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Pharmaceutical Sciences,**

Established 1907

*We celebrate
100th Years
Anniversary !!*



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Graduate Student	200
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謝 辞

金沢大学教授
辻 彰先生



謝 辞



金沢大学
名誉教授
山名月中先生

格に入りて格を出でざる時は狭く、また格入らざるときは邪路にはしる。

格に入り、格を出でて、初めて自在を得べし。

松尾芭蕉

日本薬物動態学会
第22年会
日本薬物動態学会賞
受賞講演

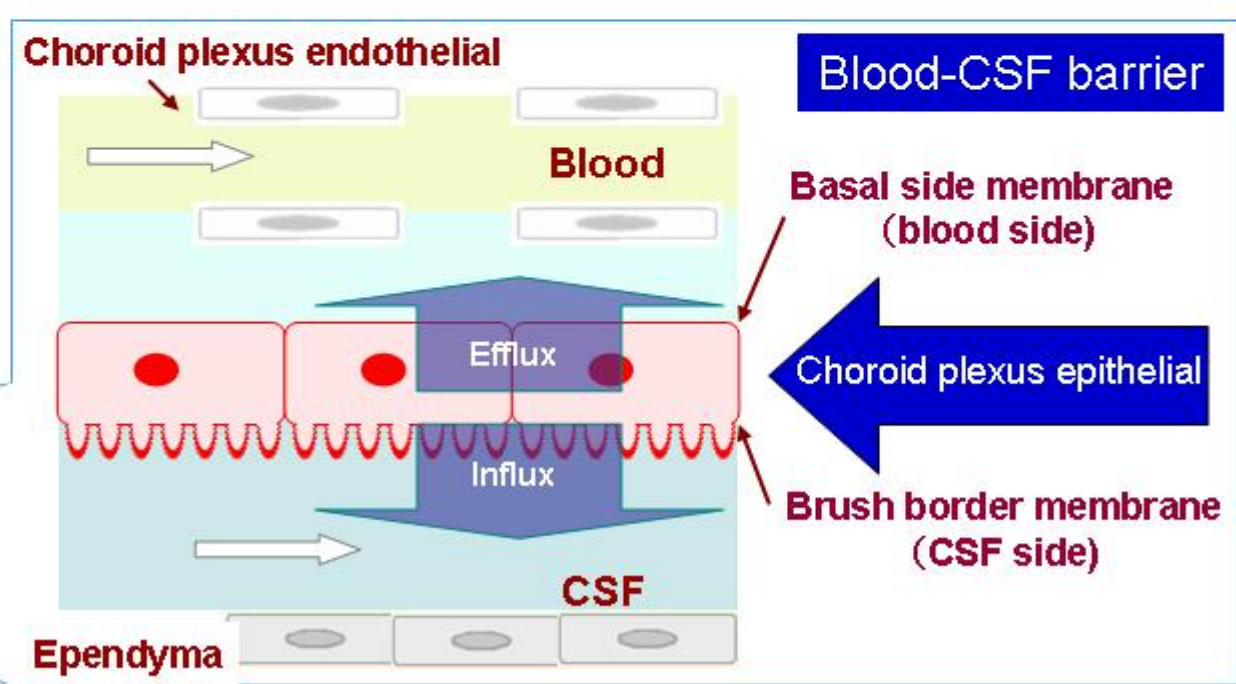
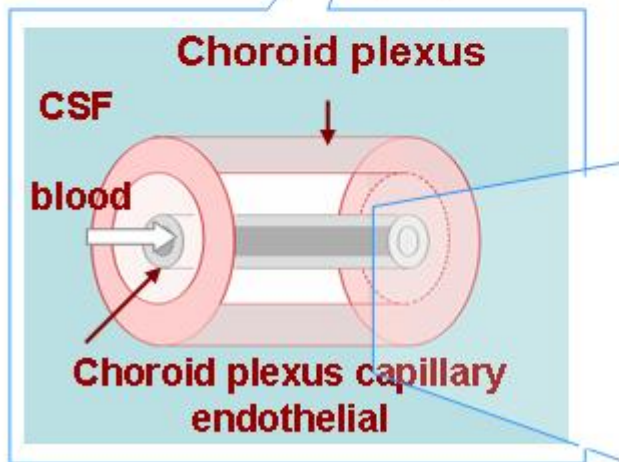
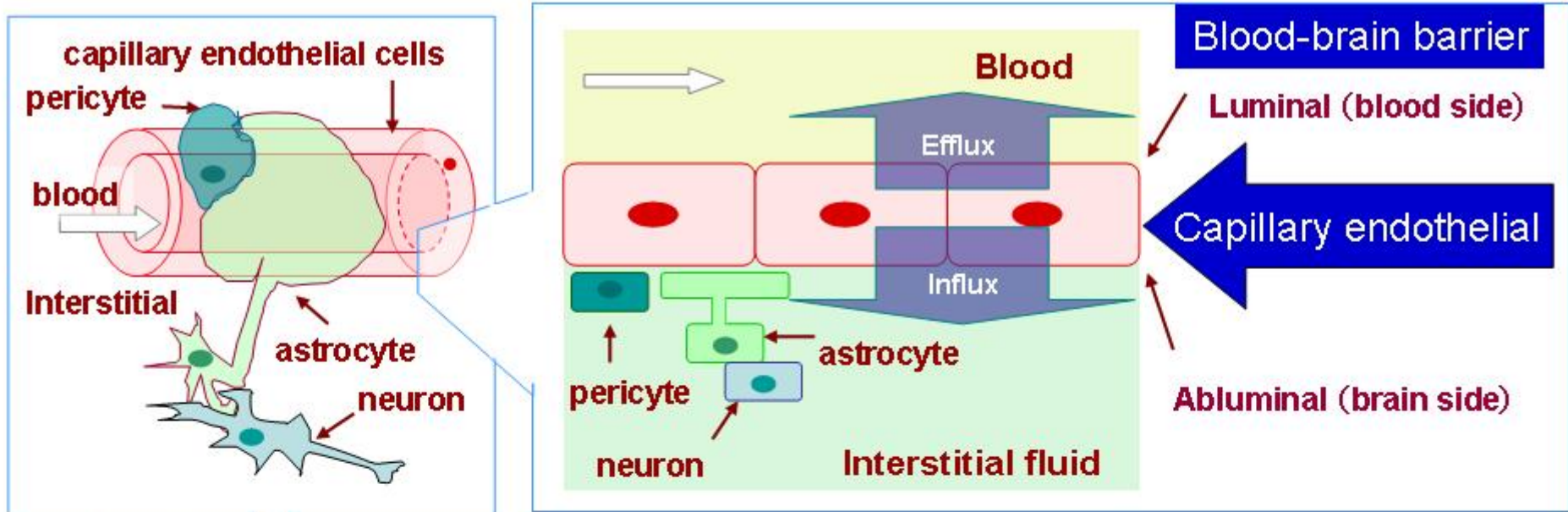


14:45–15:10,
Oct. 8, 2007, 25 min
大ホール
仙台国際センター

脳関門輸送研究の新技術開発 と脳支援防御機構解明

寺崎哲也

東北大学大学院薬学研究科、
SORST, 科学技術振興機構



Structure of the Blood-Brain Barrier and Blood-CSF Barrier



Paul Ehrlich

Web siteから写真引用



Edwin Goldmann

Web siteから写真引用



William H. Oldendorf

The blood-brain barrier cellular and molecular biology, WMPardridge, Raven Press, 1993から写真引用

**PET, CT-scan,
Brain Uptake Index**



Ronald T. Borchardt

Primary culture



Quentin R. Smith

Brain perfusion



Pierre O. Couraud

Human immortalized cell

血液脳関門を提唱

始まりは自分の過去の論文の否定から

Distributed Model Analysis of 3'-Azido-3'-Deoxythymidine and 2',3'-Dideoxyinosine Distribution in Brain Tissue and Cerebrospinal Fluid¹

KATSUKO TAKASAWA, TETSUYA TERASAKI², HIROSHI SUZUKI, TSUYOSHI OOIE and YUICHI SUGIYAMA
 Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113,

AZTの脳内濃度が低い原因は、脳から血液方向のBBB排出速度が大きいいため

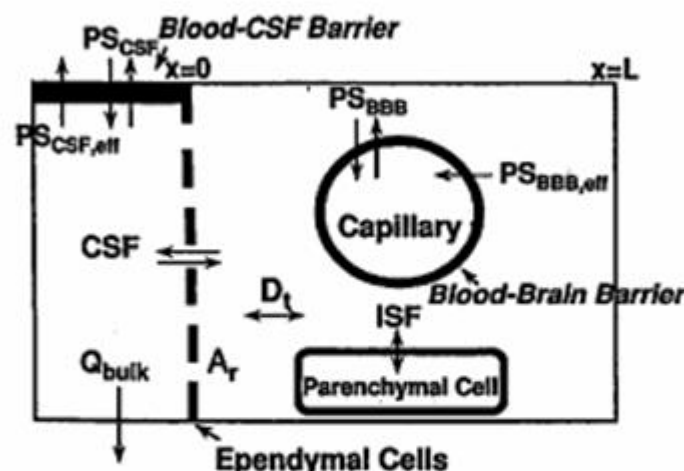
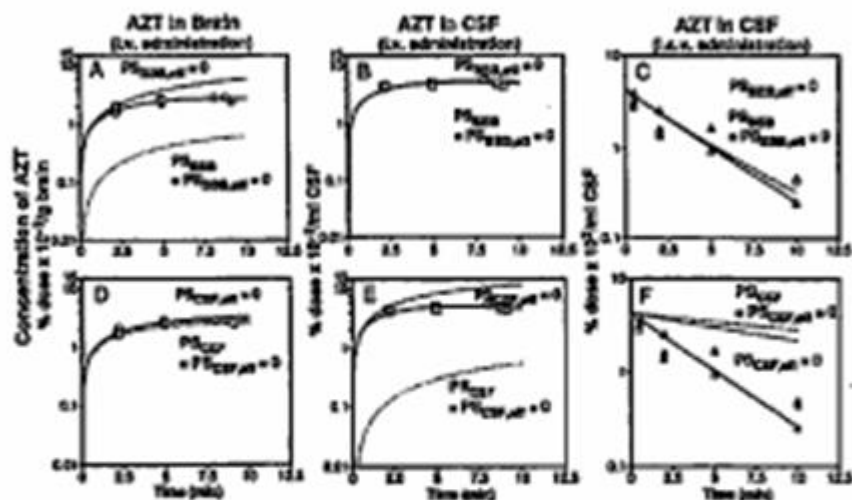


Fig. 1. Distributed model for pharmacokinetic analysis of AZT and DDI distribution in brain and CSF.

TABLE 1
 Kinetic parameters for the model analysis of AZT and DDI distribution in brain and CSF

Parameter	Unit ^a	AZT		DDI			
		Fixed	Variable		Fixed	Variable	
			Initial	Fitted		Initial	Fitted
PS _{BBB}	μl/min/g brain		3.43 ^b	10.3 ± 2.1		0.724 ^b	2.03 ± 0.42
PS _{BBB,eff}	μl/min/g brain		30.5 ^c	180 ± 77		18.0 ^c	79.3 ± 38.5
PS _{CSF}	μl/min/ml CSF		1.03 ^b	6.43 ± 0.58		0.363 ^b	1.34 ± 0.15
PS _{CSF,eff}	μl/min/ml CSF		68.2 ^d	51.5 ± 3.6		53.9 ^d	44.6 ± 3.7
V _{tr}	ml/g brain	1.07			0.727		
D _w	cm ² /min	4.60 × 10 ⁻⁴			4.99 × 10 ⁻⁴		
D _s	cm ² /min	5.38 × 10 ⁻⁵			6.63 × 10 ⁻⁵		

^a PS_{CSF} (μl/min/ml CSF) was calculated assuming that the volume of CSF equals 250 μl/rat.

^b Estimated by the integration plot described in the text.

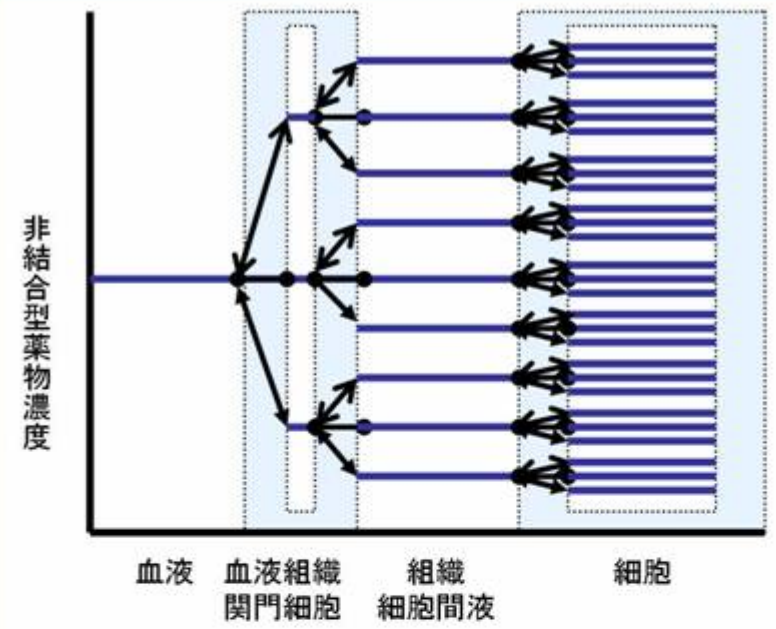
^c The efflux rate constant obtained from a previous report (Takasawa et al., 1997) was multiplied by the volume of distribution in brain to calculate the PS product.

^d PS_{CSF,eff} was estimated from the efflux clearance from the CSF (CL_{CSF}) by subtracting the CSF bulk flow rate and the component of drug diffusion through parenchymal tissue and PS_{CSF} using equation 18.

J. Pharmacol. Exp. Ther.,
 282: 1509-1517 (1997)

新規実験法開発の必要性を痛感して

中枢効果を左右する脳細胞間液中濃度は、Influx rateとefflux rateの比で決まる。排出速度が大きいと中枢効果が低い可能性が大きい。

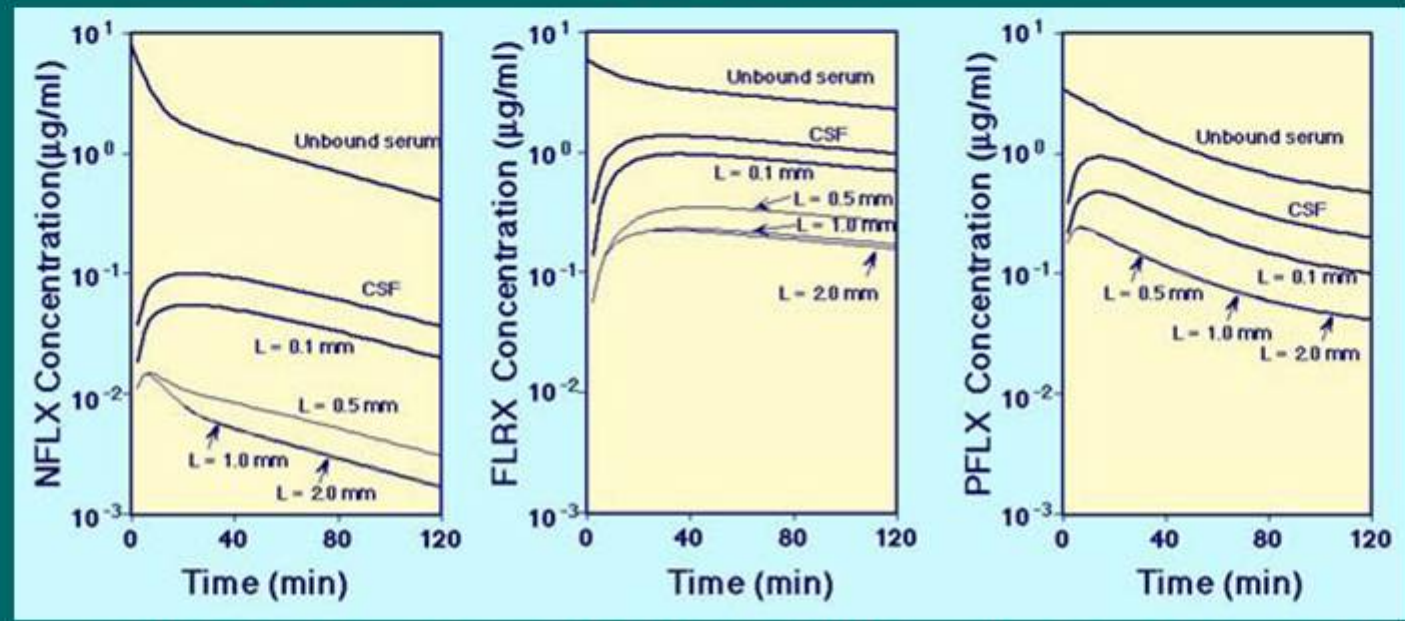


$$\frac{C_{\text{brain,interstitial}}}{C_{\text{blood,unbound}}} = \frac{PS_{\text{BBB,influx}}}{PS_{\text{BBB,efflux}}}$$

ノルフロキサシン

フレロキサシン

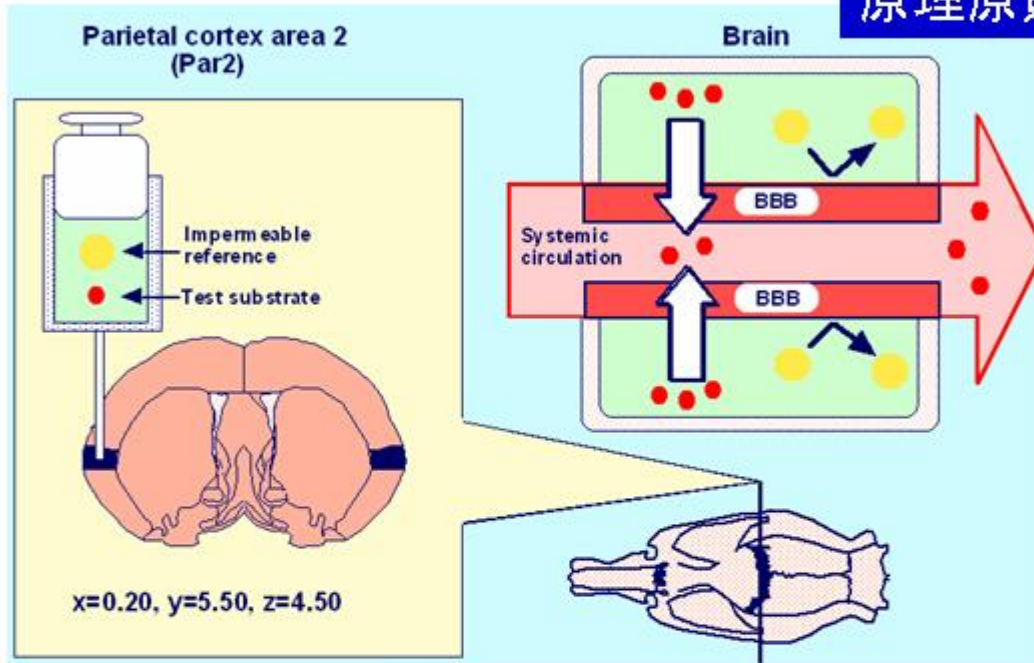
ペフロキサシン



J. Pharmacol. Exp. Ther., 283: 293-304 (1997) *Drug Metab. Dispos.*, 25: 784-789 (1997)

脳から血液方向のBBB輸送機構を解析する Brain Efflux Index (BEI) methodの開発

原理原則に戻って考えることの大切さを知って

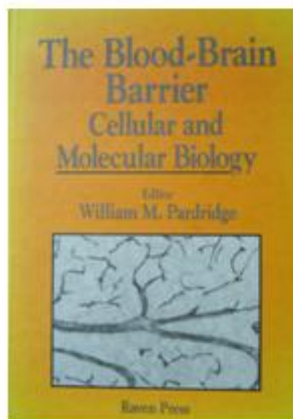


Brain Efflux Index (BEI)

Amount of drug
effluxed from the brain

$$= \frac{\text{Amount of drug effluxed from the brain}}{\text{Amount of drug injected in the brain}} \times 100$$

Amount of drug
injected in the brain



BBB efflux rate constant (k_e)

= Slope of $\log(100 - \text{BEI})$ versus time plot

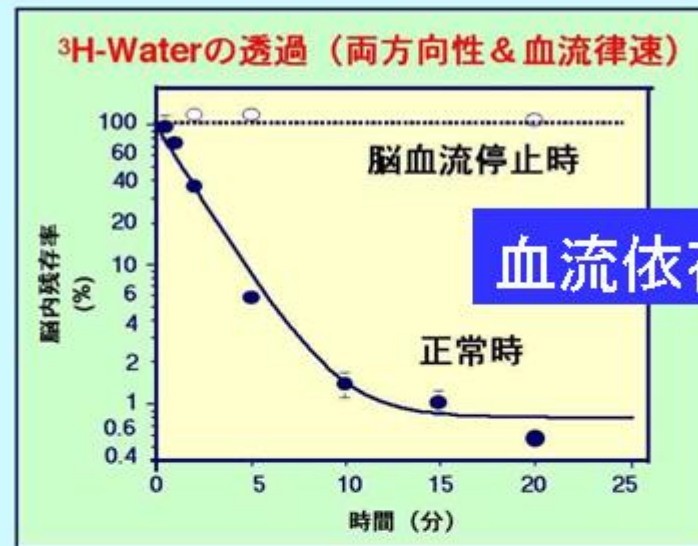
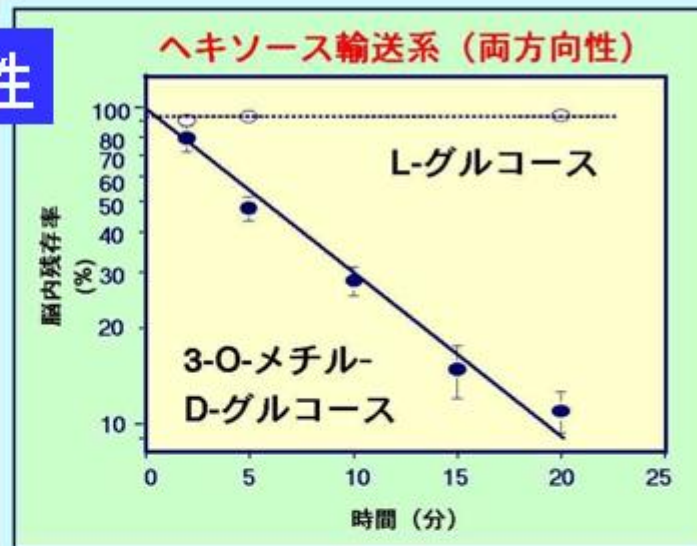
Brain-to-blood efflux permeability clearance (CL_{BBB})

= $k_e \times V_{\text{br}}$ (distribution volume determined by steady state brain slice uptake)

手法の完成にはcriticalに、そして美しく

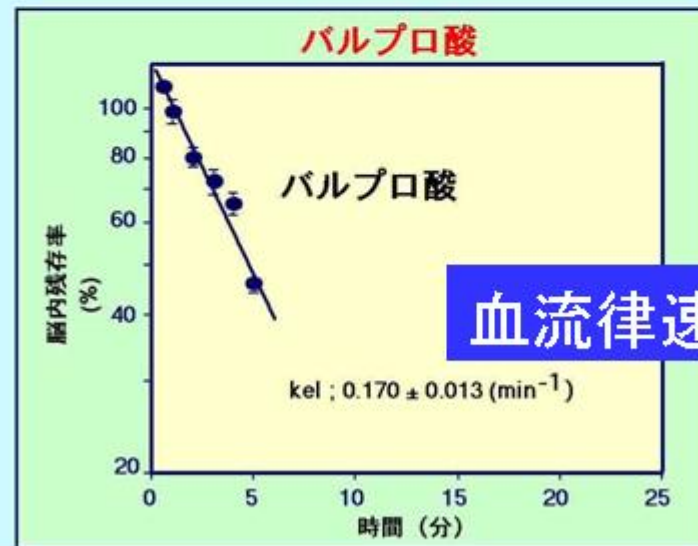
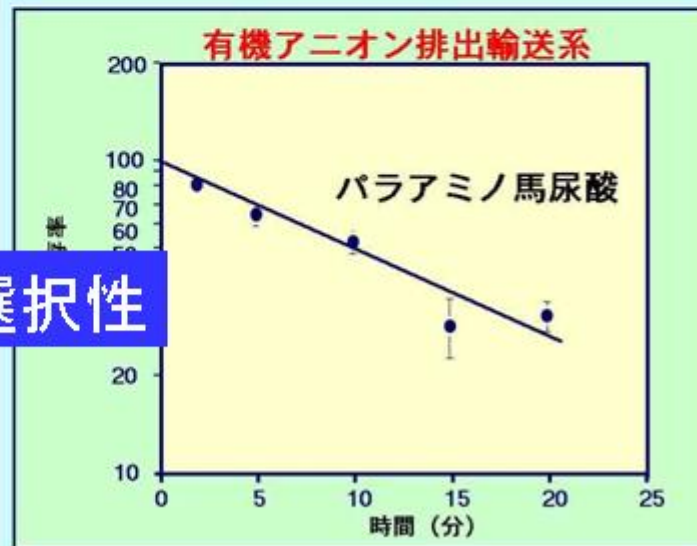
Brain Efflux Index法のvalidation

立体選択性



血流依存性

排出方向選択性

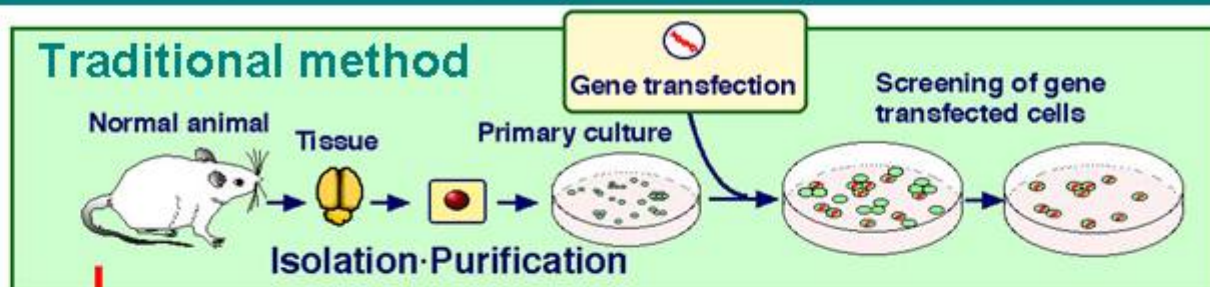


血流律速型

Microv. Res., 63: 233-238 (2002). *J. Pharmacol. Exp. Ther.*, 277: 1550-1559 (1996)
J. Pharmacol. Exp. Ther., 283: 1018-1025 (1997)

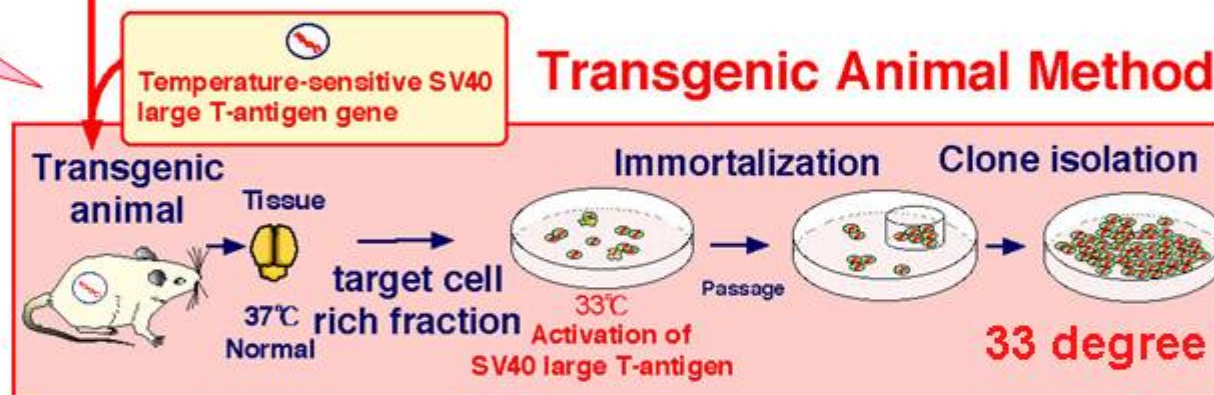
異分野との出会いを大切に、新しい領域開拓の始まり

Conditionally immortalized brain capillary endothelial cell line



tsA58 SV40 large T antigen is **inactive at 37 degree.**

Phenotype of the transgenic rat was **normal.**



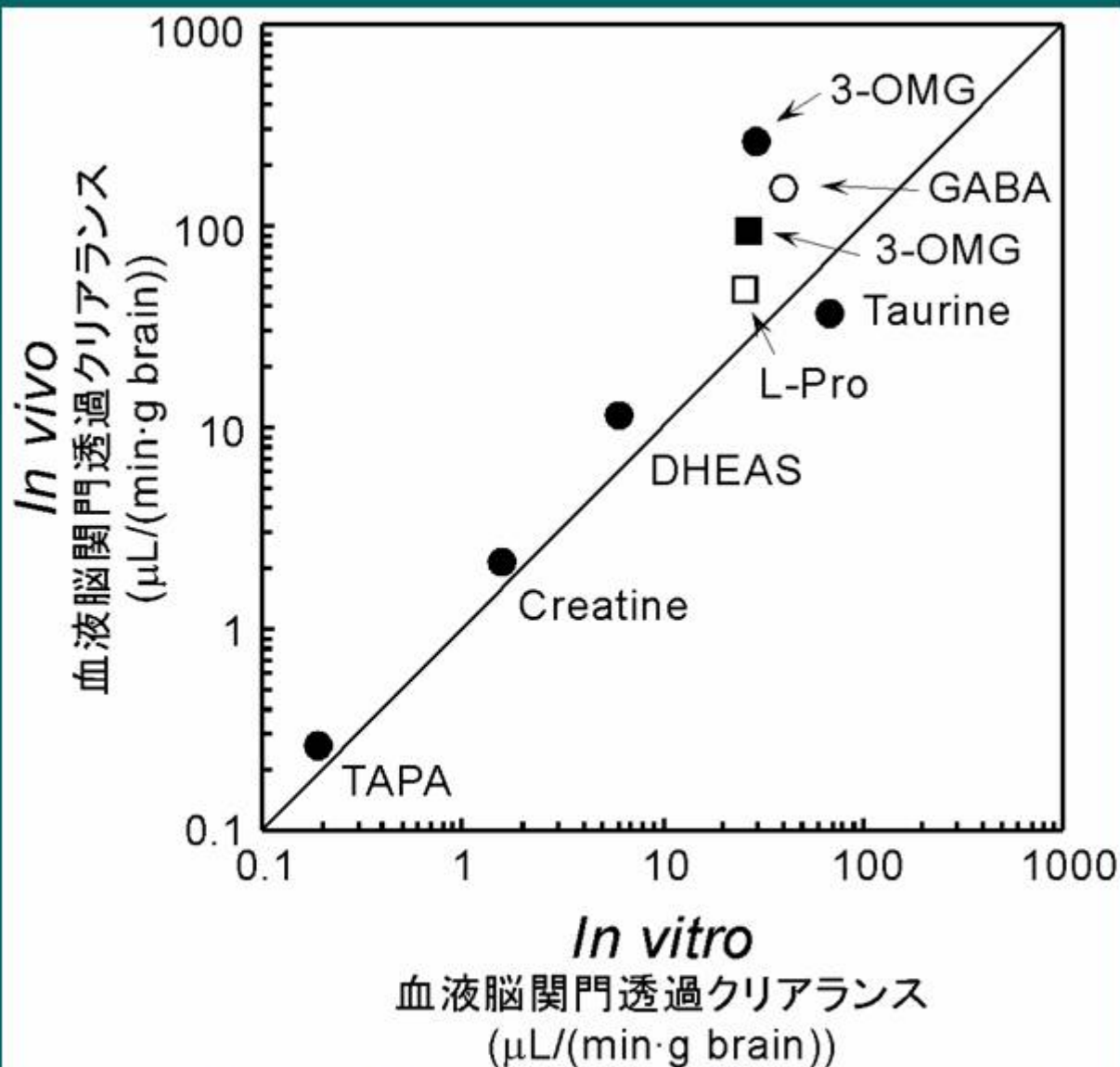
tsA58 large T antigen is **active at 33 degree.**
Conditionally immortalized **cell lines**
maintained most of in vivo functions.



TR-BBB

血液脳関門輸送の分子実体と調節機構を解明するために、新規in vitro系として条件的不死化脳毛細血管内皮細胞株を樹立

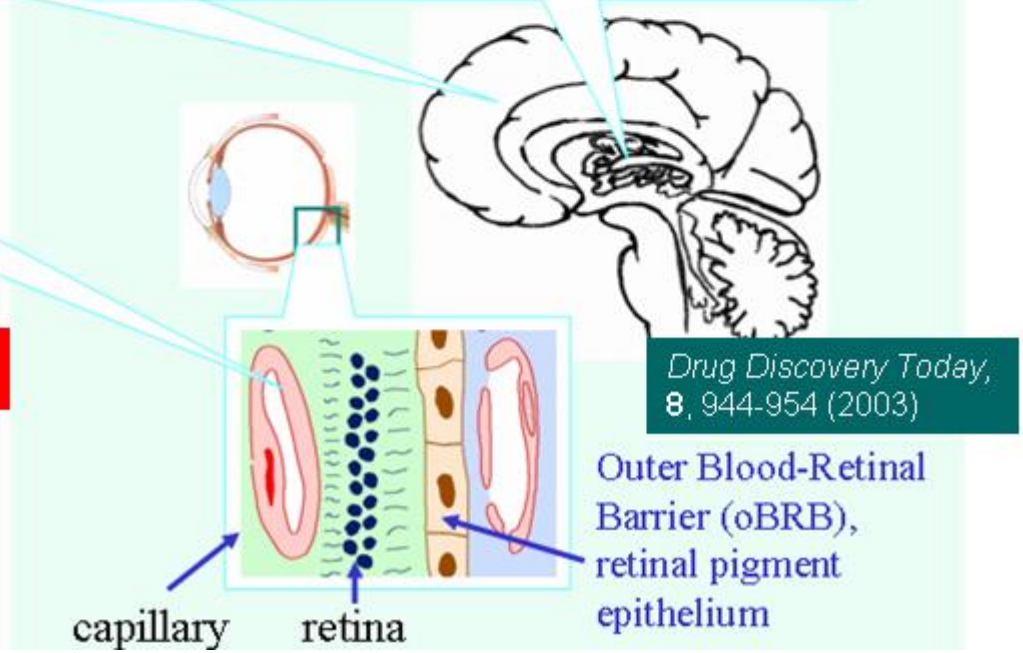
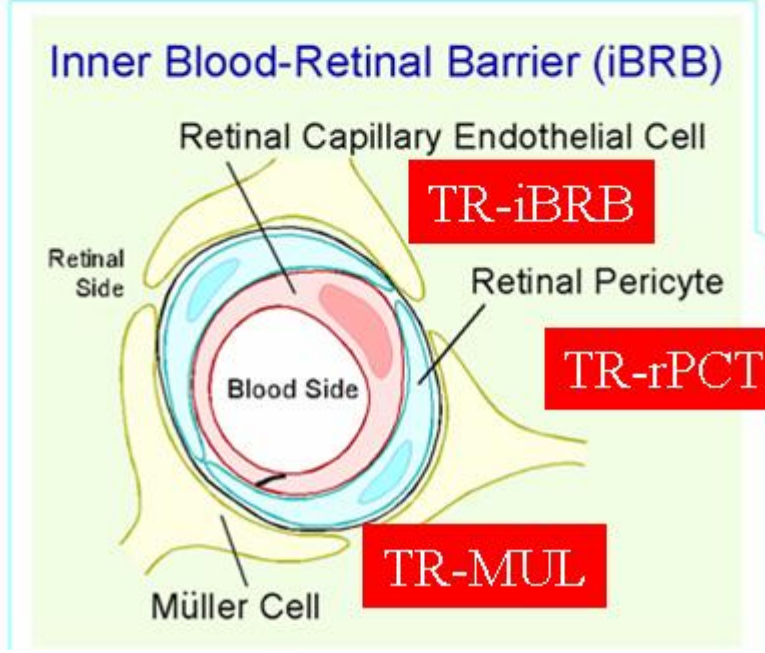
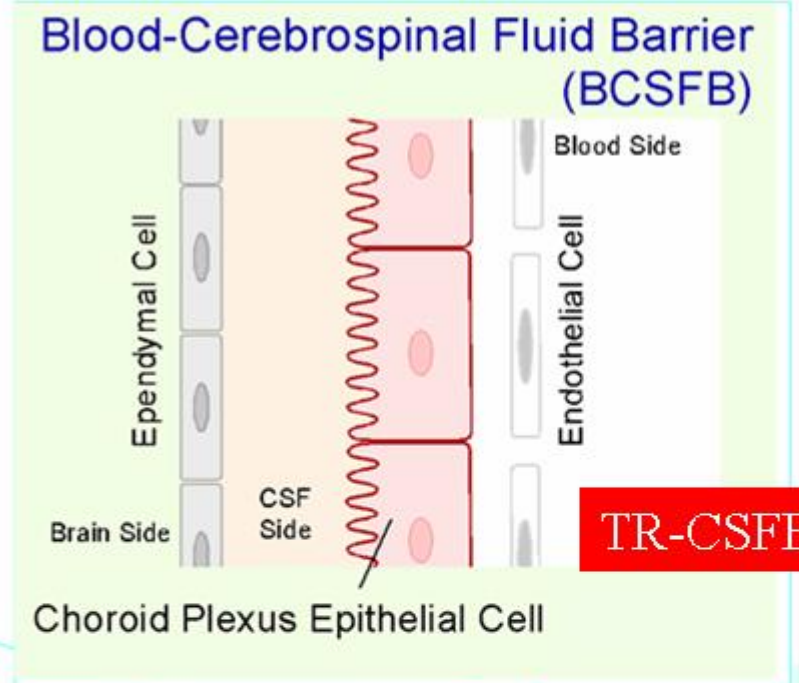
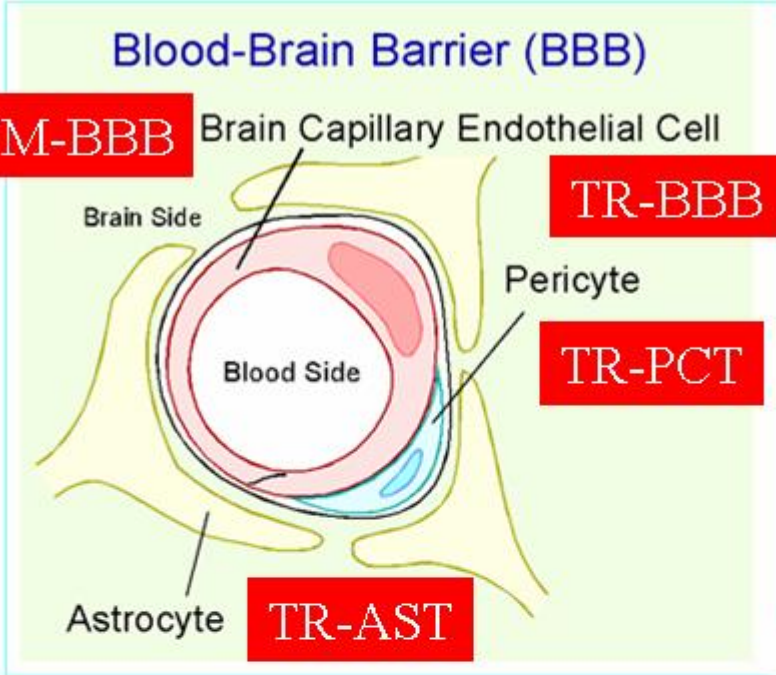
AAPS Pharmsci, 2: article 27 (2000) J. Drug Target., 8: 357-370 (2000)



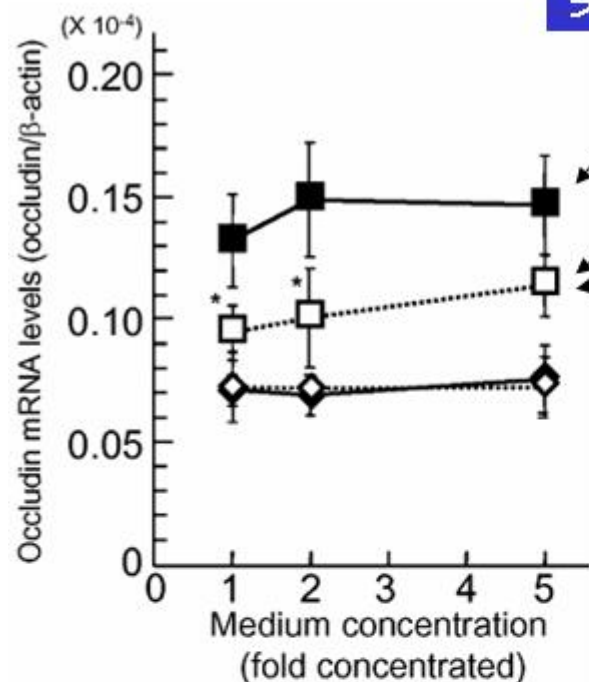
Drug Discovery Today,
8, 944-954 (2003)

In Vivo輸送活性とほぼ同程度のIn Vitro輸送活性を保持する条件的不死化脳毛細血管内皮細胞株が樹立

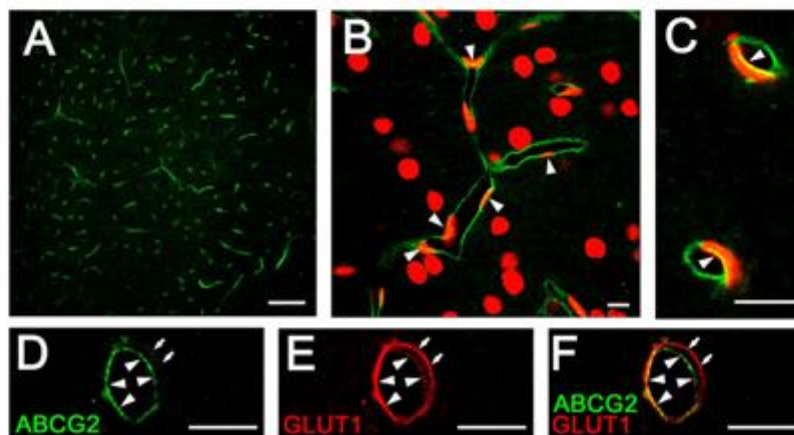
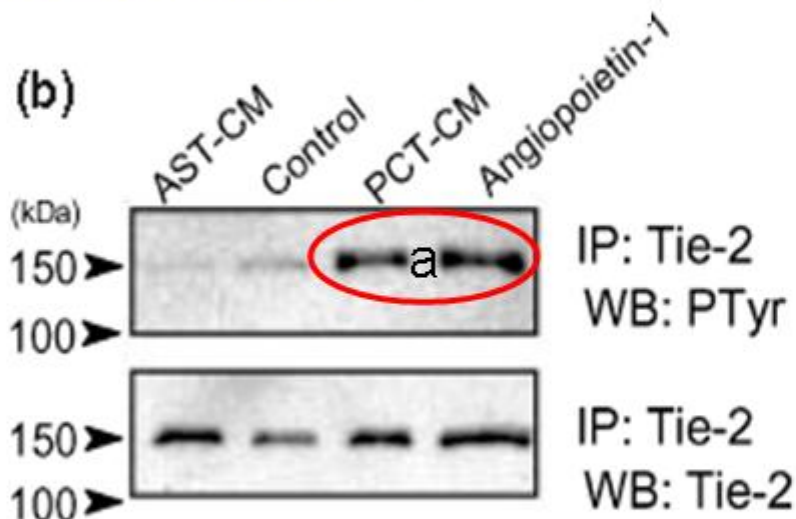
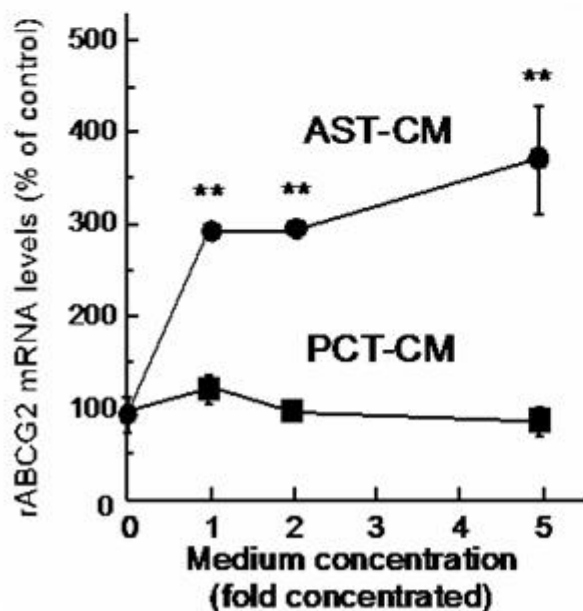
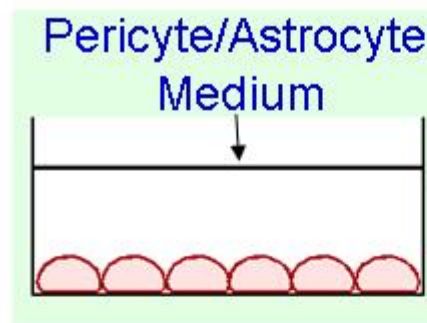
多くの共同研究者の力をお借りして、網羅的・系統的研究展開の大切さを



実体解明の限界を感じて。質量分析の手法導入の必要性を



Pericyte CM
anti-angiopoietin-1 antibody
Control medium

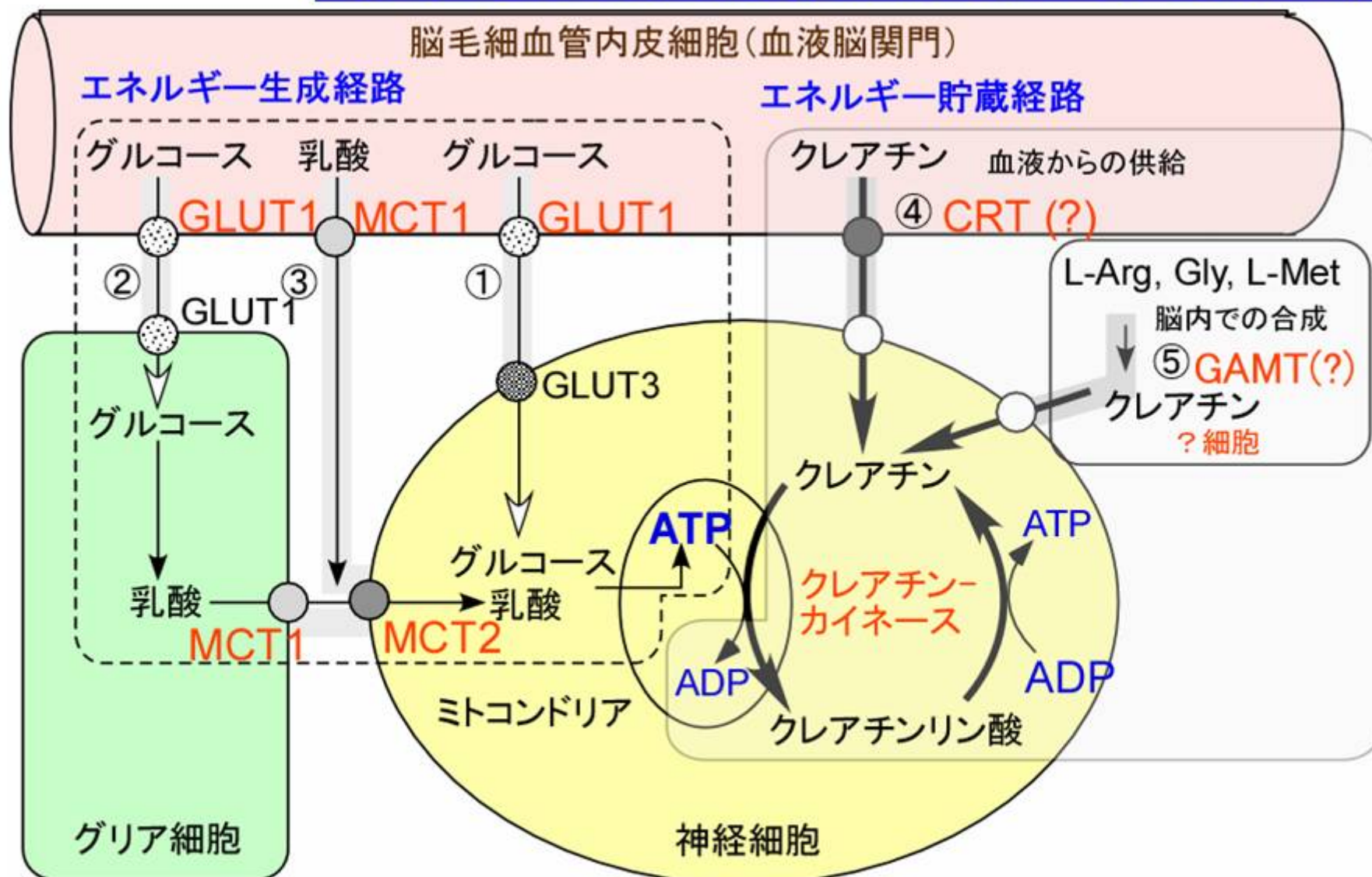


J. Neurochem.,
89: 503-513 (2004)

J. Neurochem.,
90: 526-536 (2004)

J. Neurochem.,
93: 63-71 (2005)

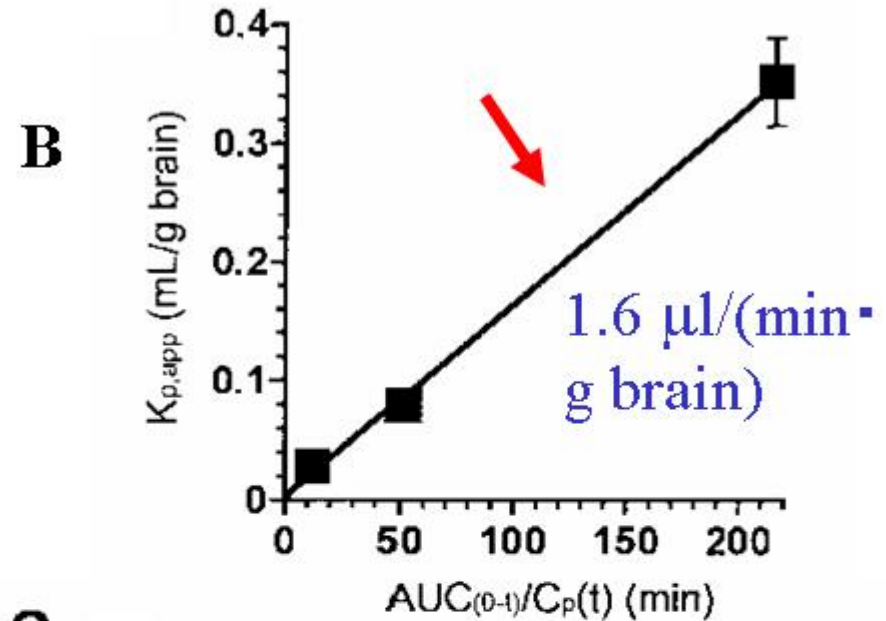
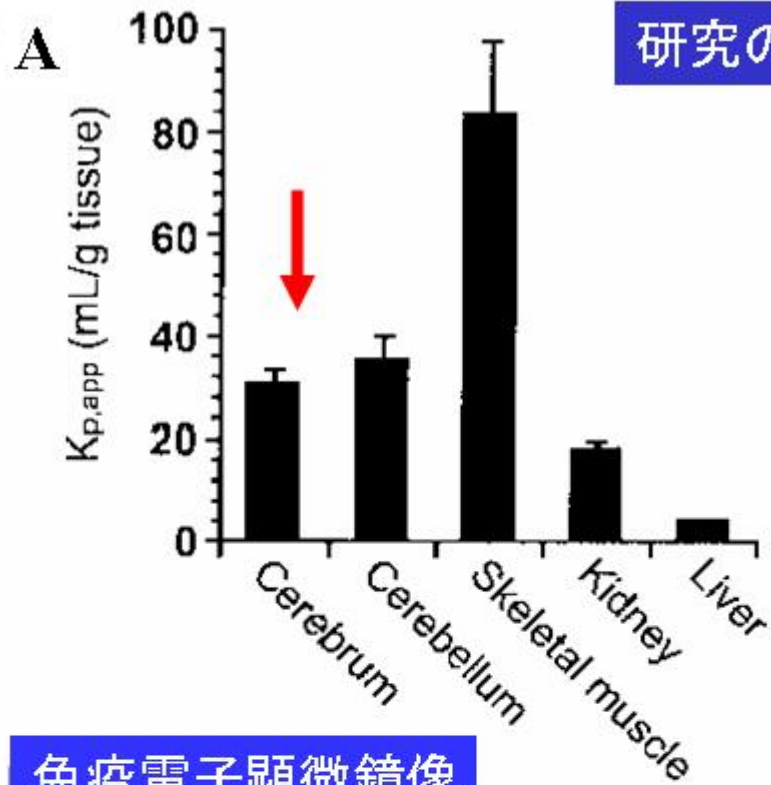
想定外のささいな実験結果を無視しない。真実への大きな扉。



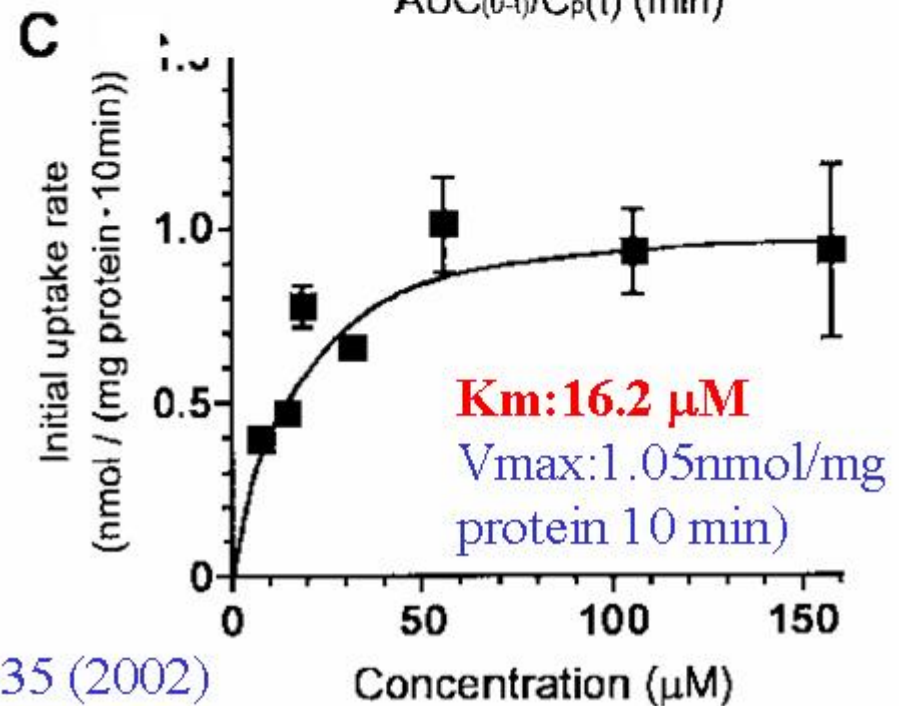
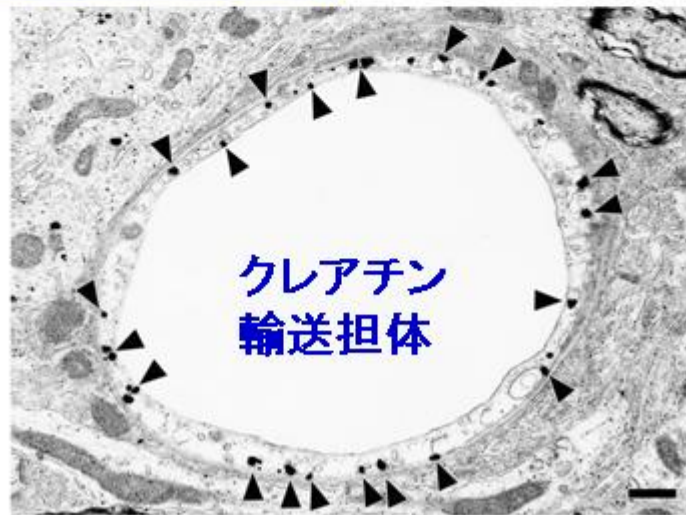
・血液脳関門の役割は、グルコースやケトン体の供給輸送のみか？

・脳内のクレアチン濃度は血液の180倍も高いが、由来は？

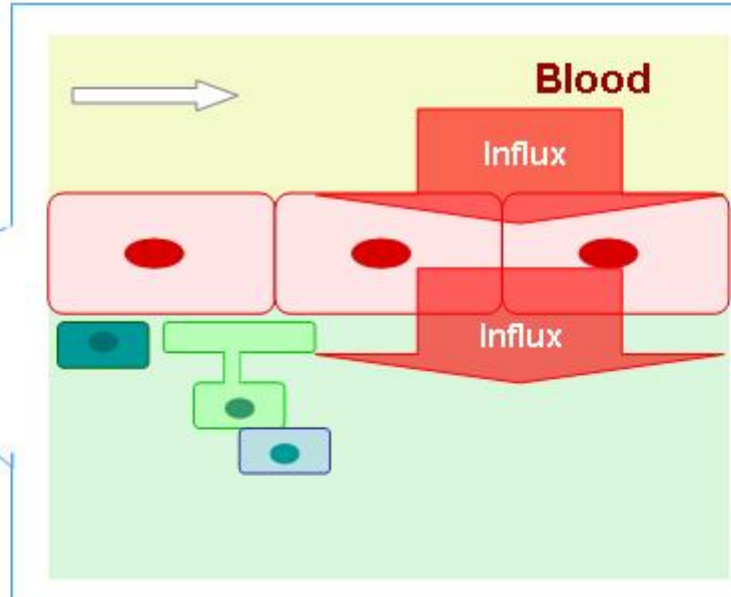
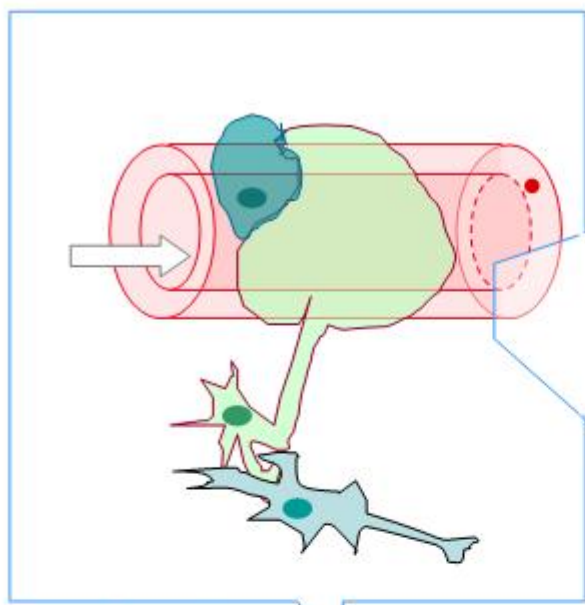
研究の始点と終点in vivoを忘れず、積極的共同研究を



免疫電子顕微鏡像



J. Cereb. Blood Flow Metab., 22: 1327-1335 (2002)



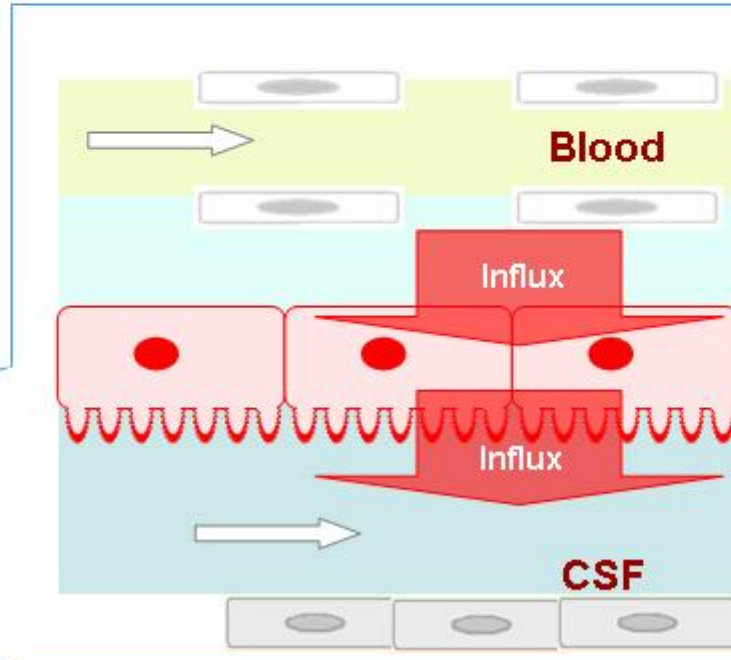
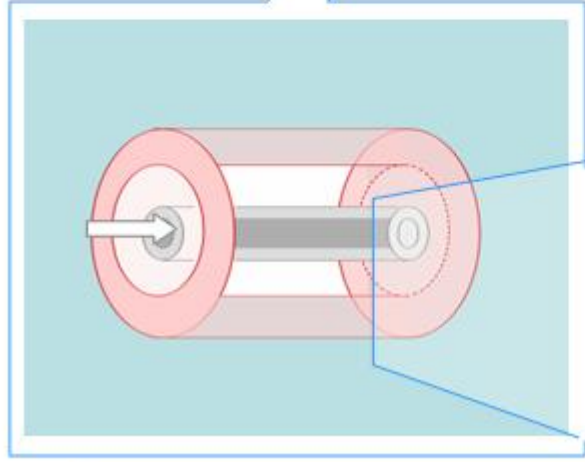
Blood-brain barrier

Energy source and buffer are supplied by GLUT1, MCT1 and CRT



cerebral cortex

ventricular

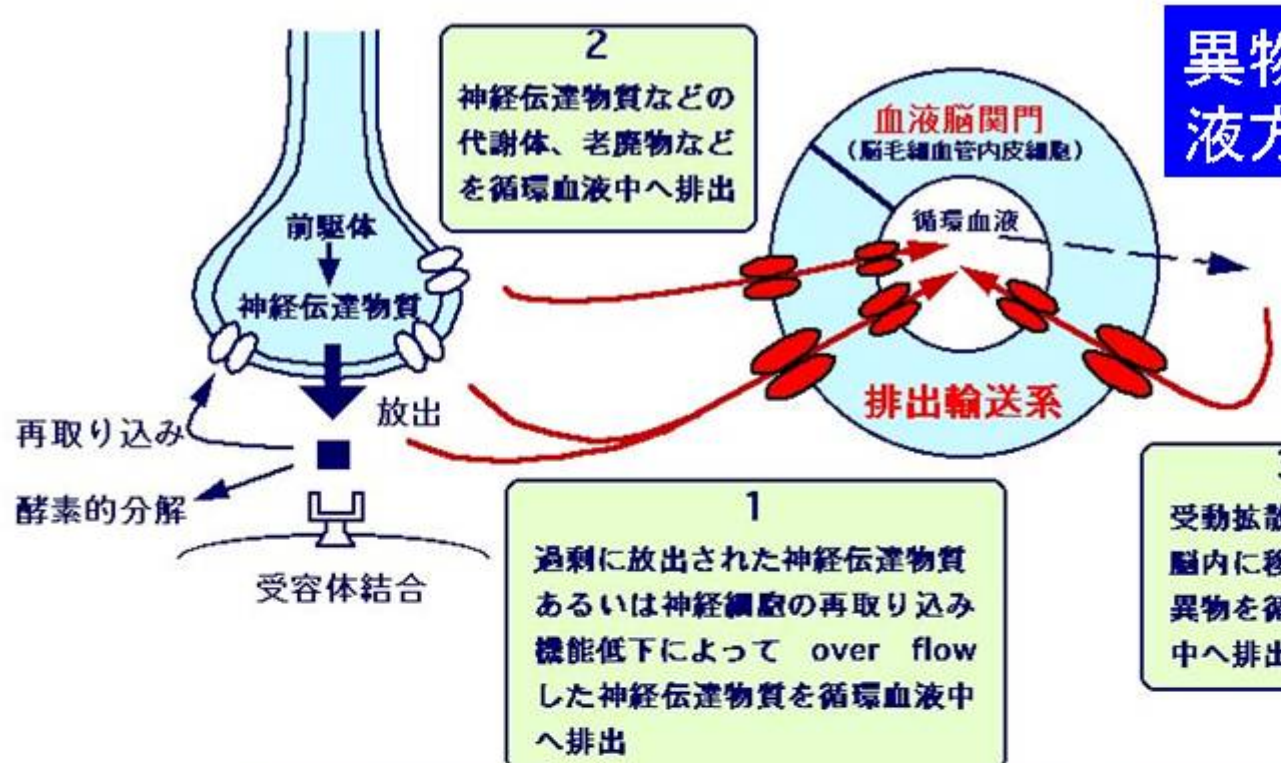


Blood-CSF barrier

Nutrients are supplied

BBBとBCSFBの役割

1. 脳を支援 (供給輸送)



異物解毒以外に脳から血液方向に働く輸送系は？

素朴な疑問を原点に

血液脳関門中枢解毒機構仮説
 脳を守る『中枢解毒機構』として、複数の排出輸送系が血液脳関門において機能している

1996年

- a) γ -Aminobutylic acid (GABA)
Neurotransmitter
- b) L-Aspartic acid (L-Asp)
Amino Acid
- c) 24s-hydroxycholesterol(24sOH-Chol)
Nuclear receptor ligand
- d) Homovanillic acid (HVA)
Neurotransmitter metabolite
- e) Indoxyl sulfate (IS)
Uremic toxin
- f) 6-Mercaptopurine (6-MP)
Xenobiotics

GABA

GAT2/BGT-1

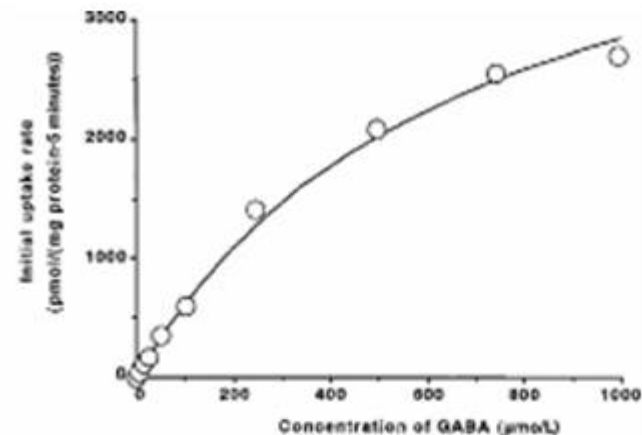
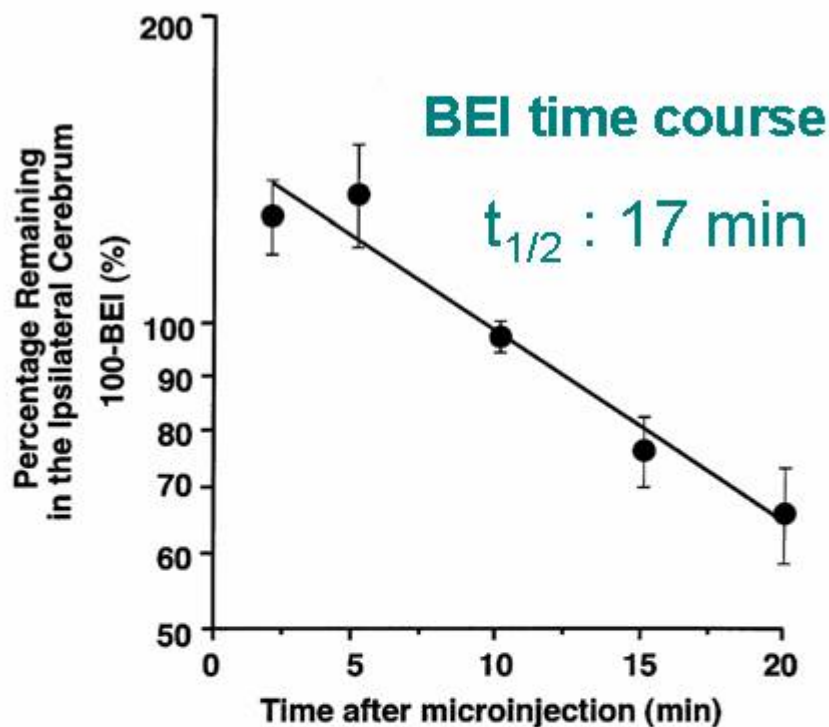
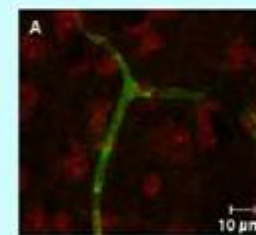
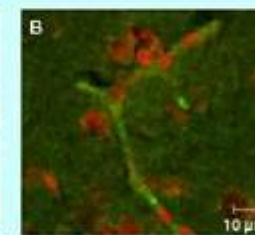


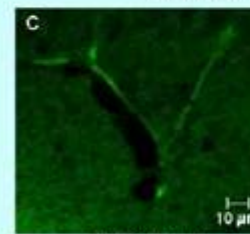
FIG. 2. Concentration-dependence of γ -aminobutyric acid (GABA) uptake by TM-BBB2 cells. [3 H]GABA uptake (10 nmol/L) was performed at 37°C for 5 minutes. Each point represents the mean \pm SD (n = 3 to 4). The deviation bar is smaller than the size of the symbol. K_m and V_{max} are $679 \pm 80 \mu\text{mol/L}$ and $4,790 \pm 494 \text{ pmol}/(\text{mg protein} \cdot 5 \text{ minutes})$, respectively.



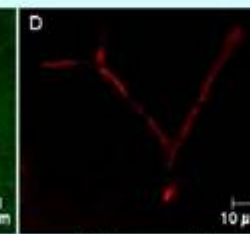
P-glycoprotein (C219) and nuclei



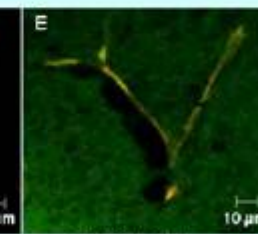
GAT2/BGT1 and nuclei



GAT2/BGT1



P-glycoprotein



GAT2/BGT1 and P-glycoprotein

Localization of GAT2/BGT1 and P-glycoprotein in mouse brain

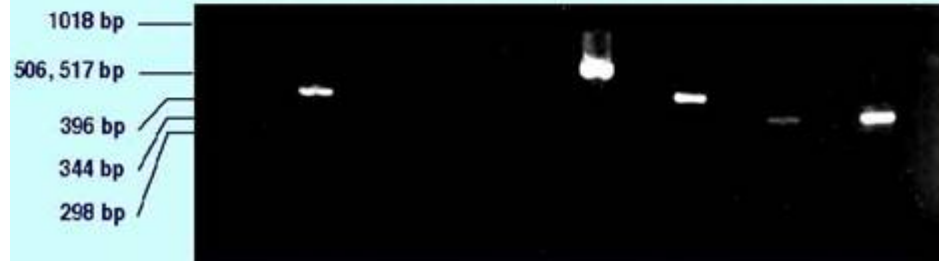
J. Cereb. Blood Flow Metab.,
21: 1232-1239 (2001)

J. Neurochem., 71: 110-118 (2001)

Conditionally immortalized mouse brain capillary endothelial cell line (TM-BBB2)

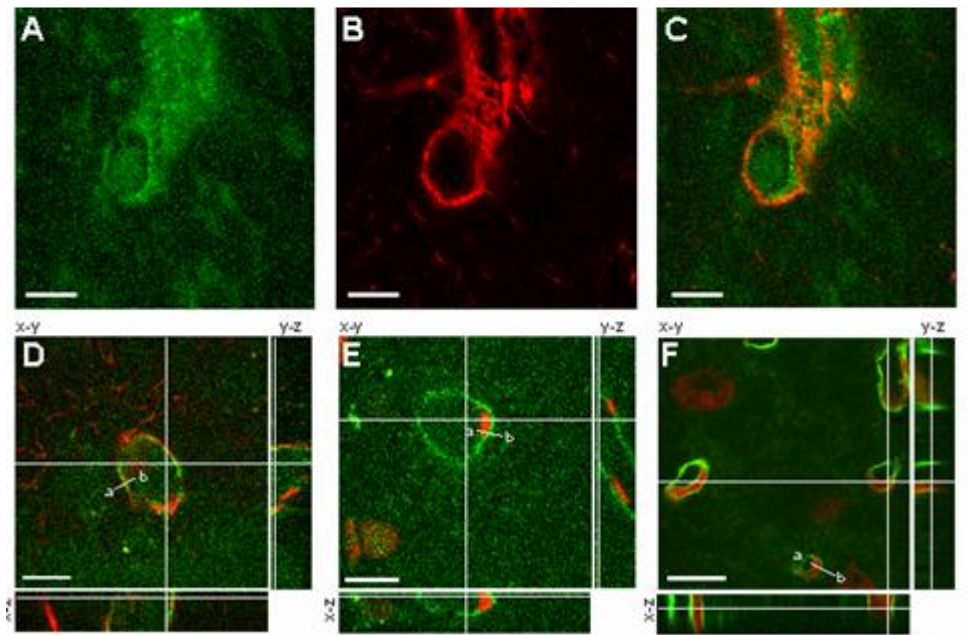
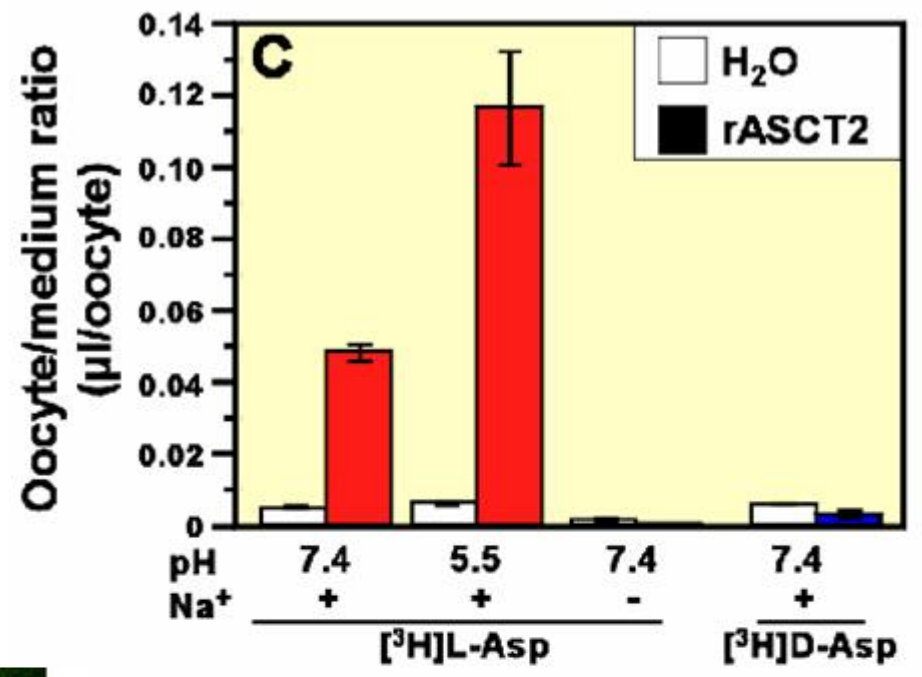
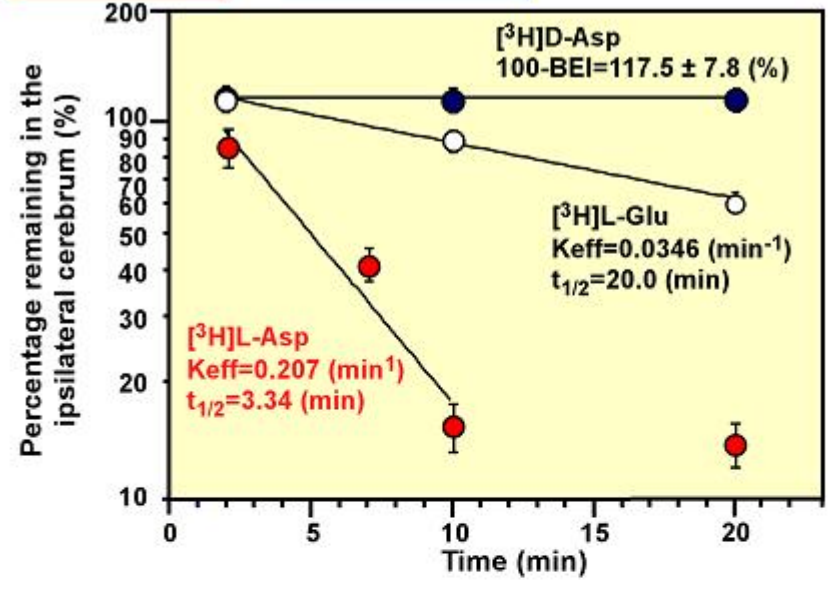
Mouse whole brain

	TM-BBB2				Mouse whole brain			
	GAT1	GAT2	GAT3	GAT4	GAT1	GAT2	GAT3	GAT4
RT	+	-	+	-	+	-	+	-

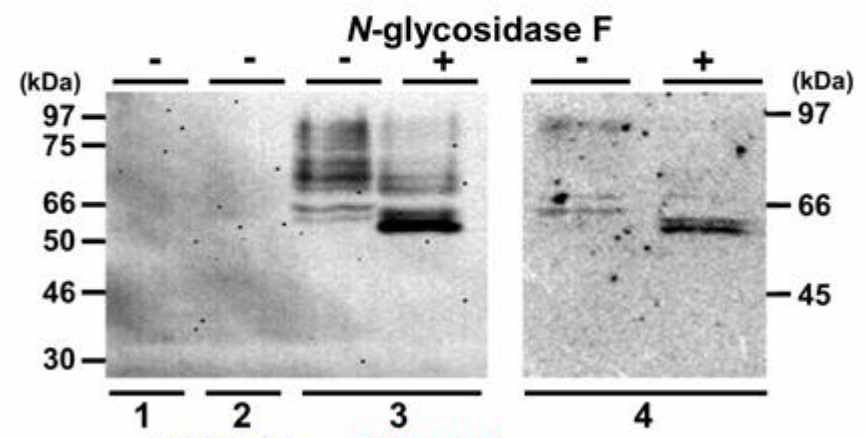


Amplification of GAT family with RT-PCR method using total RNA of mouse brain or TM-BBB2

L-Asp **ASCT2**



mASCT2 + GFAP
mASCT2 + nuclei
GLUT1 + nuclei

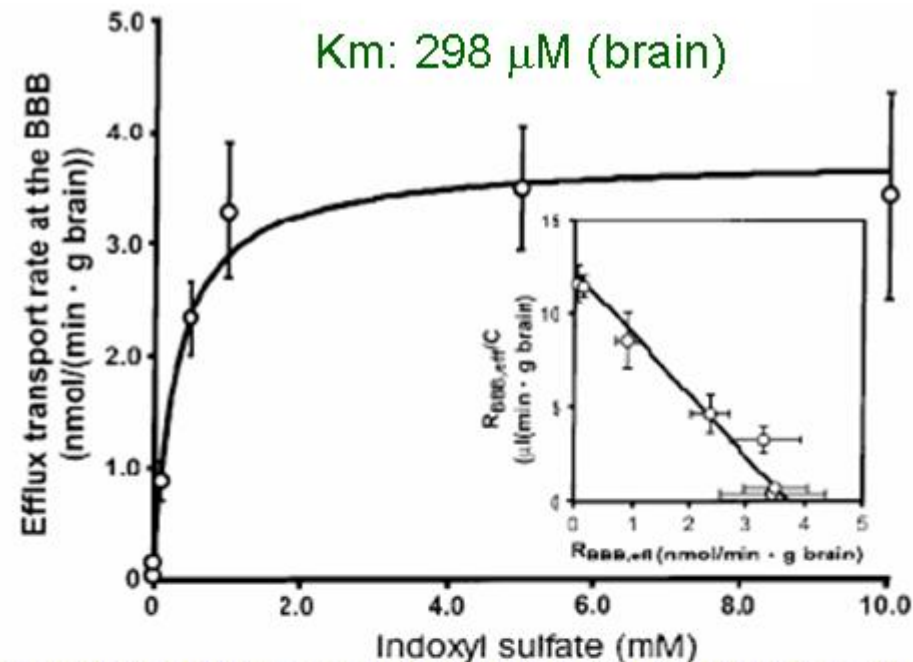
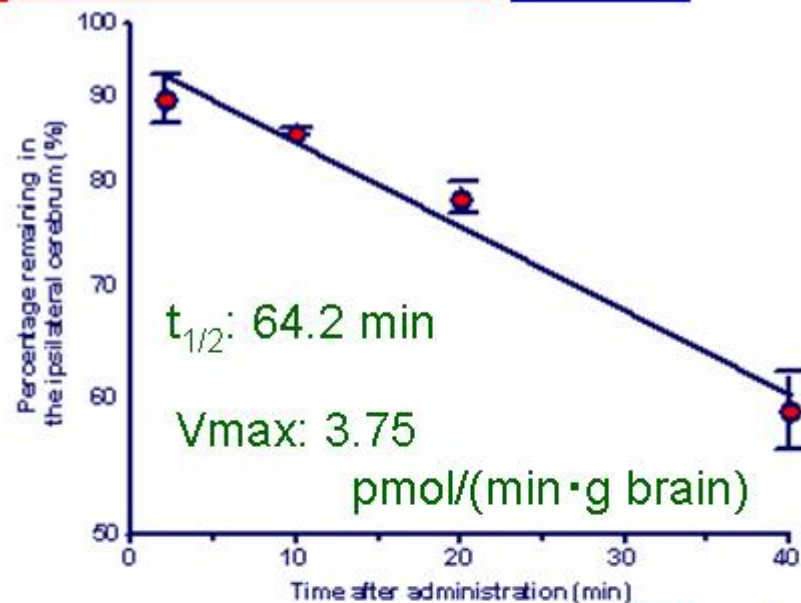


mASCT1/ **mASCT2/**
CHO-K1 **CHO-K1** **TM-BBB**

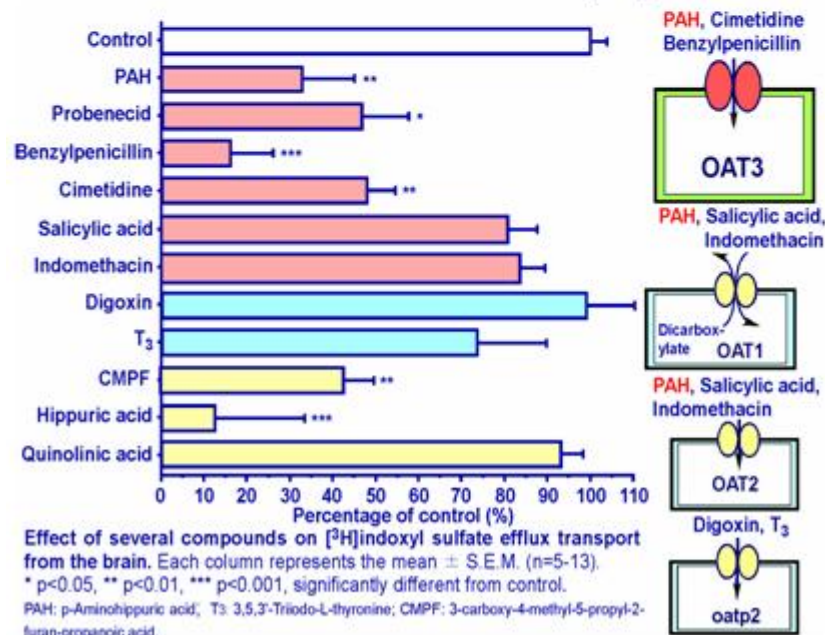
J. Neurochem., 73: 1206-1211 (1999)
J. Neurochem., 87: 989-901 (2003)

Indoxyl Sulfate

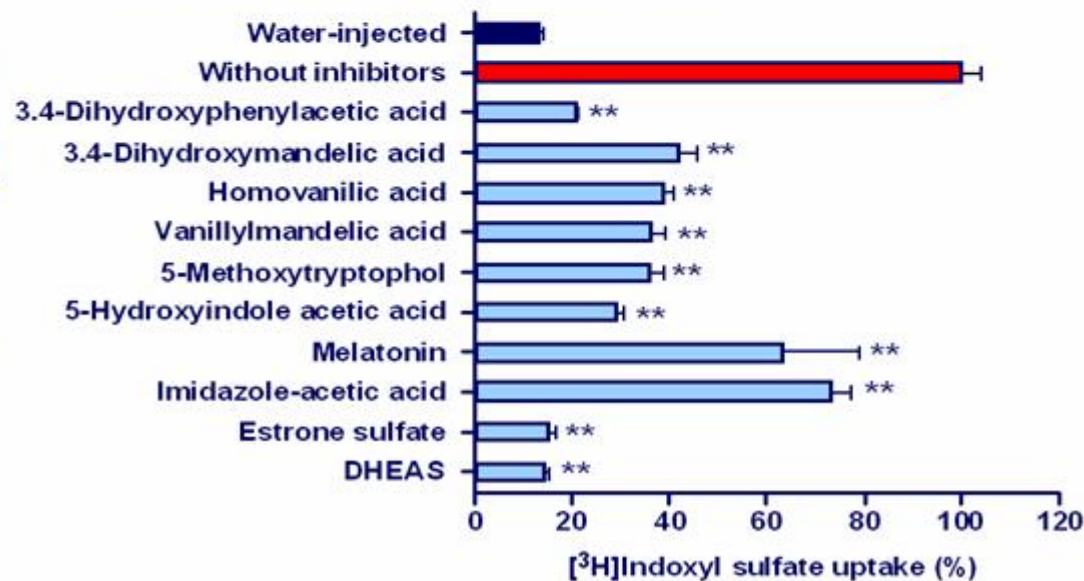
Oat3



Inhibition of OAT3-mediated $[^3H]$ indoxyl sulfate uptake by metabolites of endogenous substances

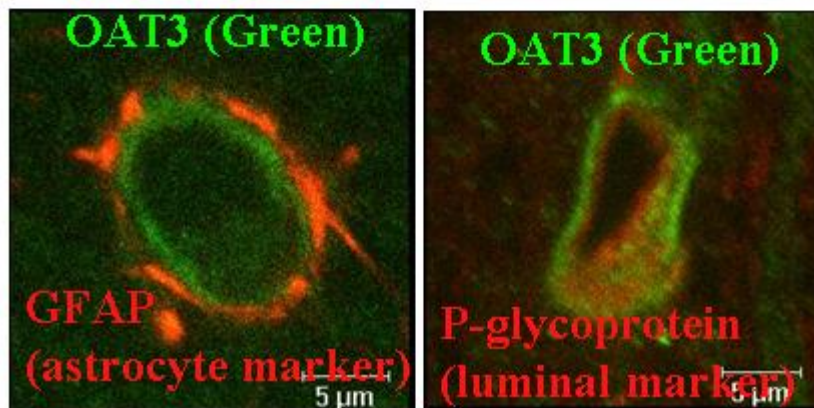
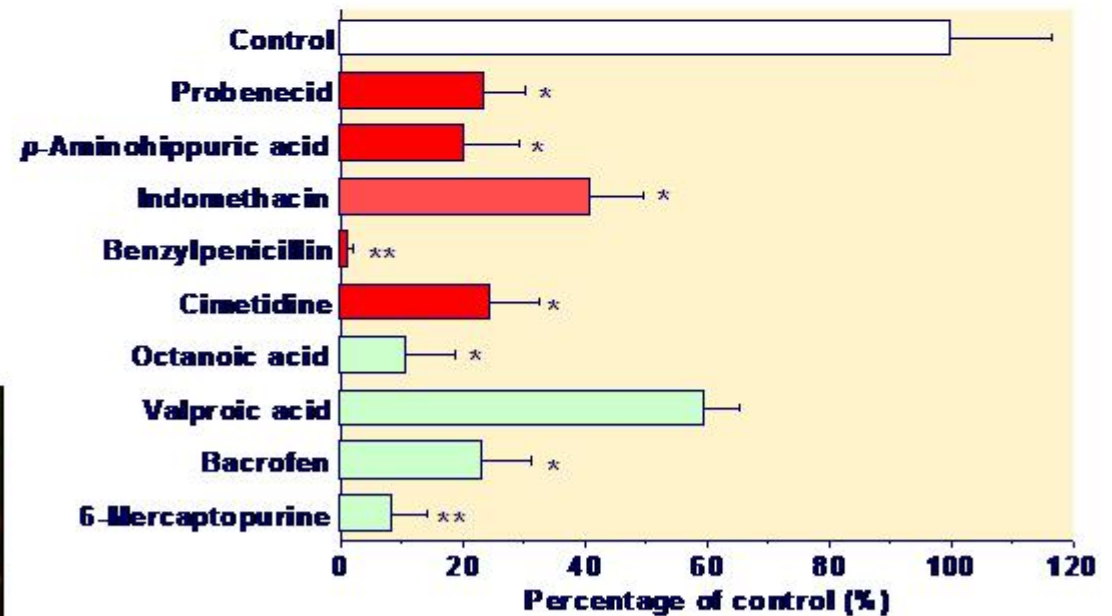
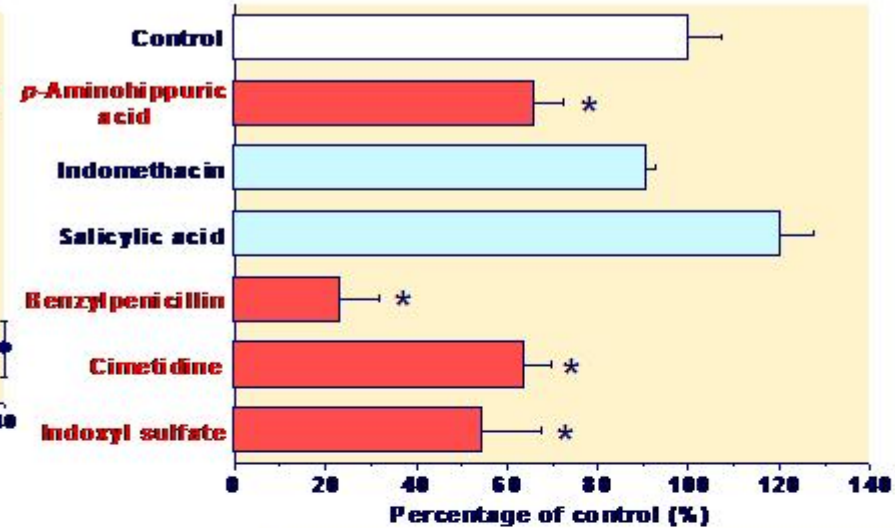
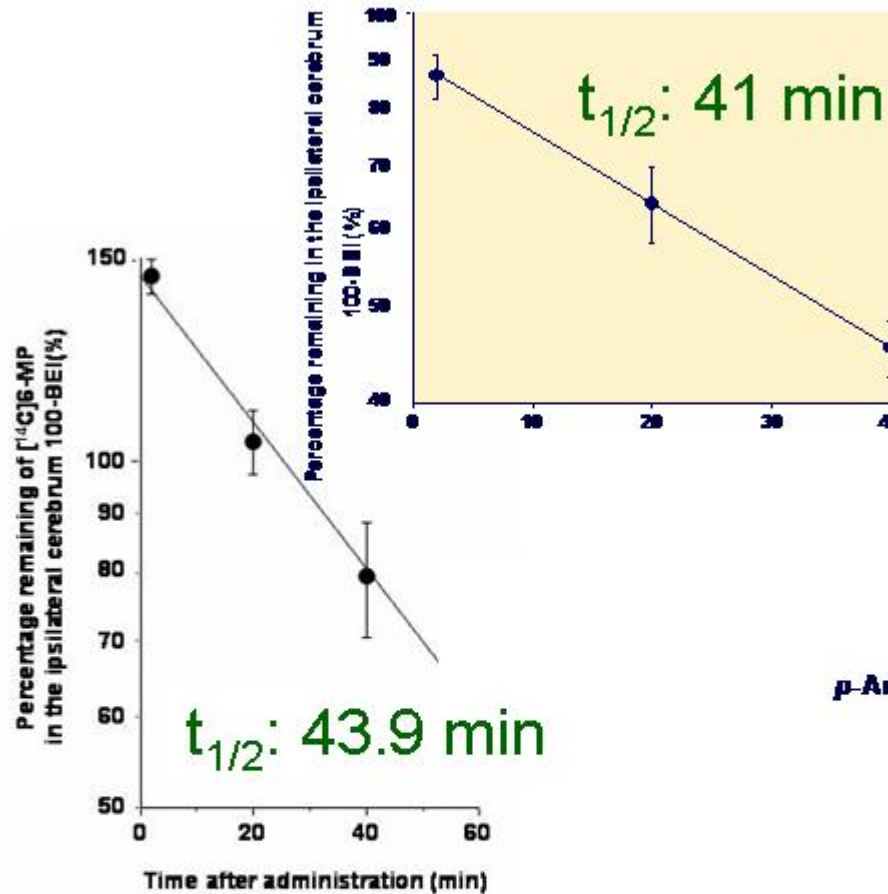


Effect of several compounds on $[^3H]$ indoxyl sulfate efflux transport from the brain. Each column represents the mean \pm S.E.M. ($n=5-13$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from control. PAH: p-Aminohippuric acid; T_3 : 3,5,3'-Triiodo-L-thyronine; CMPF: 3-carboxy-4-methyl-5-propyl-2-furan-propanoic acid.



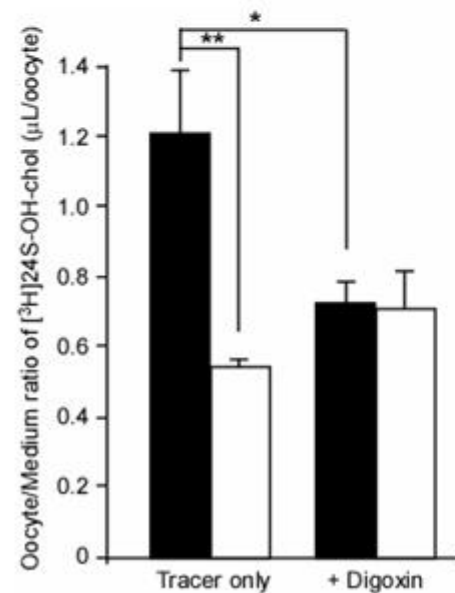
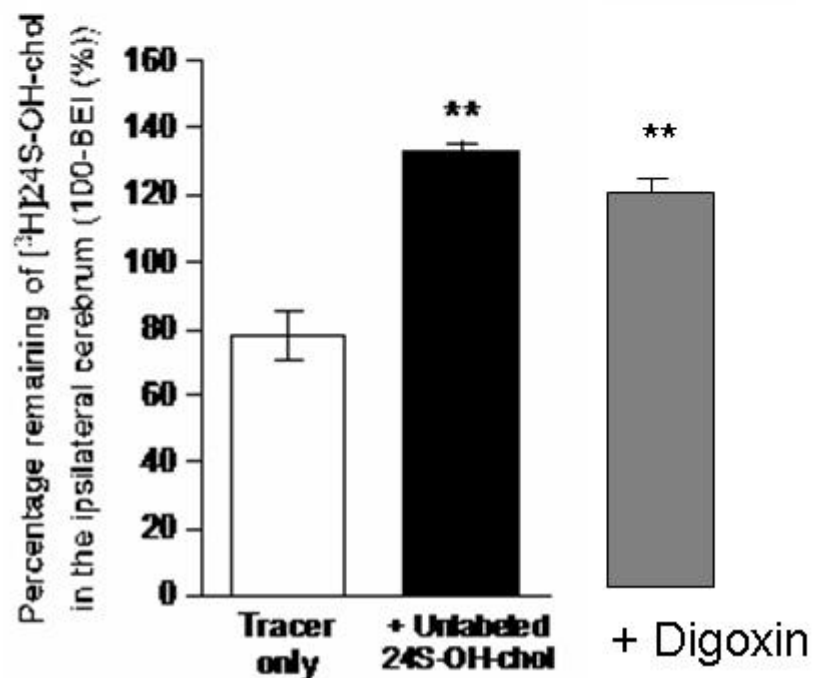
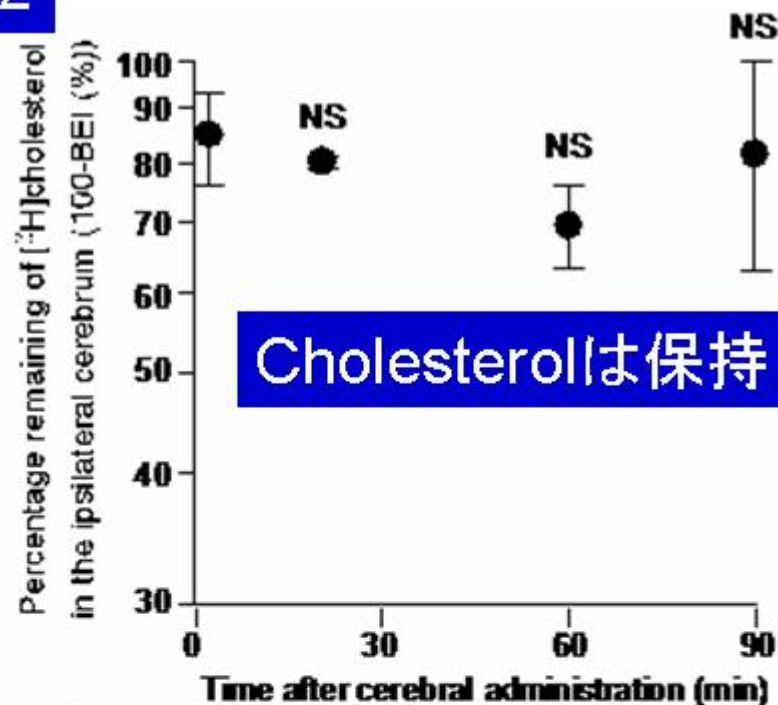
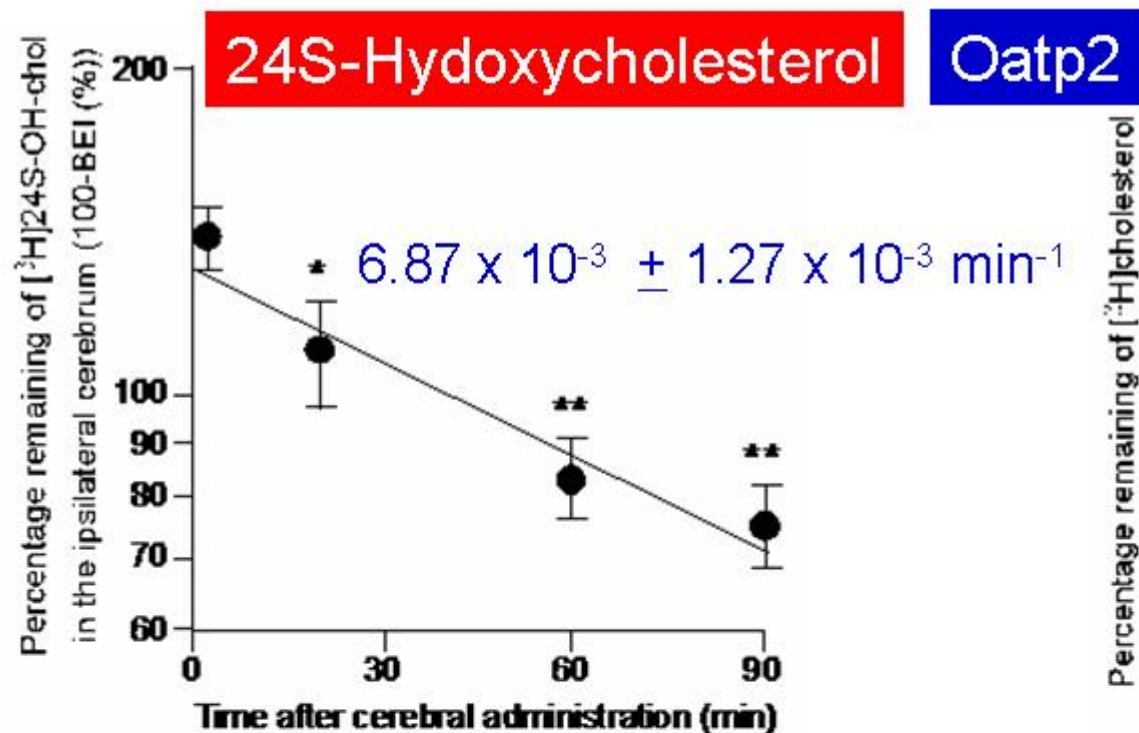
Homovanillic acid, 6-Mercaptopurine, 6-Thioguanine

Oat3

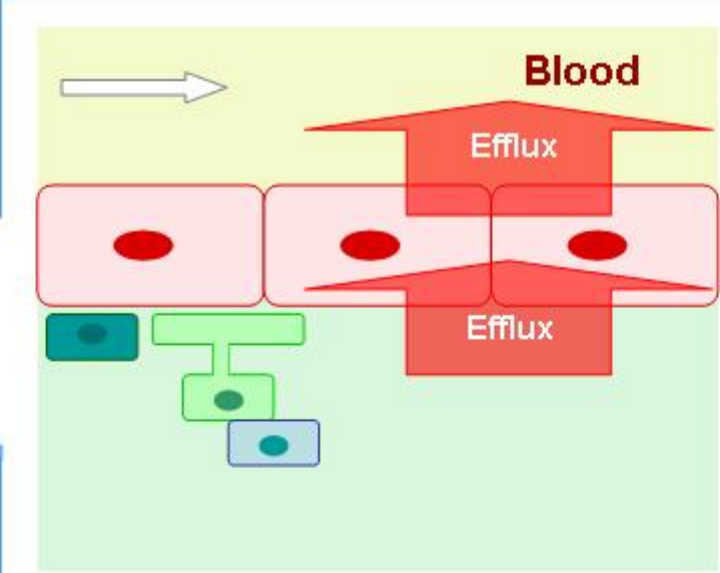
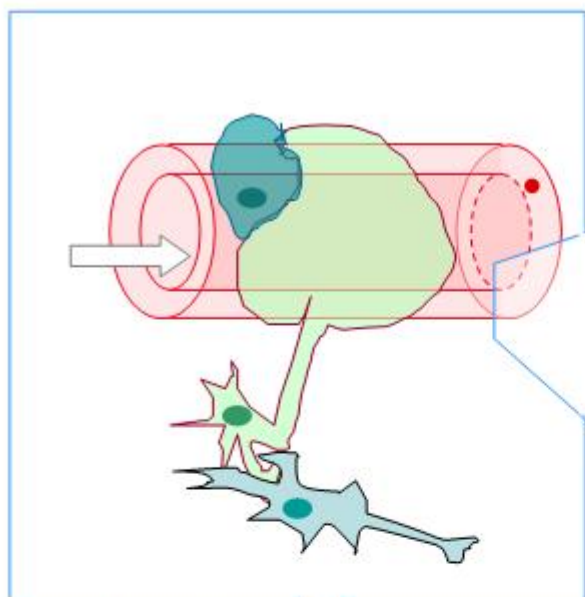


J. Cereb. Blood Flow Metab., 23: 432-440 (2003)

J. Neurochem., 90: 931-941 (2004)

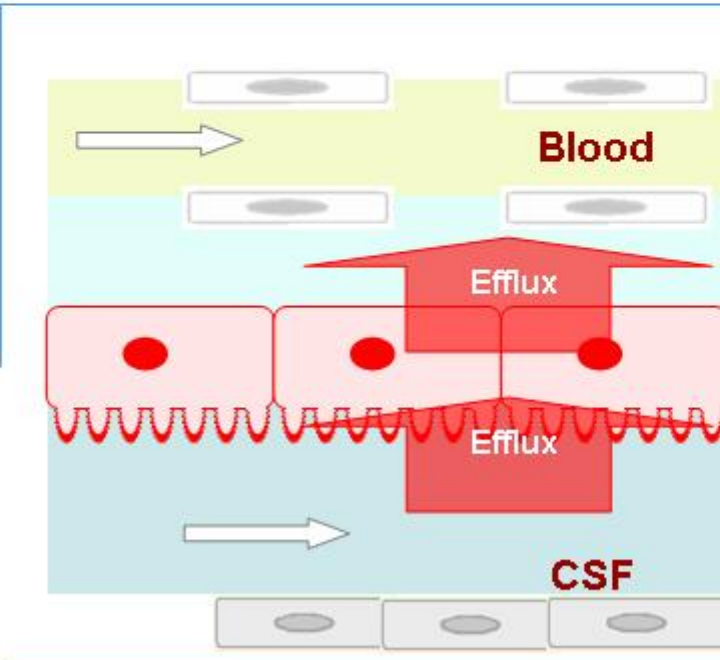


J. Neurochem., 103: 1430-1438 (2007)



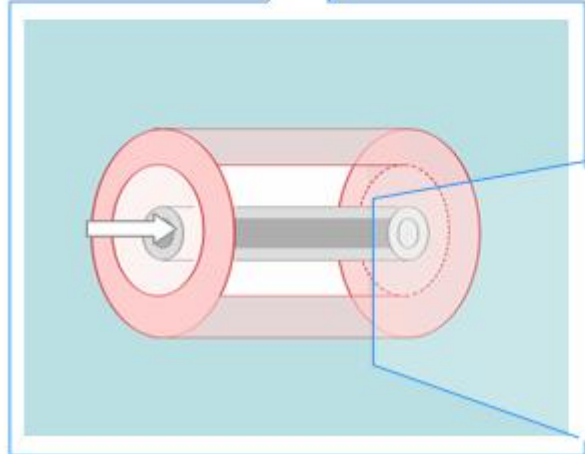
Blood-brain barrier

Xenobiotics, PGs, neuro-transmitter metabolite, uremic toxins are effluxed by **MDR1**, **BCRP**, **MRP4**, **Oat3**, **Oatp2**



Blood-CSF barrier

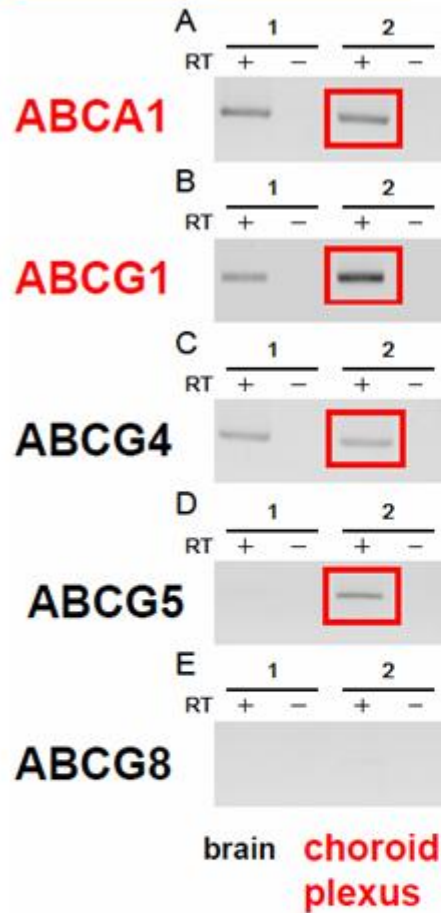
Xenobiotics, PGs, toxins are effluxed by **MRP1**, **MRP4**, **MRP5**, **OAT3**, **Oatp2**, **Oatp3**



BBBとBCSFBの役割

2. 脳を防御 (排出輸送)

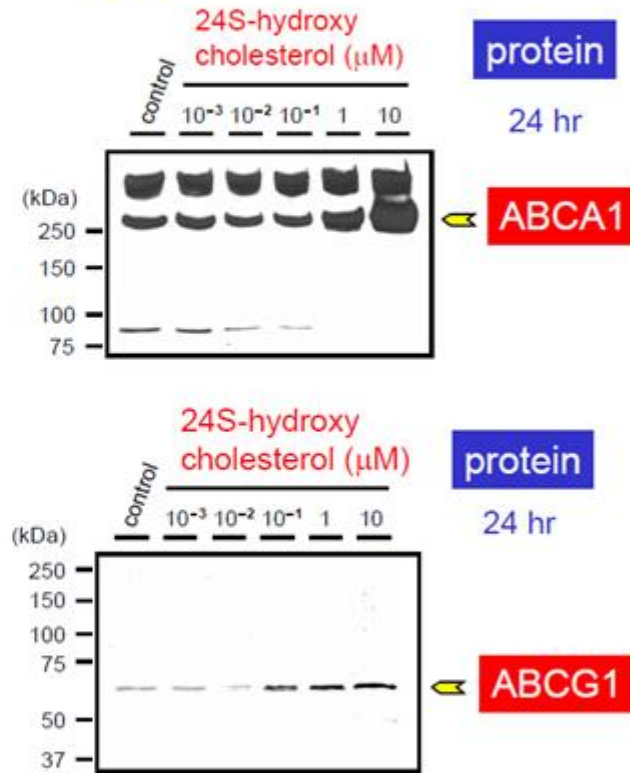
Cholesterol



BCSFBにABCA1,G subtypesが発現している。→コレステロール輸送関連トランスポートソーム

血液脳脊髄液関門Abca1, Abcg1で脳内に保持

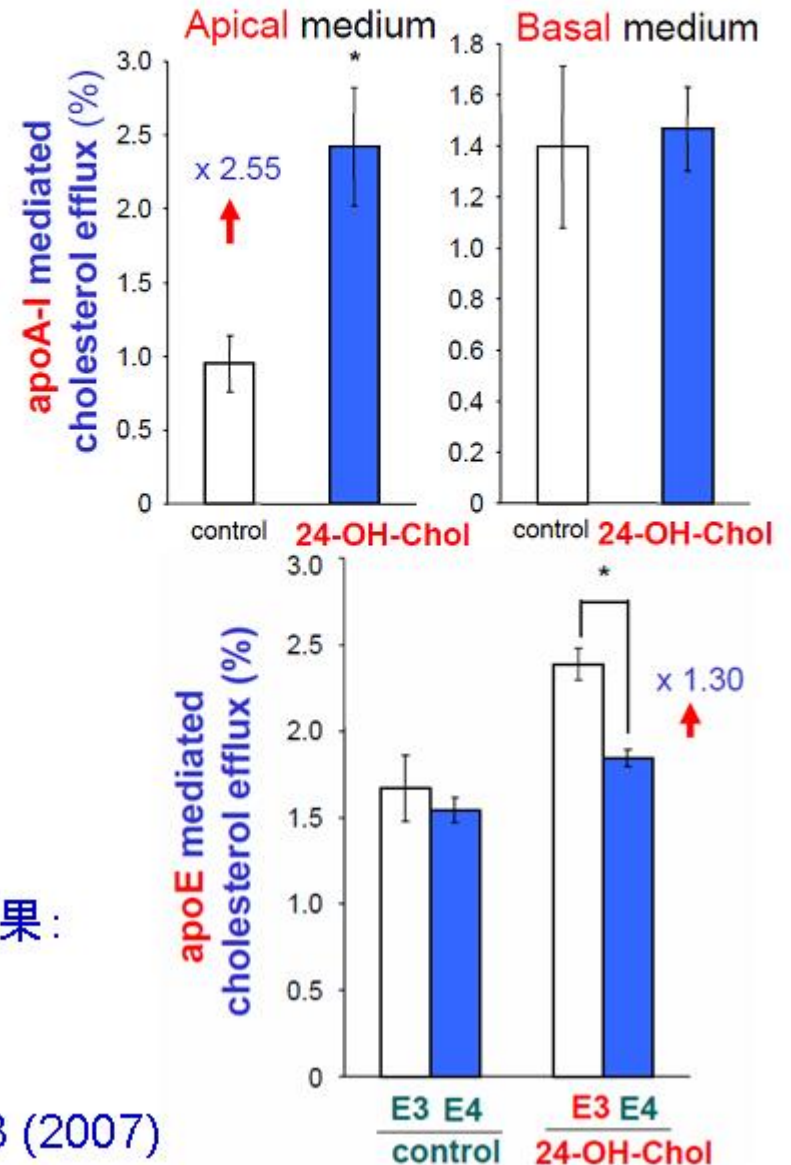
BCSFBのABCA1,G1はLXR ligandにより誘導される。



LXR ligand による誘導効果: ApoE3 > ApoE4

J. Neurochem., 100: 968-978 (2007)

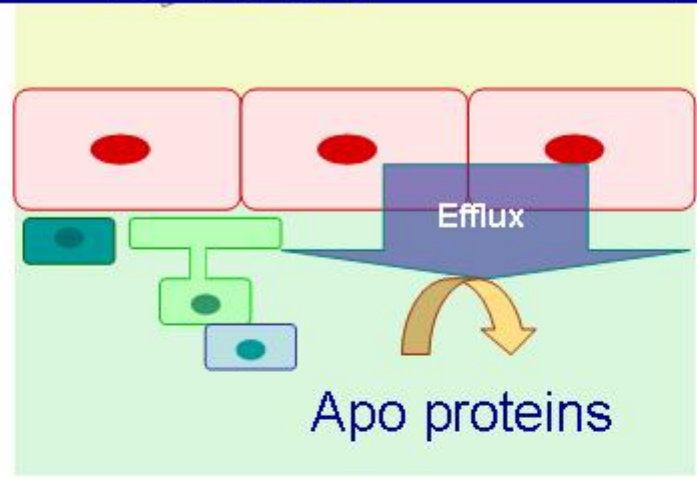
脳脊髄液側の分泌がLXR ligandにより選択的に誘導



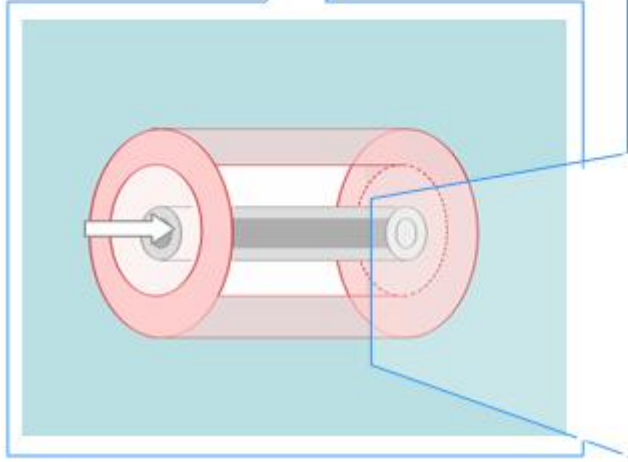
排出輸送が解毒に限らない。固定観念からの脱却

Blood-brain barrier

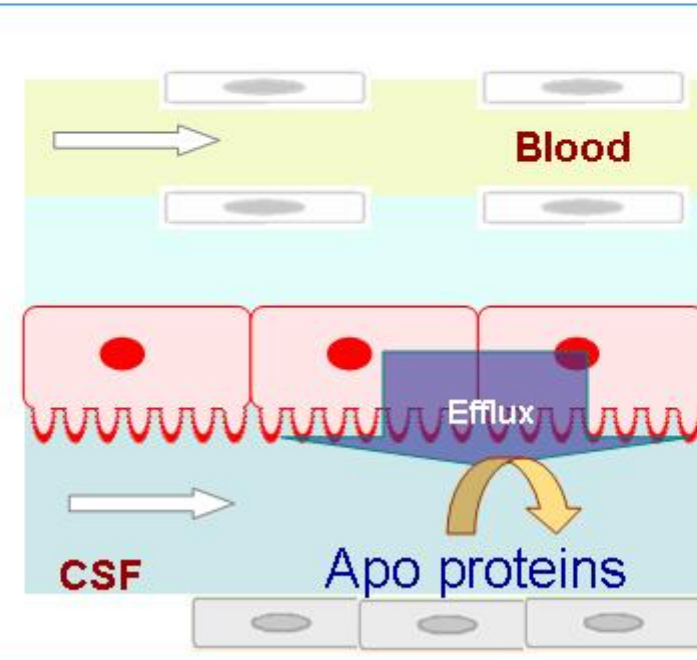
- 1) Cholesterol is synthesized in the brain.
- 2) 25% of body cholesterol (Chol) is in the brain.
- 3) There is no efflux of cholesterol



Cholesterol would be retained in the brain by Abca1



Blood-CSF barrier



Cholesterol is retained in the CSF by Abca1, Abcg1

BBBとBCSFBの役割

3. 脳を支援 (排出輸送)

定量的プロテオミクスに基づく新しい薬物動態学

Pharmacoproteomics

Quantitative Proteomics based Physiological Pharmacokinetics

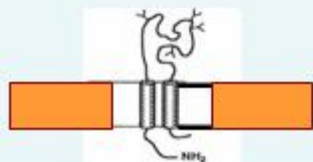
Quantitative ATLAS of
Human Protein
Expression



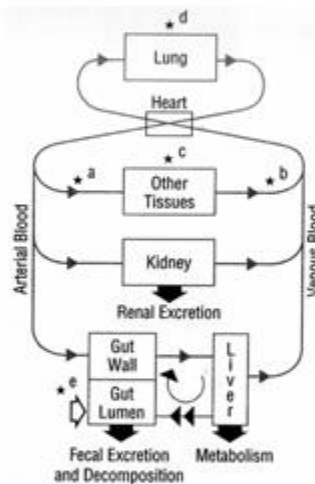
Transporter
Amount



Enzyme
Amount



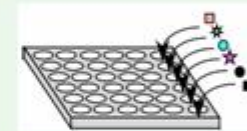
Receptor
Amount



In vivo
prediction



Activity per
protein molecule



V_{max}

Transport
Activity

V_{max}

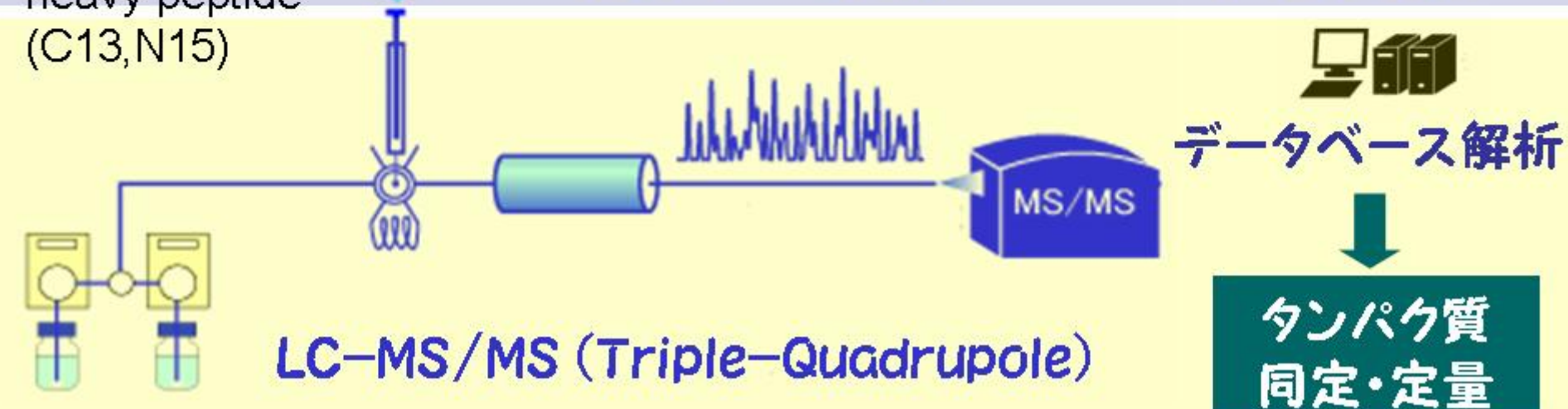
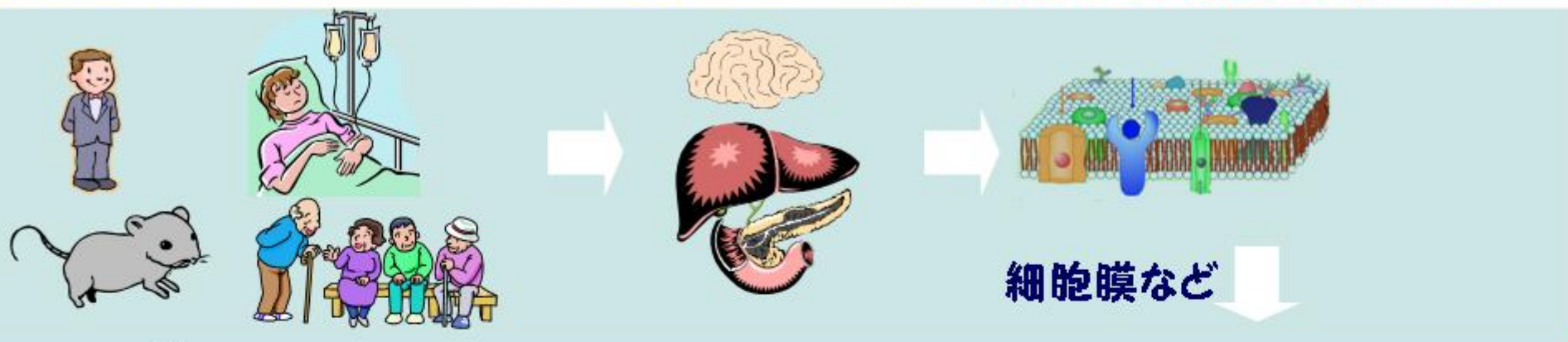
Metabolic
Activity

B_{max}

Pharmacologic
Activity

In silico peptide probe 設計法と 多検体同時タンパク質定量法の開発

格を出でて、
初めて自在を得べし



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