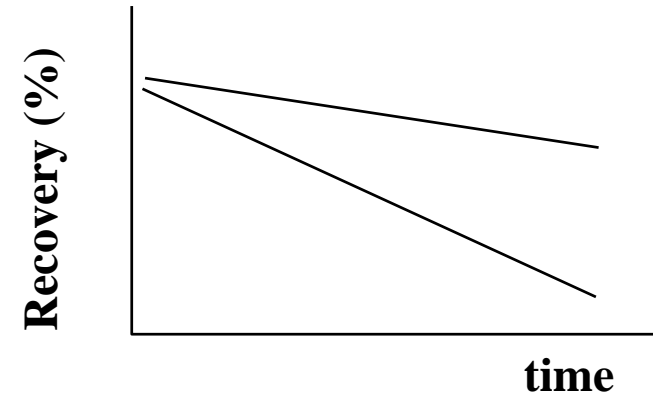
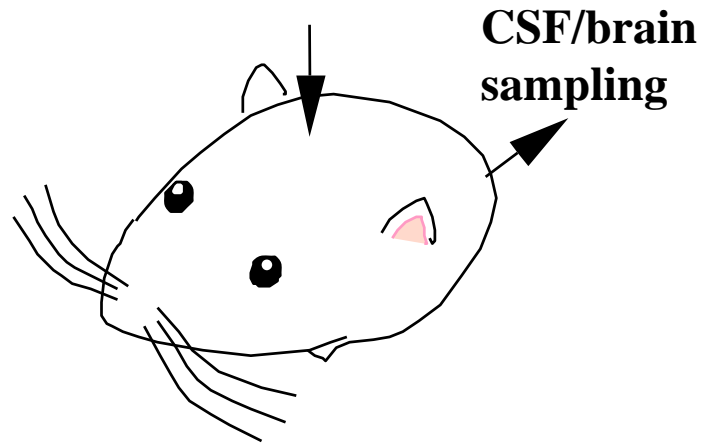


Schematic diagram of renal drug transport systems

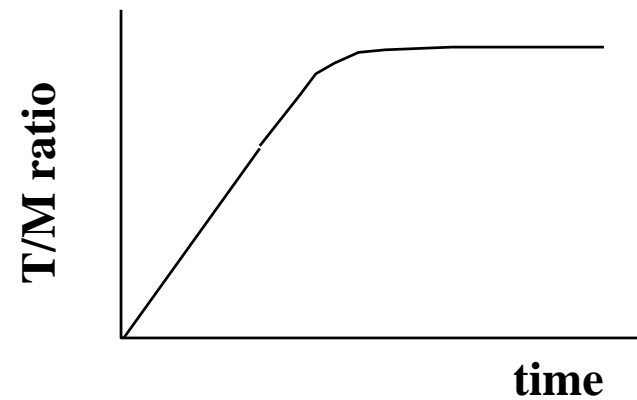
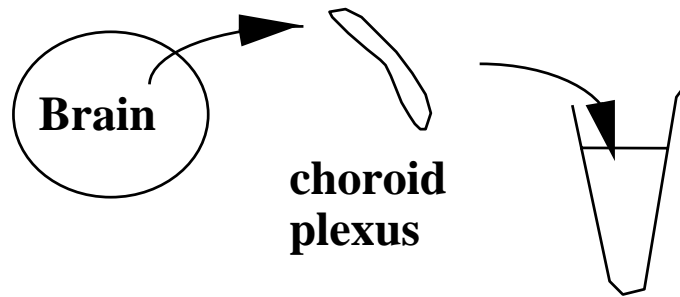
Hasegawa M, et al J Pharmacol Exp Ther, 305: 1087-97 (2003).

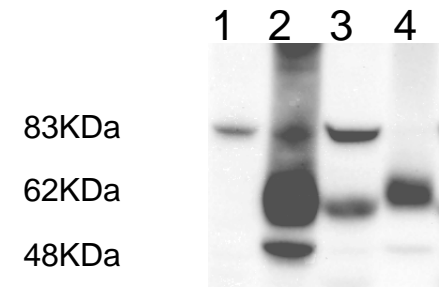
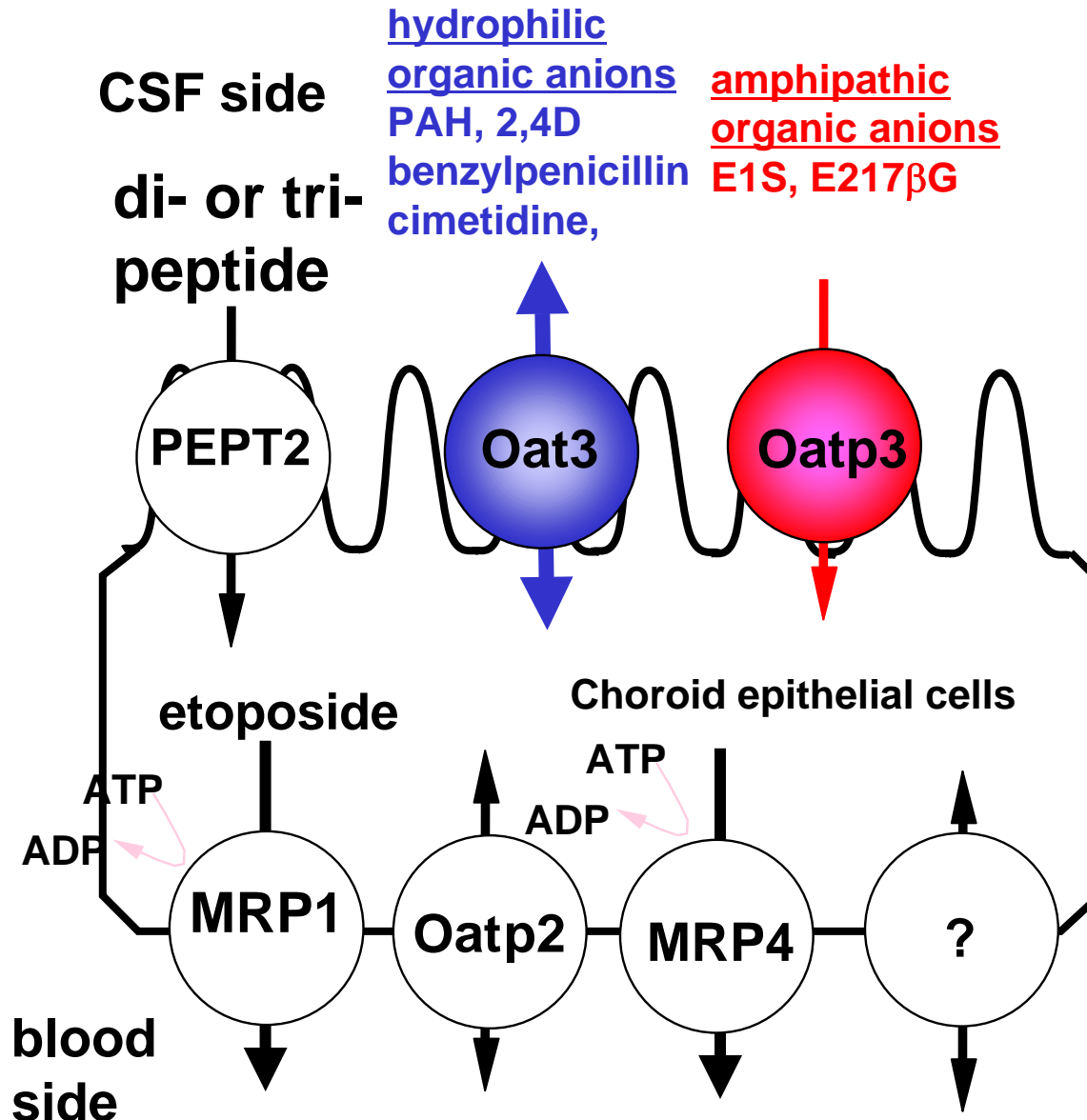
Deguchi T, et al Kidney Int, 65: 162-74 (2004).

In vivo:
intracerebroventricular administration
Brain Efflux Index method

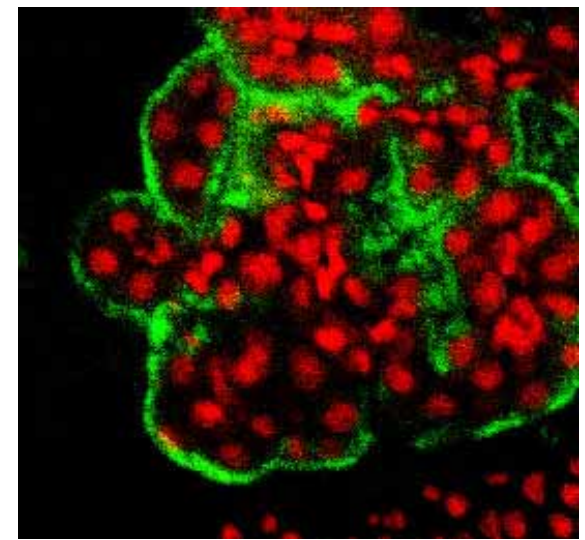


In vitro :
cDNA transfectants
isolated choroid plexus



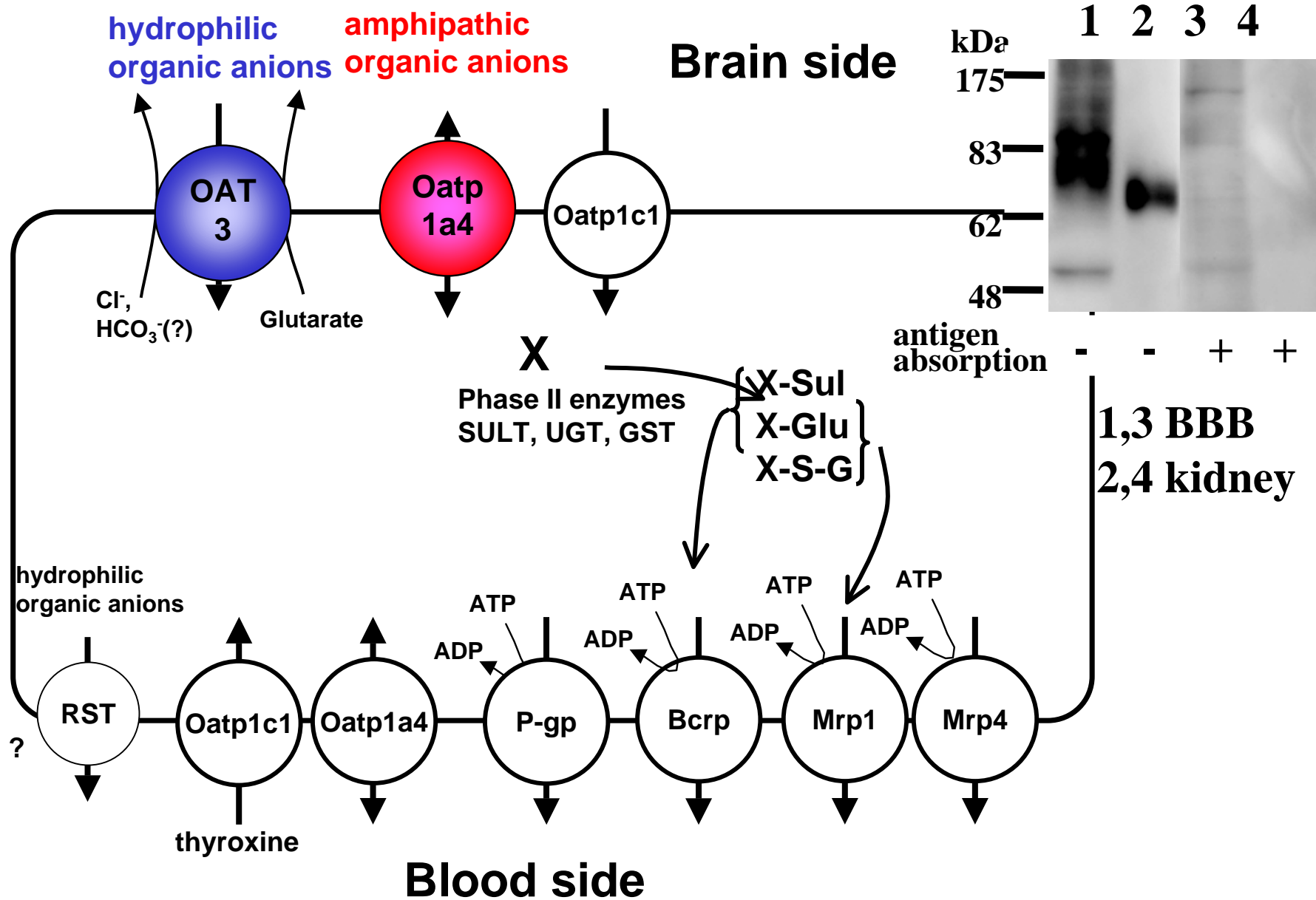


1:vector transfected cells
2:rOat3-expressed cells
3.choroid plexus
4:kidney



Drug Transport systems at the BCSFB

Nagata Y, et al Mol Pharmacol 61: 982-8 (2002).



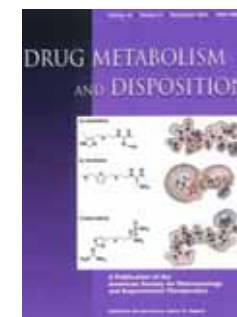
Efflux Transport Systems for Drugs at the BBB

Kikuchi R, et al J Pharmacol Exp Ther 306: 51-8 (2003).

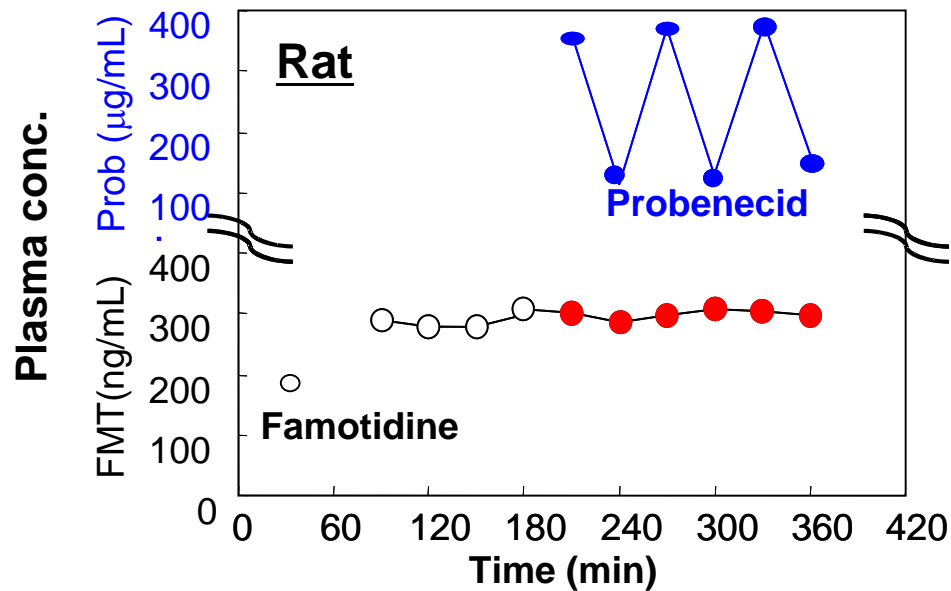
Table 1. Plasma total, plasma unbound, CSF and brain concentrations of H₂-receptor antagonists after intravenous infusion in rats treated with or without probenecid

	Cimetidine		Ranitidine		Famotidine	
	Control	+Probenecid	Control	+Probenecid	Control	+Probenecid
C _p (μM)	3.71±1.20	2.97±0.15	2.65±1.25	2.11±0.18	3.47±1.22	2.79±0.03
C _{p, fu} (μM)	2.25±0.60	1.89±0.11	1.72±0.49	1.52±0.06	2.01±0.72	1.54±0.18
C _{CSF} (nM)	39.1±19.8	116±17**	25.9±12.0	97.1±5.2***	23.1±9.2	43.9±4.1*
C _{brain} (nM)	96.5±27.1	99.9±12.5	85.7±33.8	83.9±10.9	106±30	102±18
C _{CSF} /C _{p, fu} ratio	0.0166±0.005	0.0615±0.0121**	0.0147±0.0046	0.0639±0.0046***	0.0114±0.0027	0.0289±0.0057**

Nagata Y, et al Drug Metab Dispos. 32:1040-7, 2004



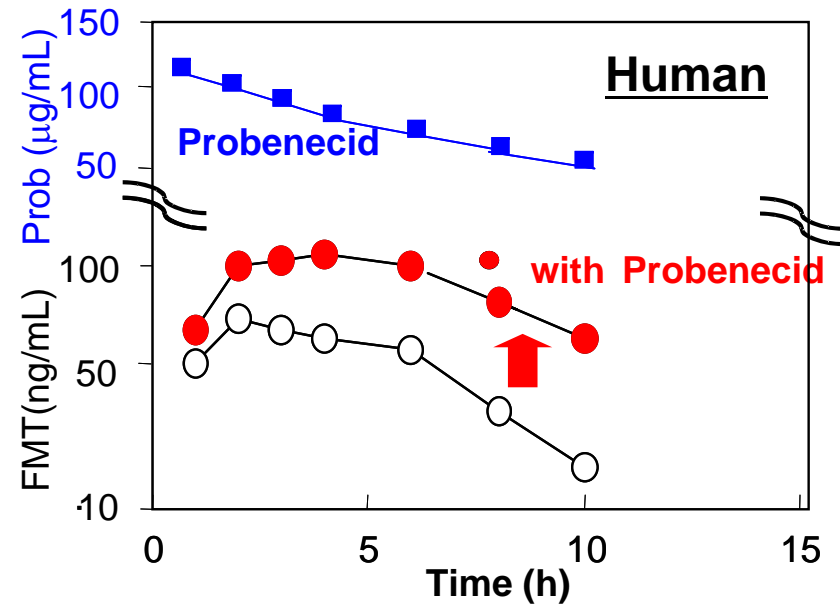
Species difference in drug-drug interaction between famotidine and probenecid



Infusion study in Rat

	CL renal (ml/min/kg)
Famotidine alone	42.3 ± 8.9
With probenecid	45.8 ± 10.3

DMD, Lin JH et al. (1988)
 Probenecid 40mg/kg(iv, every 40 min during infusion,
 L.K.Paalzow 1988)



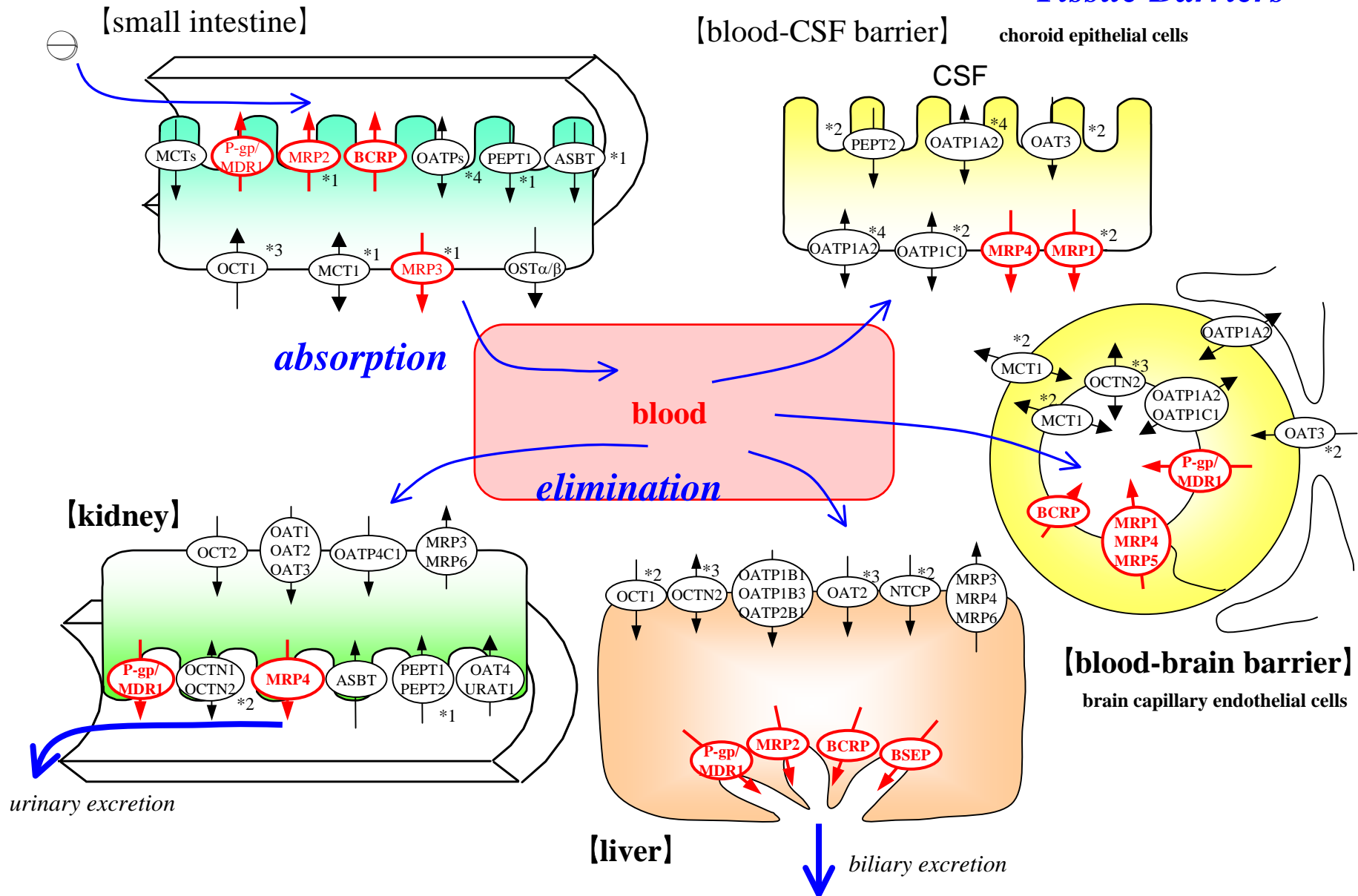
p.o. study in Human

	CL renal (ml/min)	CL secretion (ml/min)
Alone	297 ± 19	196 ± 21
With	107 ± 5	22.0 ± 4.2

J Clin Pharmacol 1990;30; 50-56
 Probenecid 1.5g (oral)

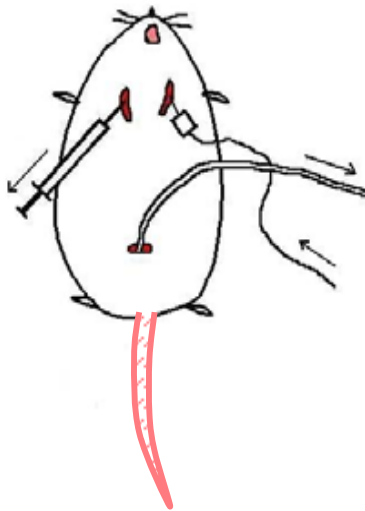
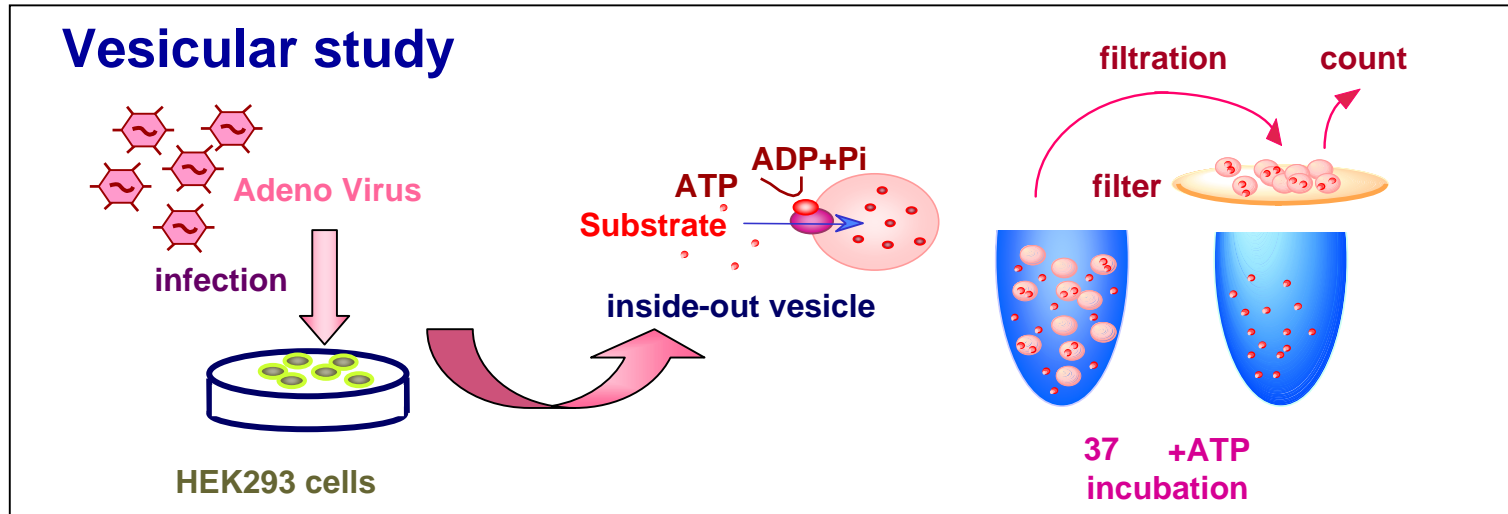
Probenecid had no effect on the renal clearance of cimetidine in humans and rats.

Tissue Barriers



Vecotrial Transports is involved in the drug disposition

Methods



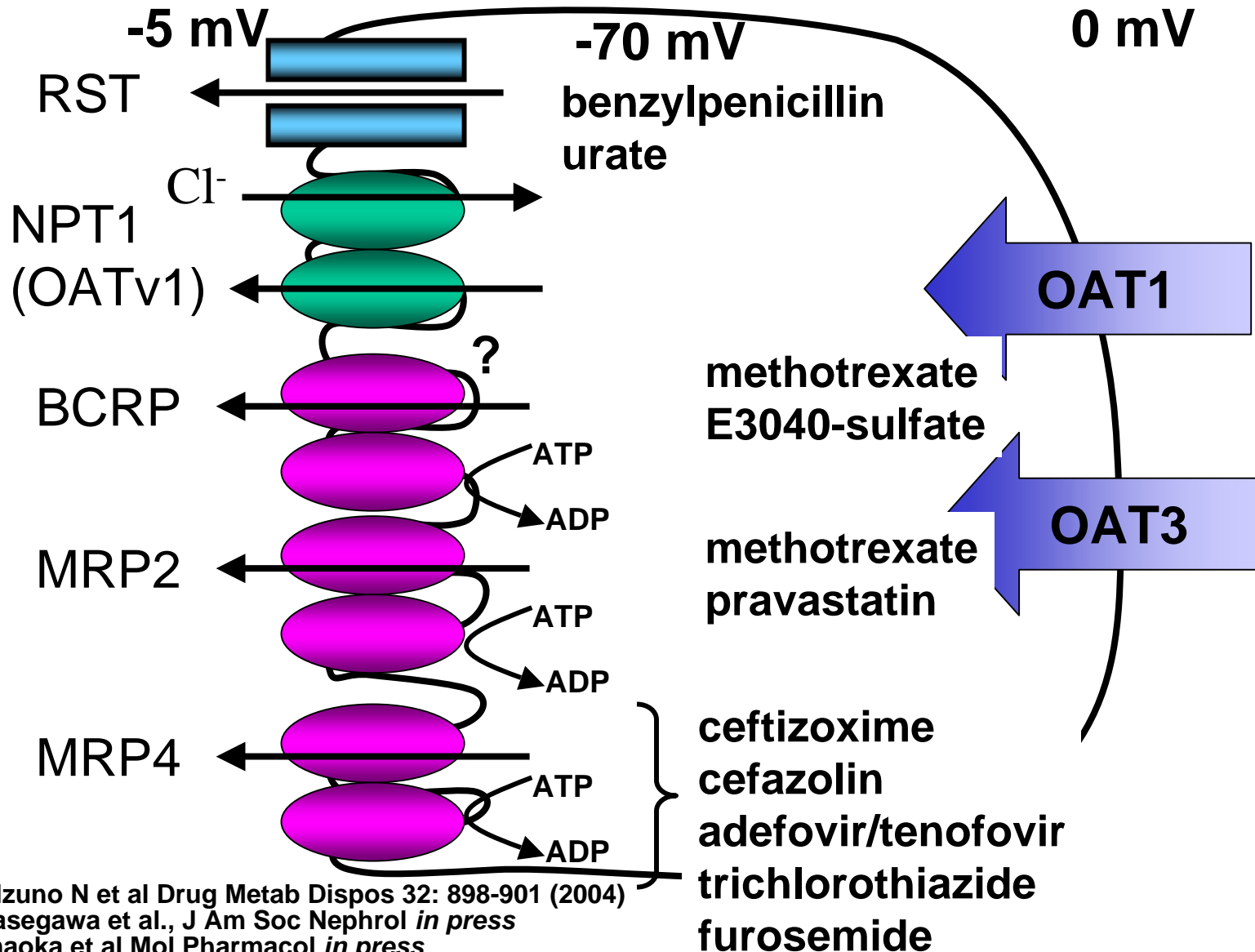
The jugular vein and bladder were cannulated. Under pentobarbital anesthesia, mice received a constant infusion.

At steady-state, plasma and kidney concentrations, and urinary excretion rate of adefovir, tenofovir and cidofovir were measured.

Efflux system of organic anions in the proximal tubules of the kidney

brush border membrane

basolateral membrane



Mizuno N et al Drug Metab Dispos 32: 898-901 (2004)
 Hasegawa et al., J Am Soc Nephrol *in press*
 Imaoka et al Mol Pharmacol *in press*

血漿中濃度の時間推移や腎クリアランスでは、刷子縁膜側の排出能力低下の検出感度が悪い



分子イメージング(PET・SPECT・MRI)を用いた分子イメージング技術で、組織中濃度を非侵襲的に測定することができる。トランスポーター選択的なプローブ薬の整備を進めていく必要がある。

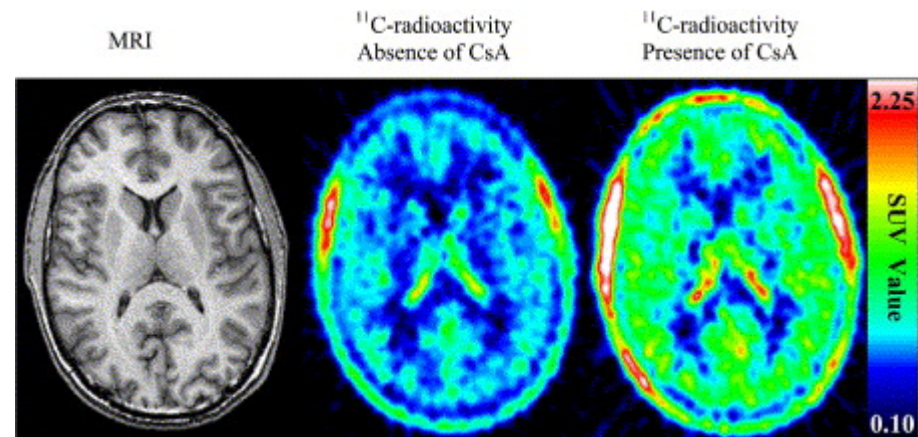
P-gpのイメージング(脳)に、 ^{11}C verapamilが使われている。

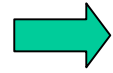
CysAとの薬物間相互作用:

Sasongko L, et al *Clin Pharmacol Ther.* 77:503-14, 2005.

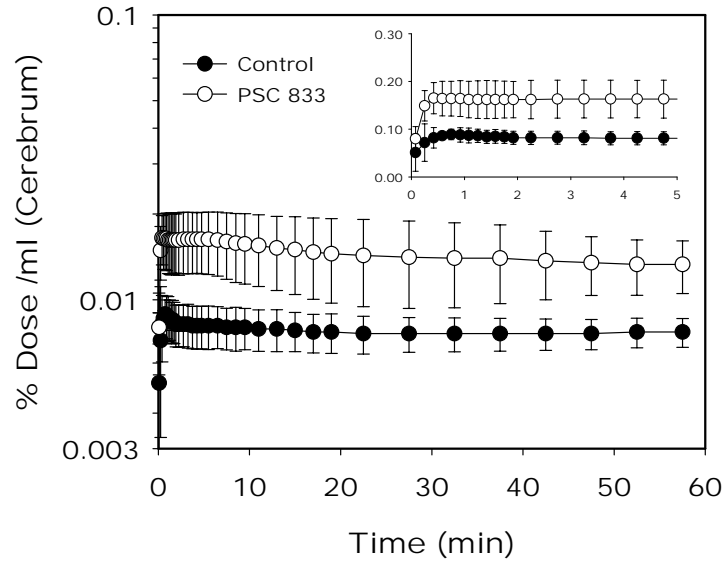
P-gpのSNP:

Brunner M et al *Clin Pharmacol Ther.* 78:182-90, 2005.

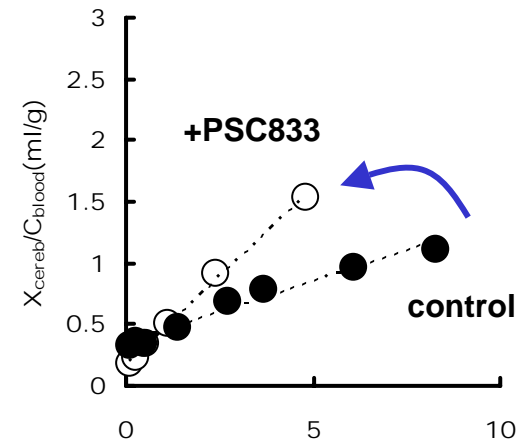
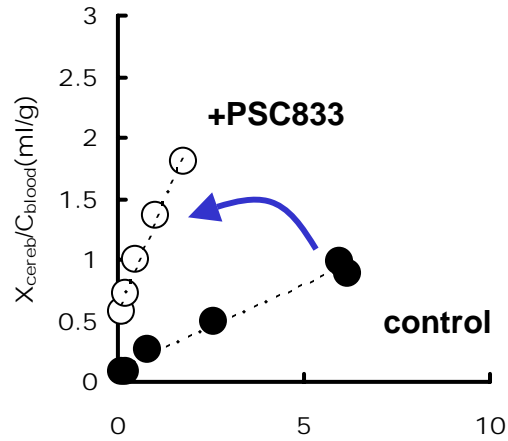
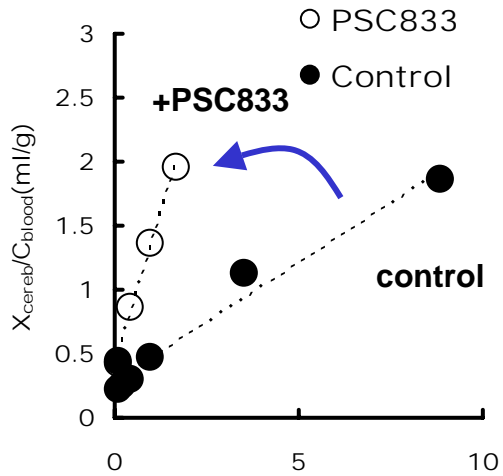
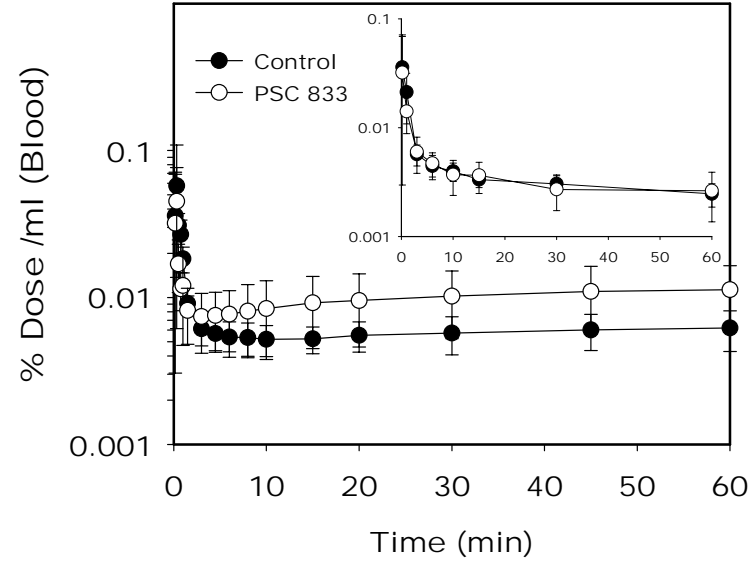




脳内濃度



血漿中濃度



PSC833が¹¹C verapamilの脳移行性に与える効果

もし、分子イメージングで、リアルタイムでの組織中濃度が決定できたら

腎クリアランス($CL_{\text{renal},p}$)

$$CL_{\text{renal},p} = A / AUC_p$$

腎分泌クリアランス($CL_{\text{sec},p}$)

$$CL_{\text{sec},p} = CL_{\text{renal},p} - f_{u,b} \text{GFR}$$

Integration plot

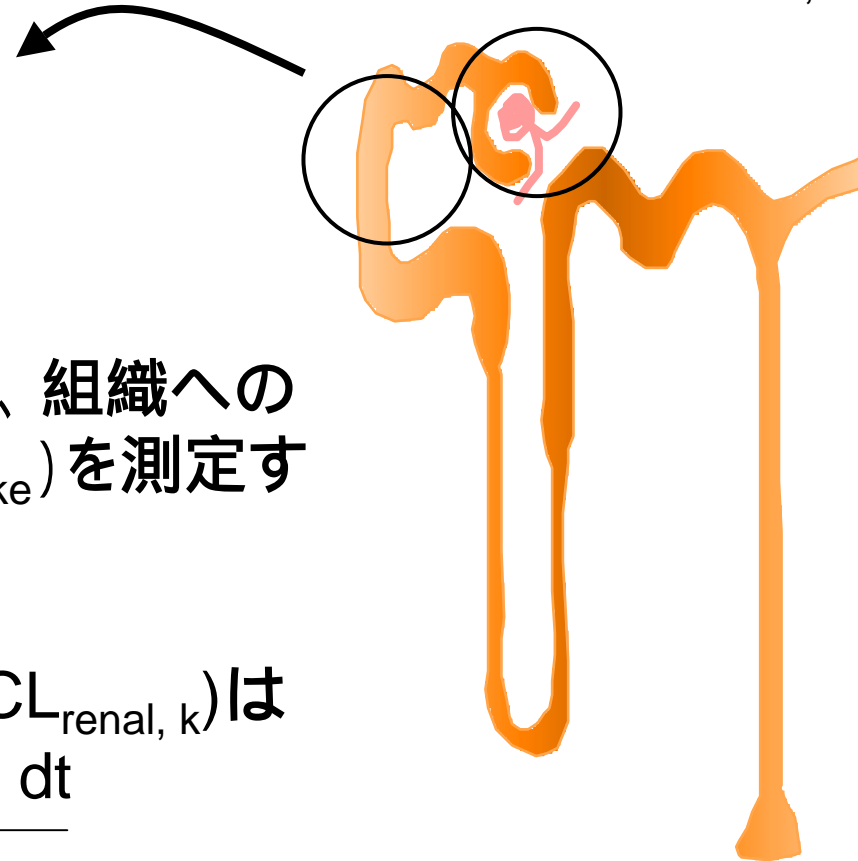
ある時間(t)の AUC_p と X_t から、組織への取り込みクリアランス(CL_{upake})を測定することができる。

刷子縁膜側のクリアランス($CL_{\text{renal},k}$)は

$$CL_{\text{renal},k} = \frac{A - \text{GFR} f_{u,b} C_p dt}{C_{\text{kidney}} dt}$$

SNPを持っている健常人、刷子縁膜側トランスポーター選択的な阻害剤の併用で、パラメーターの測定。

糸球体ろ過 ($f_{u,b} \text{GFR}$)



謝辞

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正野知行
岡本基継
榎園淳一



分子薬物動態学教室

2006.4.

ご静聴ありがとうございます。

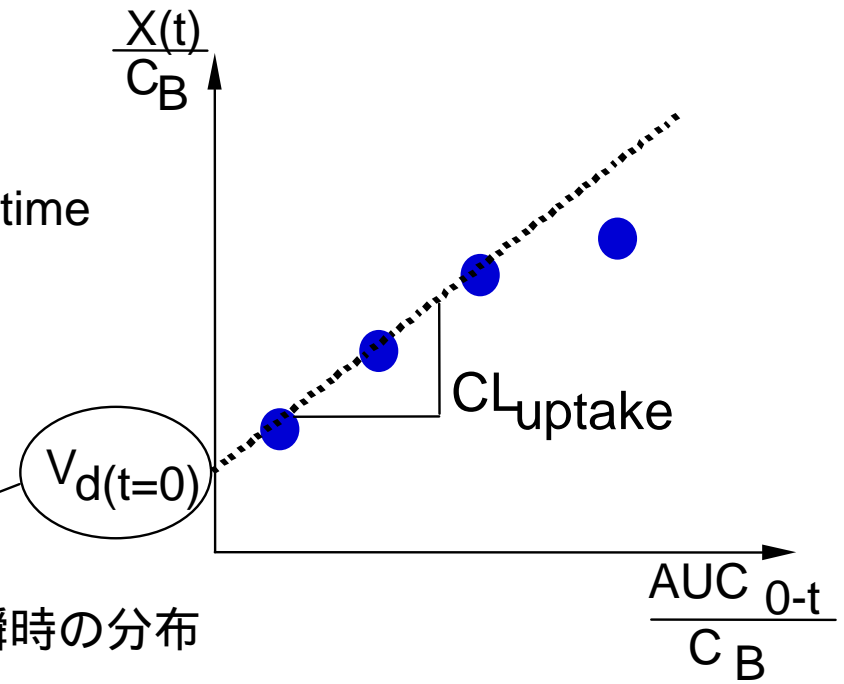
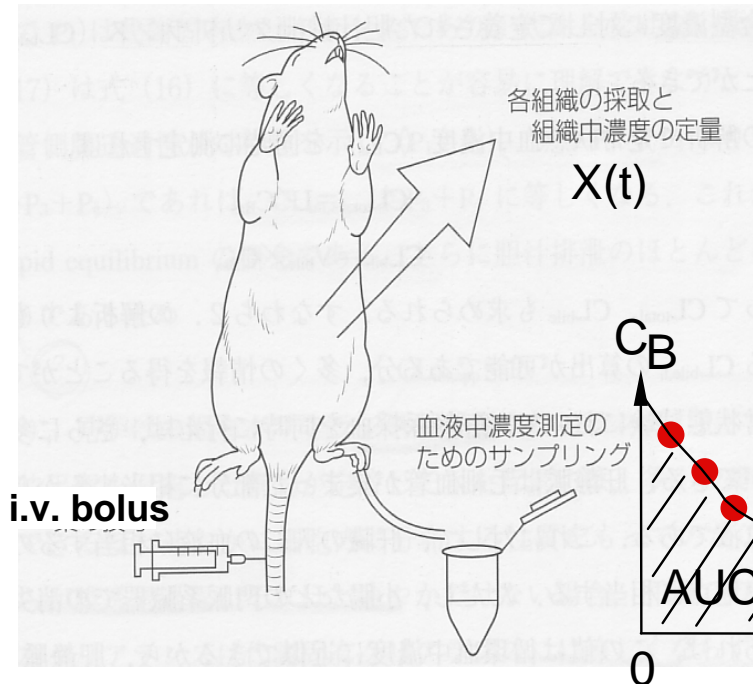
Integration plot法

肝臓からの排出が無視できる程の短時間においては、

$$\frac{dX_{\text{tissue}}}{dt} = CL_{\text{uptake}} \cdot C_B$$

これを両辺積分して、 $C_B(t)$ で除すると、

$$\frac{X_{\text{tissue}}(t)}{C_B(t)} = CL_{\text{uptake}} \cdot \frac{AUC_{0-t}}{C_B(t)} + \frac{X(0)}{C_B(t)}$$



- 1) 組織毛細血管、細胞外液スペースへの瞬時の分布
- 2) 組織表面への吸着

