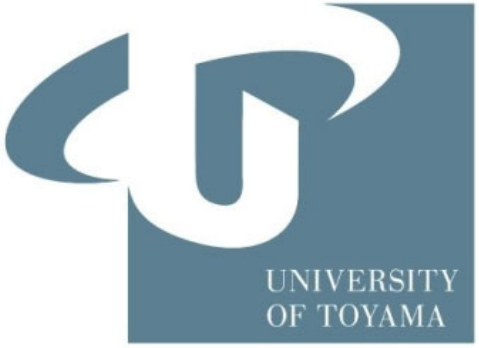
JSSX Award Lecture
The 36th Annual Meeting of JSSX in Takasaki

16:10-16:30
November 18, 2021
Web

新たな血液網膜関門輸送研究手法の開発に基づく本関門を介した血液-網膜間薬物動態の制御分子機構解明

Elucidation of the molecular mechanism of barrier transport based on the development of a novel blood-retinal barrier transport method



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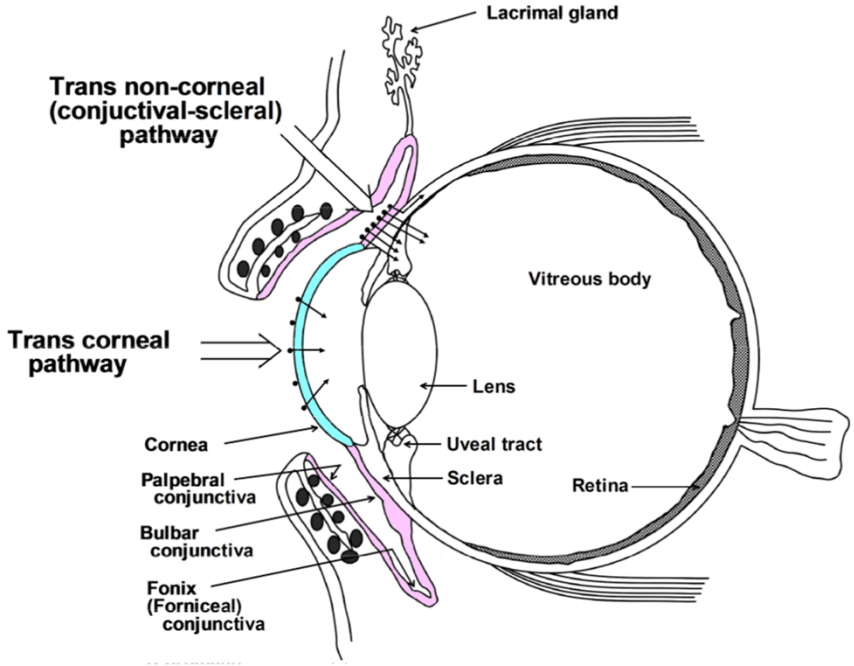
36th JSSX Annual Meeting
COI disclosure information

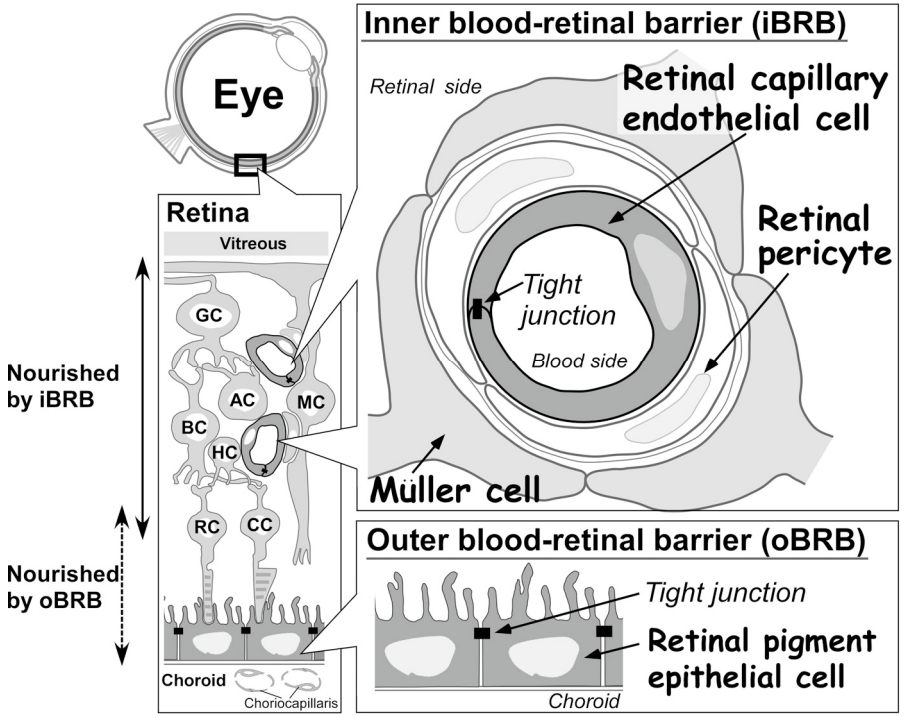
Author : Ken-ichi Hosoya

I have no financial relationship to disclose for my presentation contents.

Concept of my research



Hosoya, Lee, Kim. ***Eur J Pharm Biopharm*** 60: 227-240 (2005).



Hosoya, Tomi. ***Biol Pharm Bull*** 28:1-8 (2005).

Development

- **Elucidation of transporters and transport systems at the BRB**
- **Establishment of the *in vitro* model of BRB**
- **Evaluation of efflux transport across the BRB**

Therapy of retinal diseases by systemic drug administration （飲む目薬）

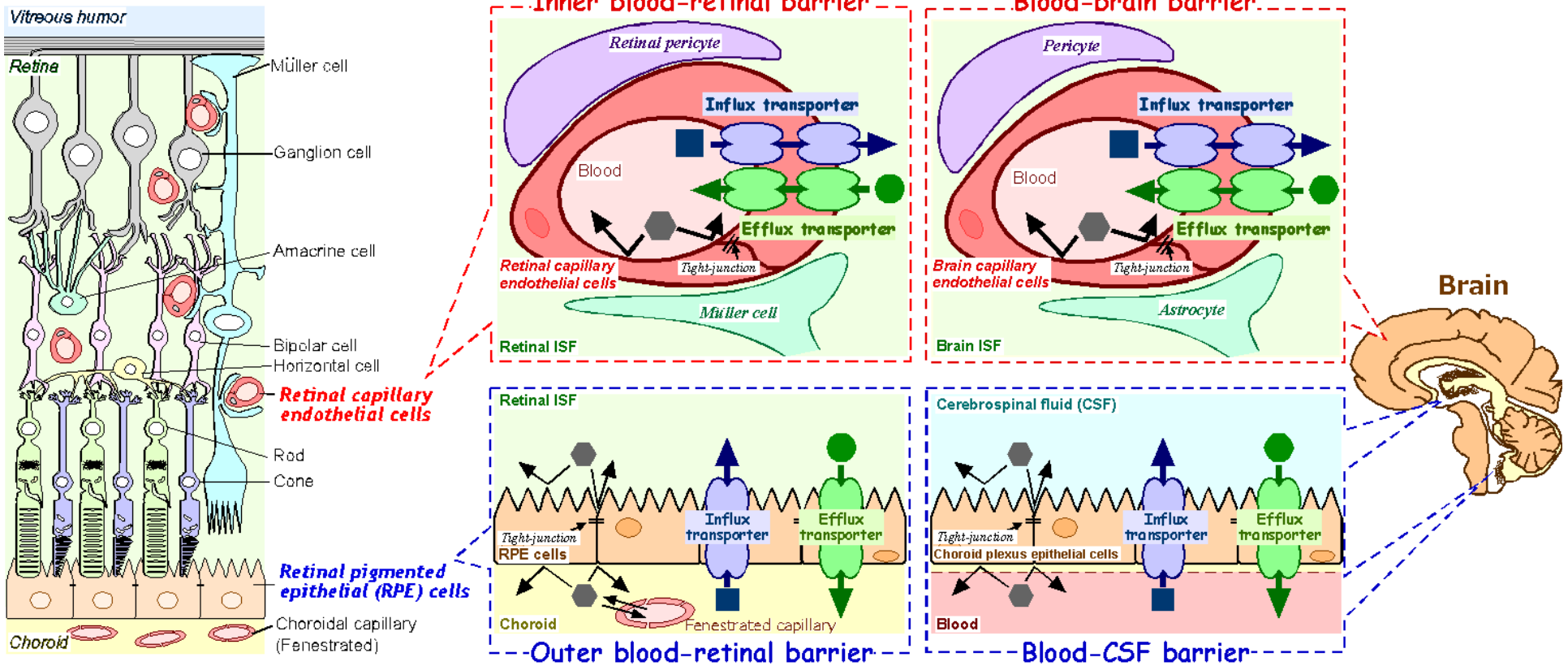
BRB vs. brain barriers | drug transport manner

Pharm Res (2010) 27:2715–2724
DOI 10.1007/s11095-010-0272-x

RESEARCH PAPER

Lipophilicity and Transporter Influence on Blood-Retinal Barrier Permeability: A Comparison with Blood-Brain Barrier Permeability

Ken-ichi Hosoya • Atsushi Yamamoto • Shin-ichi Akanuma • Masanori Tachikawa



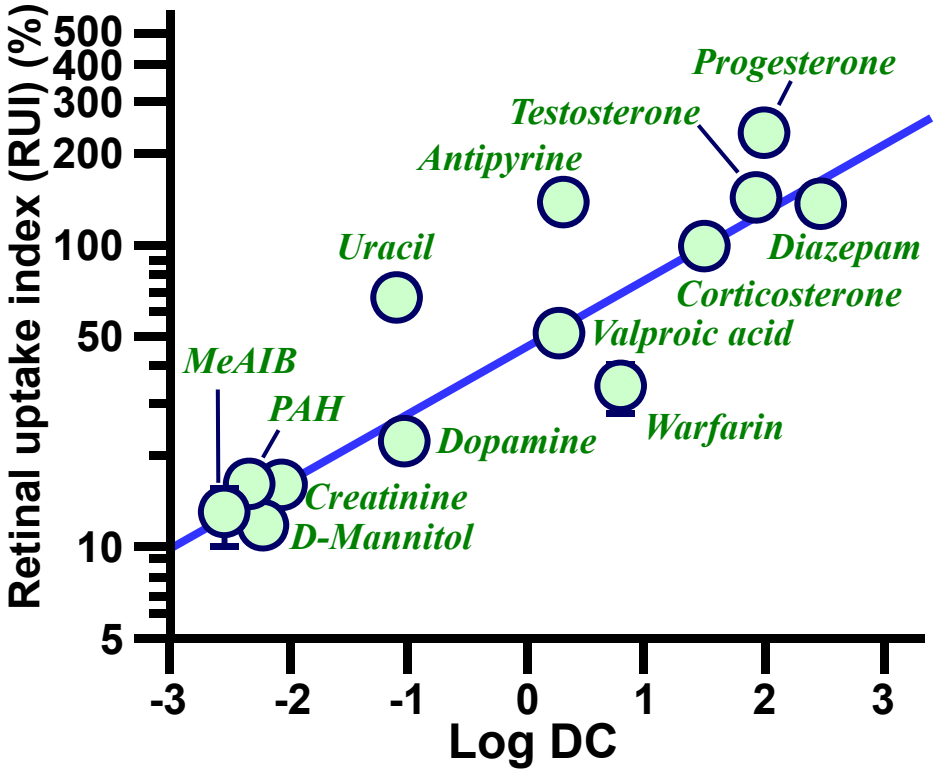
Kubo et al. 細胞工学 32:950-954 (2013).

Correlation of RUI and BUI with DC of compounds

Transportable via passive diffusion

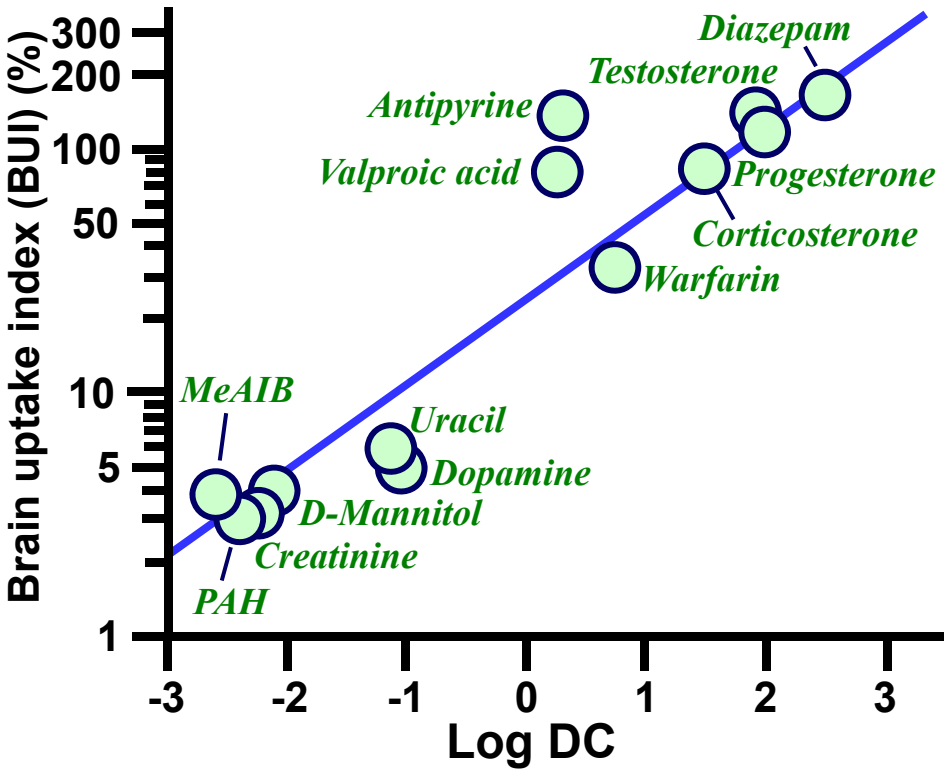
Retina

$RUI = 46.2 \times \exp(0.515 \times \log DC)$



Brain

$BUI = 24.2 \times \exp(0.816 \times \log DC)$

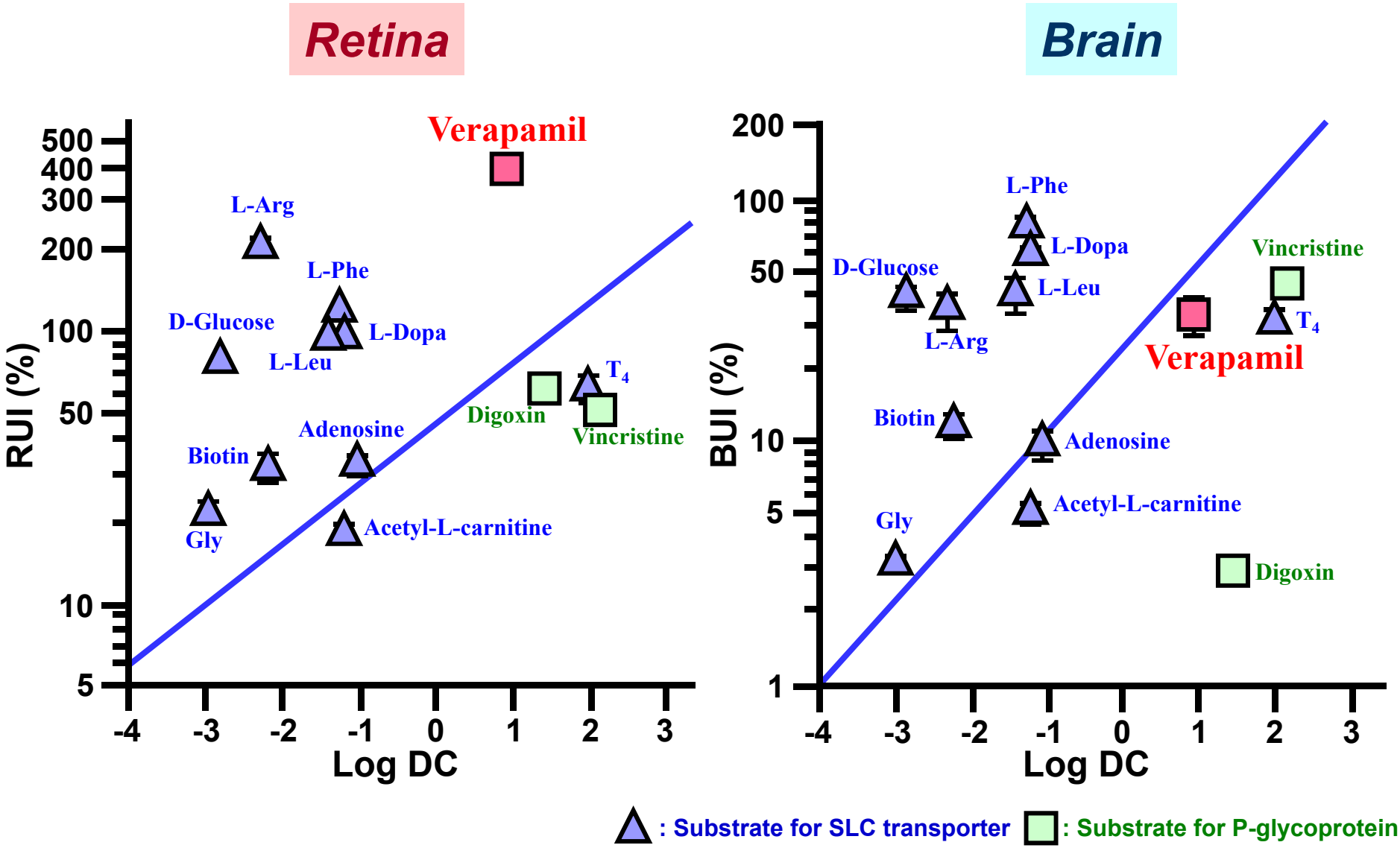


Each point represents the mean ± SEM (n = 3-9). DC, n-octanol/Ringer buffer (pH 7.4) distribution coefficient.

Hosoya et al. ***Pharm Res*** 27:2715-2724 (2010)

Correlation of RUI and BUI with DC of compounds

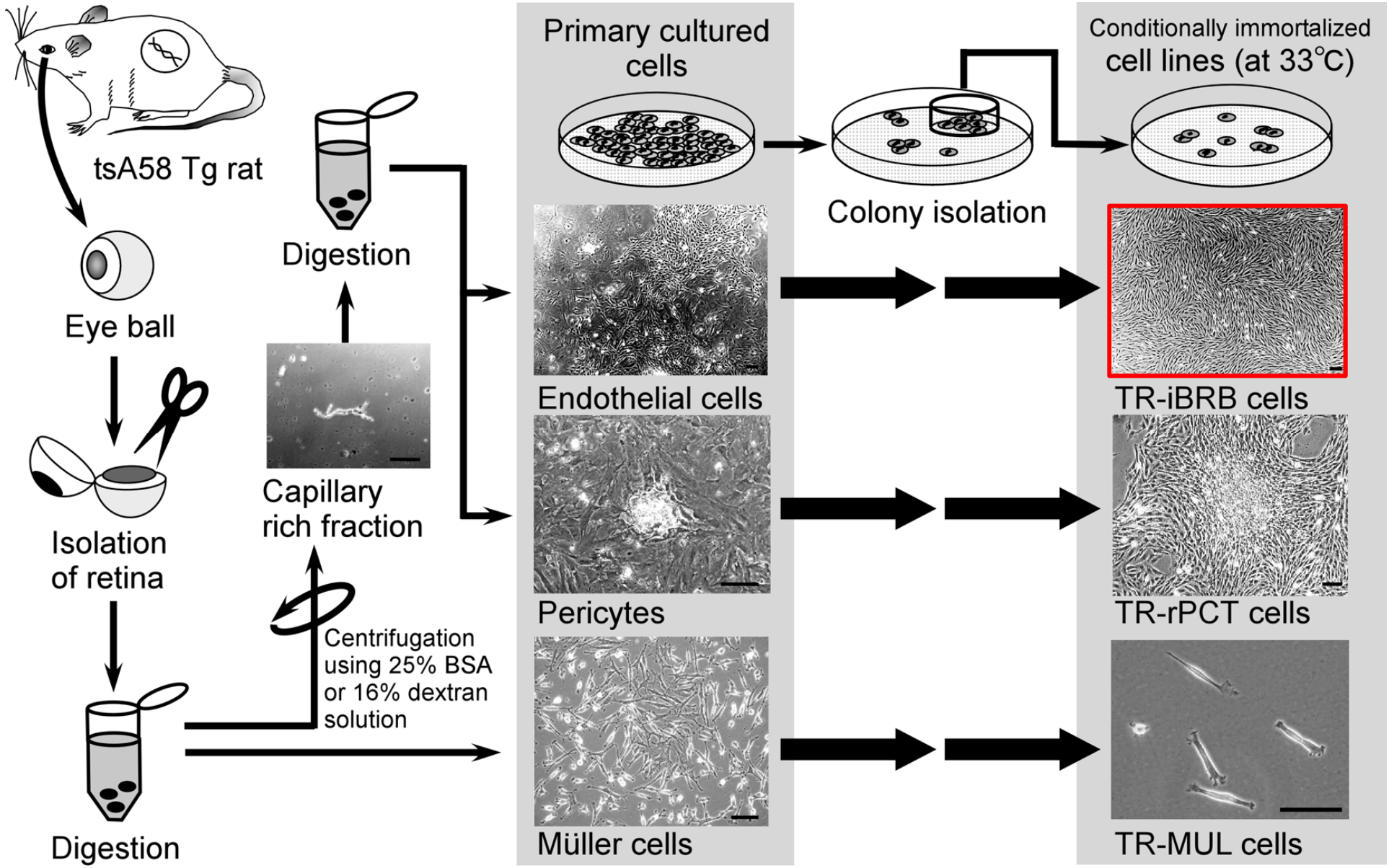
Transporter substrates



Each point represents the mean ± SEM (n = 3-6).

Hosoya et al. ***Pharm Res*** 27:2715-2724 (2010)

Conditionally-immortalized inner BRB cell lines

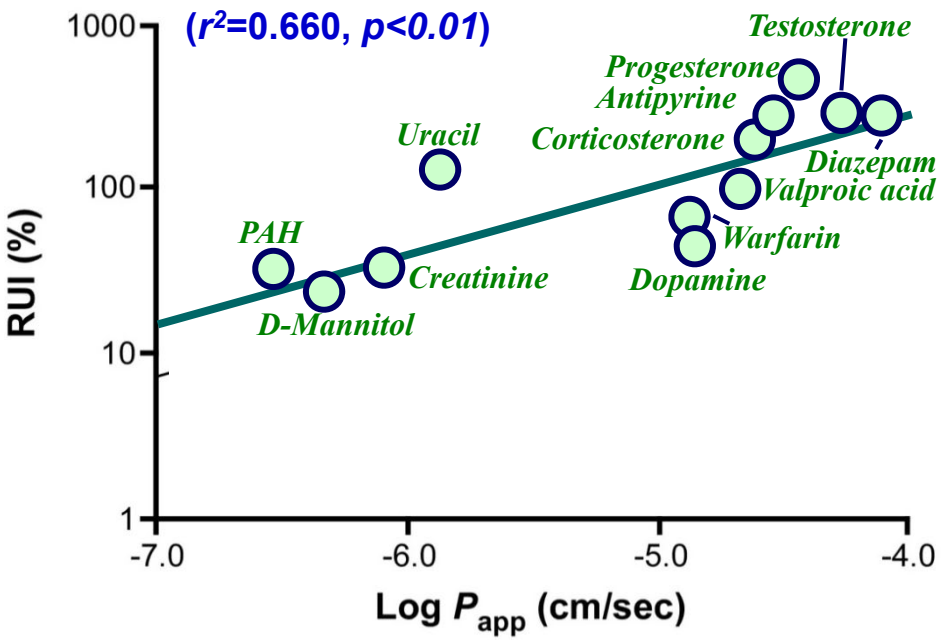


Hosoya et al. ***Exp Eye Res*** 72:163-172 (2001). Hosoya, Tomi. ***Biol Pharm Bull*** 28:1-8 (2005).

Compounds and drugs: **Passive diffusion**

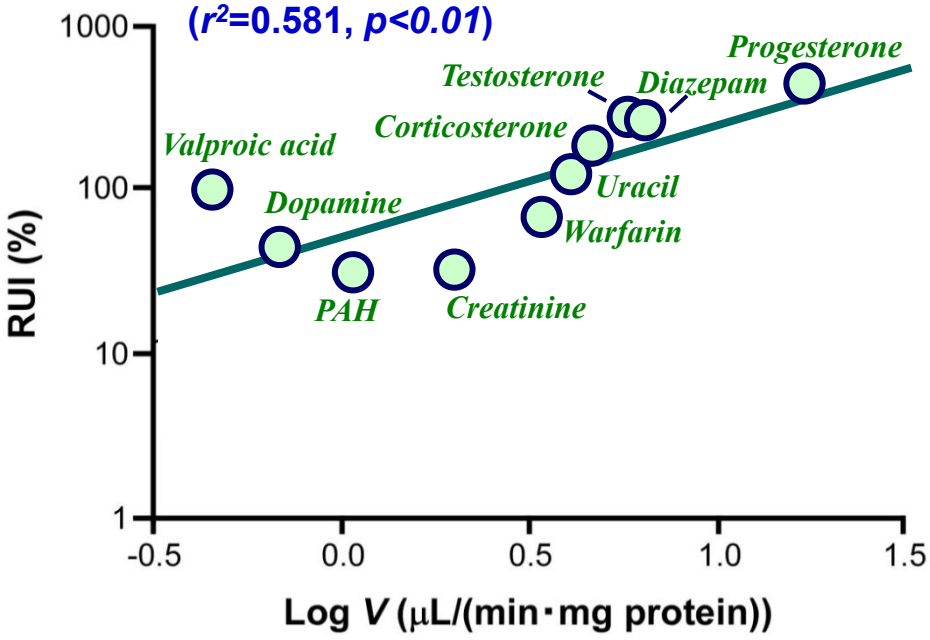
PAMPA vs. RUI

RUI = 7.93 x 10^3 x exp(0.994 x log P_{app})



TR-iBRB2 uptake vs. RUI

RUI = 26.5 x exp(1.55 x log V)



Comparison of the apparent permeability (P_{app}) and initial uptake rate (V), and the RUI value of compounds and drugs

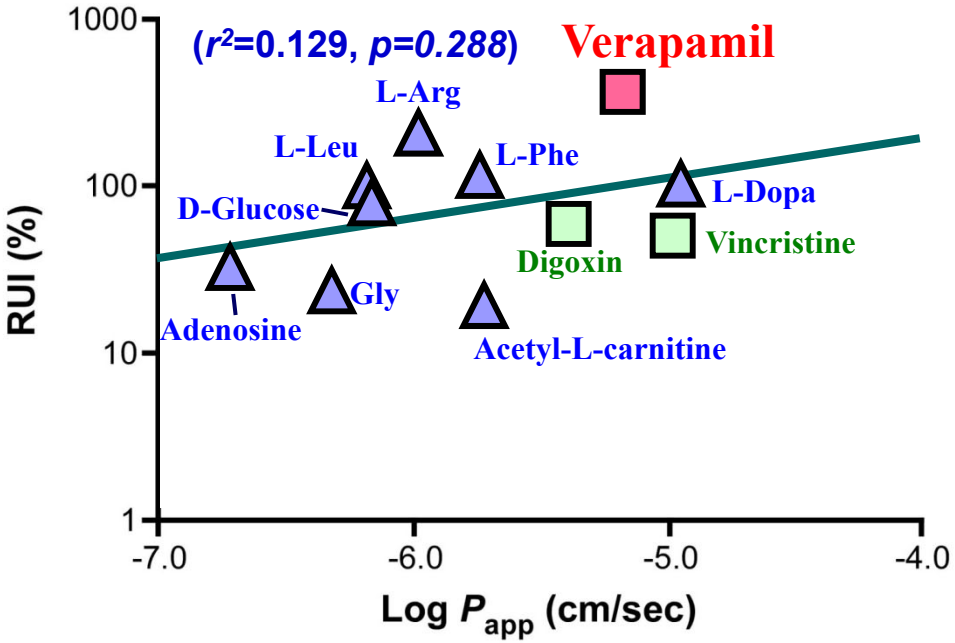
Kubo et al. *J Pharm Sci* 101:2596-2605 (2012).

In vivo vs. in vitro

Compounds and drugs: **Carrier-mediated transport type**

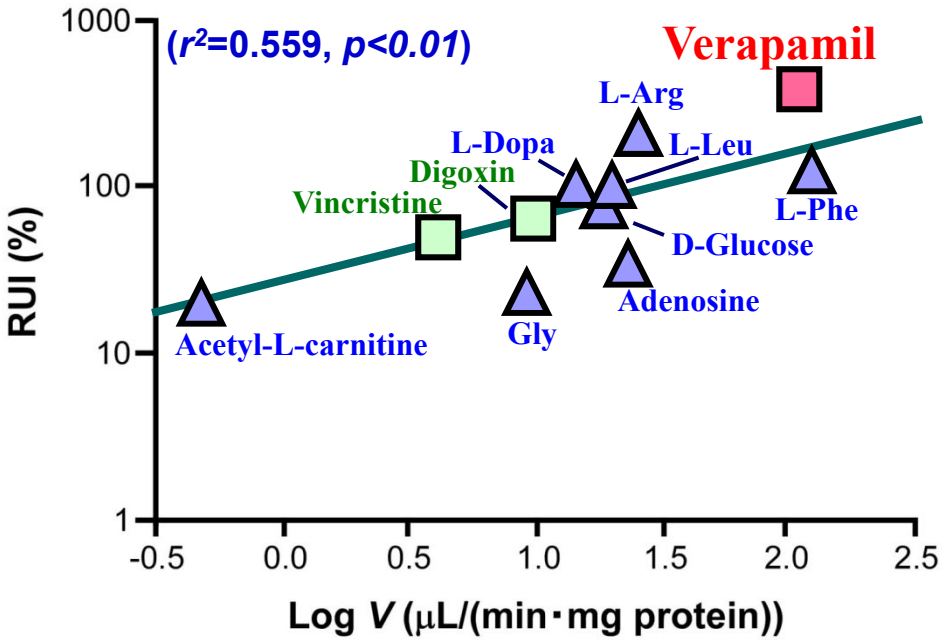
PAMPA vs. RUI

RUI = 1.83x 10^3 x exp(0.564 x log P_{app})



TR-iBRB2 uptake vs. RUI

RUI = 26.5 x exp(0.887 x log V)



△ : Substrate for SLC transporter □ : Substrate for P-glycoprotein

TR-iBRB2 cells: good tool for prediction of *in vivo* BRB influx transport

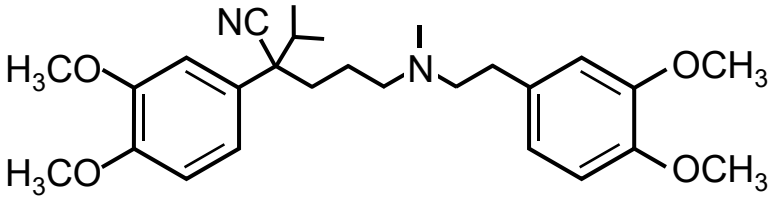
Comparison of the apparent permeability (P_{app}) and initial uptake rate (V), and the RUI value of compounds and drugs

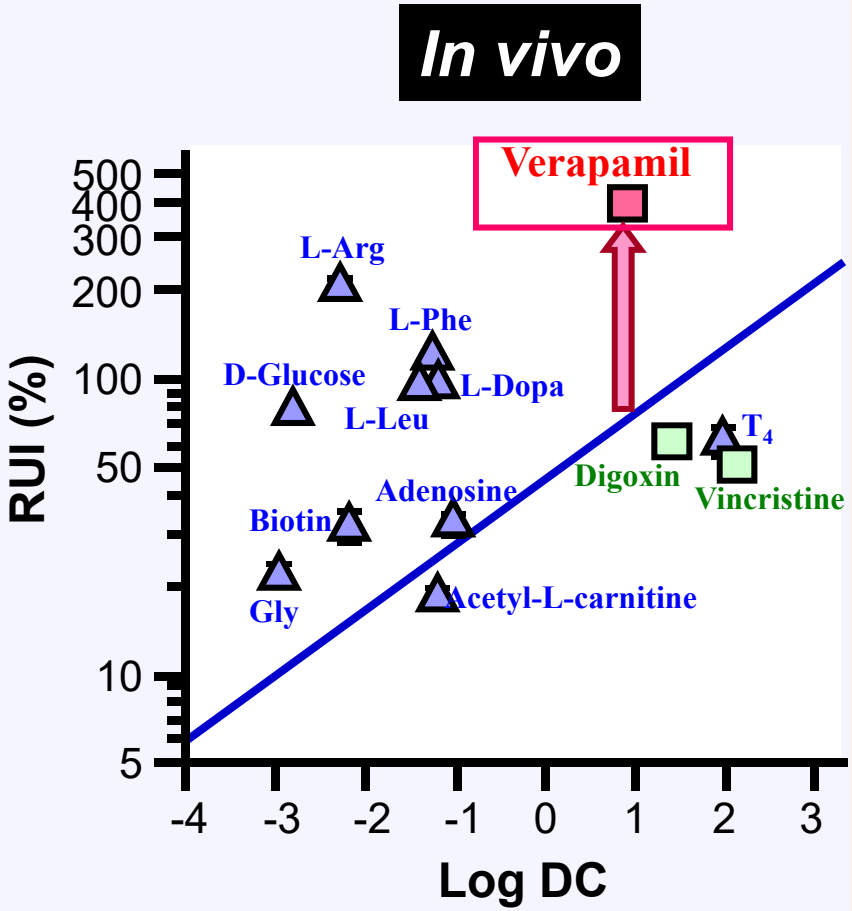
Kubo et al. *J Pharm Sci* 101:2596-2605 (2012).

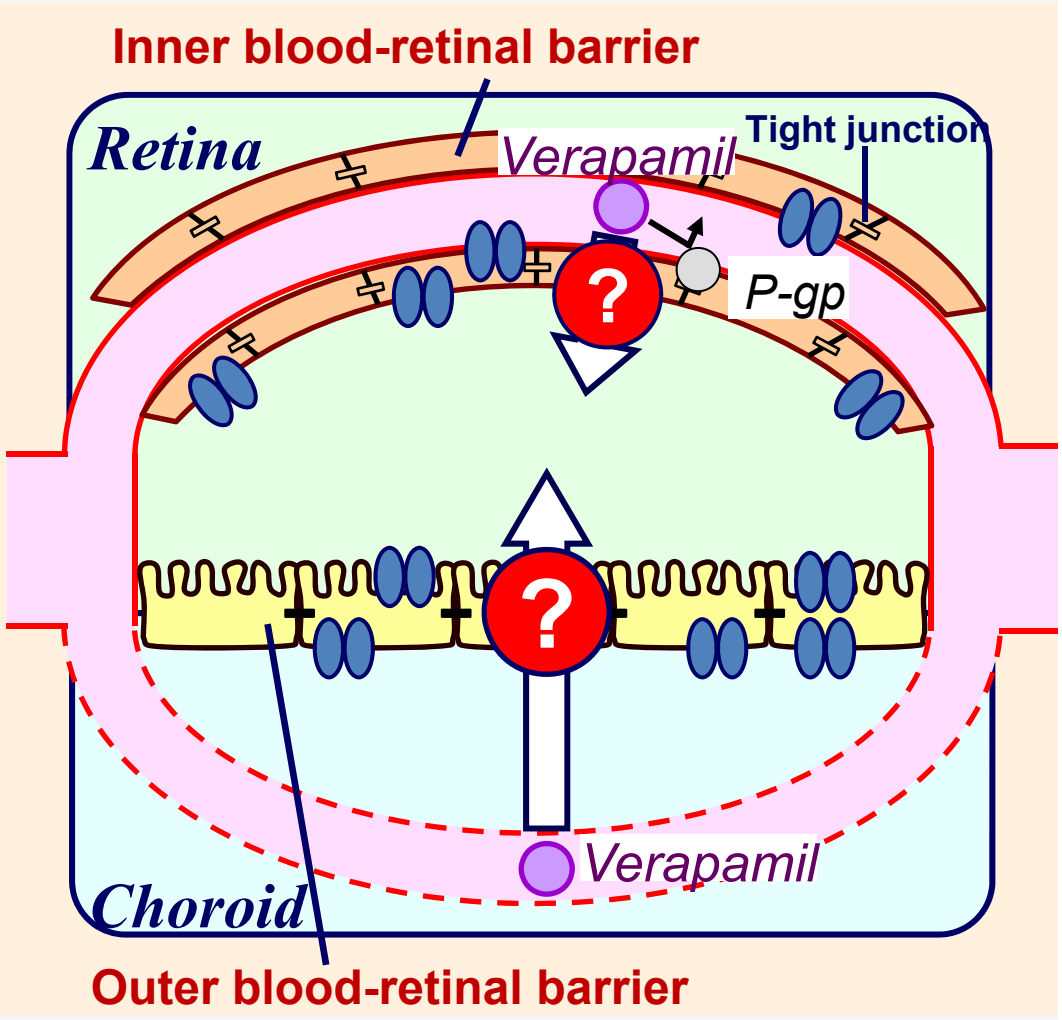
Verapamil | Great *in vivo* retinal transport activity at the BRB

Verapamil (Ca^{2+}-channel blocker)

➡ **Anti-glaucoma effect**

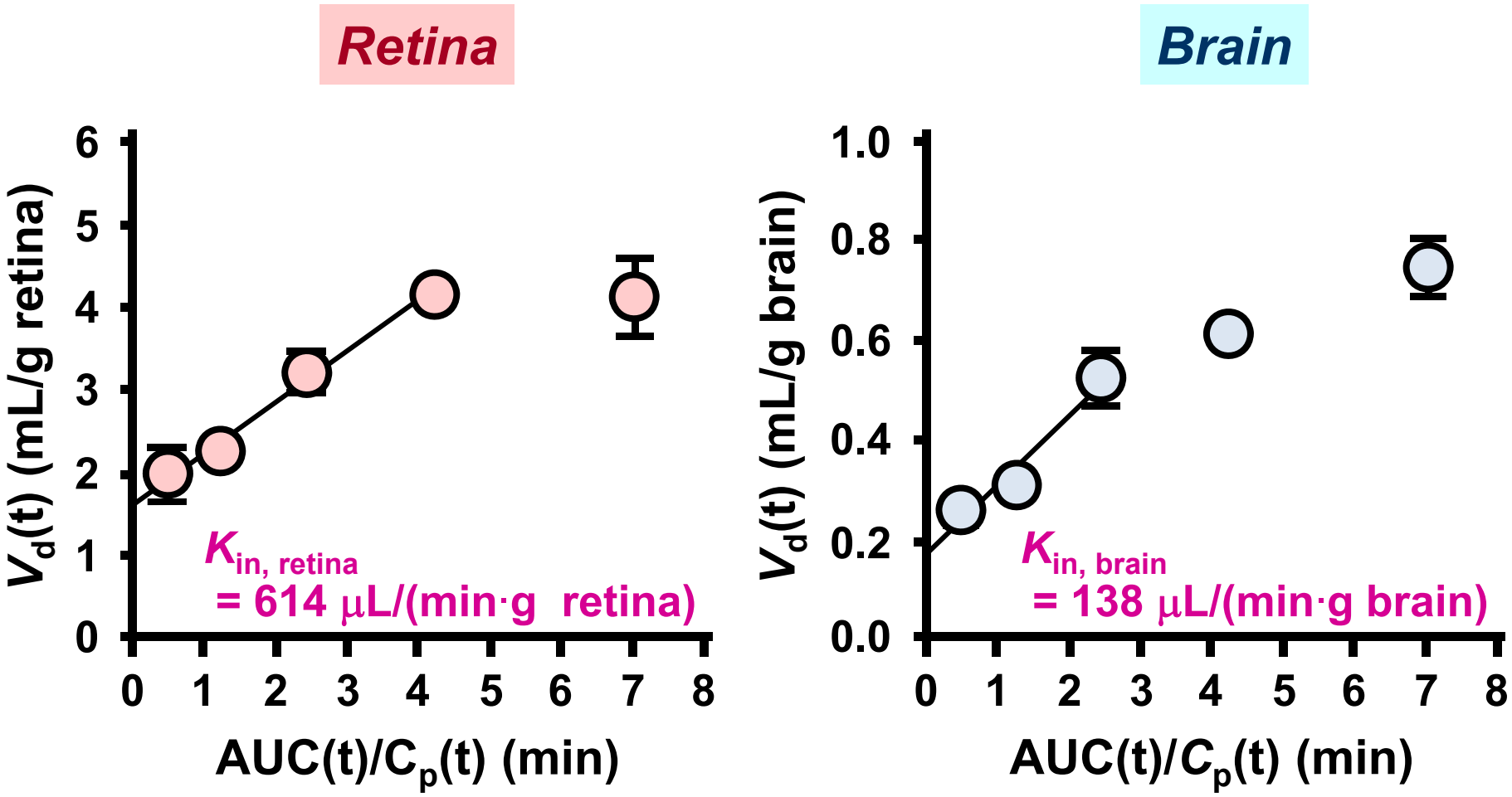






△ : Substrate for SLC transporter ▭ : Substrate for P-glycoprotein

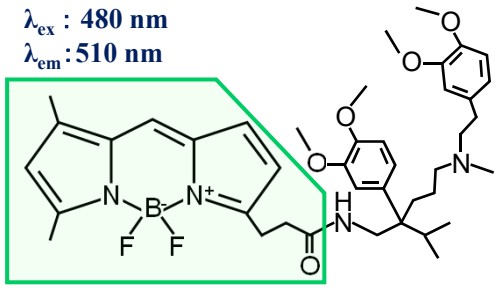
In vivo integration plot analysis of [^{3}H]verapamil



Each point represents the mean ± SEM (n = 3-4).

Kubo et al. ***Pharm Res*** 30:847-856 (2013).

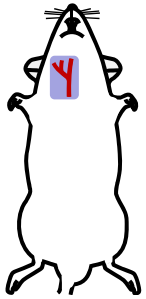
In vivo retinal distribution of BODIPY® FL verapamil



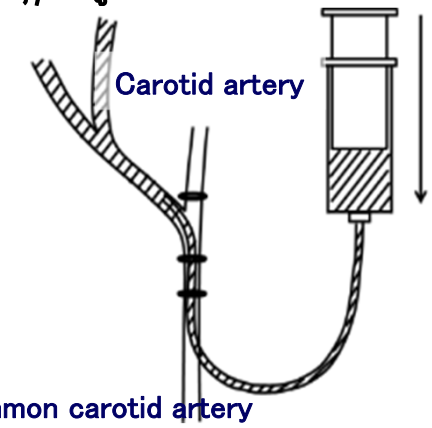
BODIPY® FL verapamil

Infusion

Wistar rat

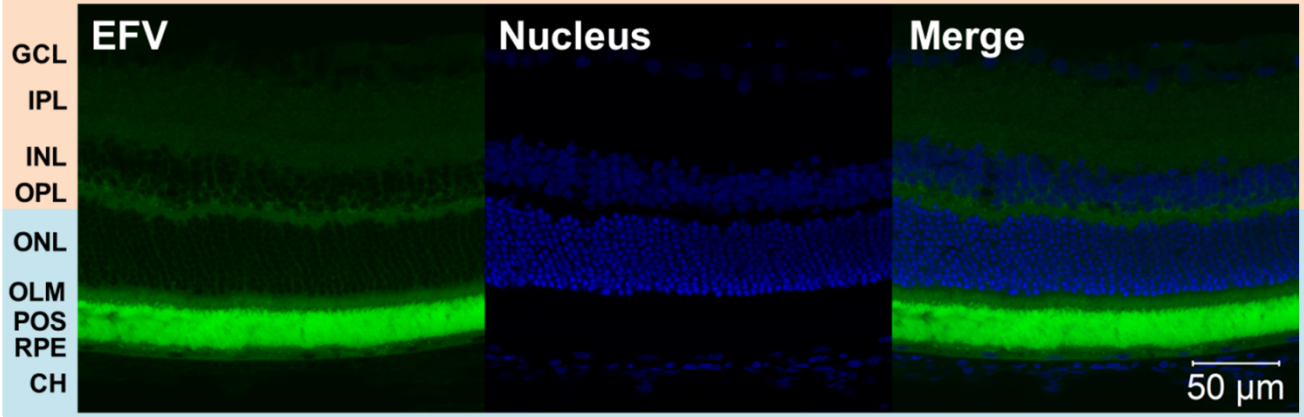


Total amount : 400 µg
Infusion: 0.85 mL/min
for 4 min



BODIPY® FL verapamil (EFV) infusion (400 µg)

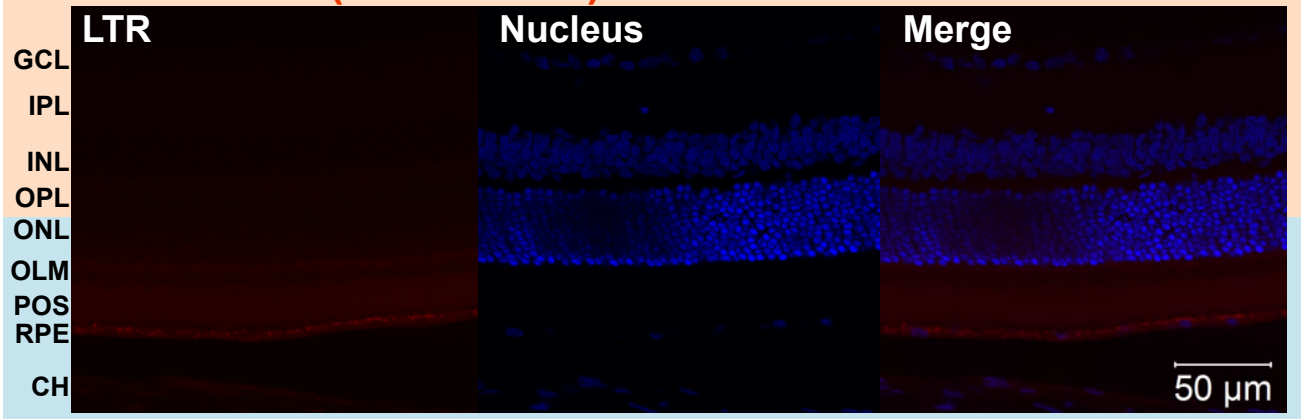
Inner BRB (ILM - OPL)



Outer BRB (ONL - RPE)

Lysotracker®Red (Lysosome marker) infusion

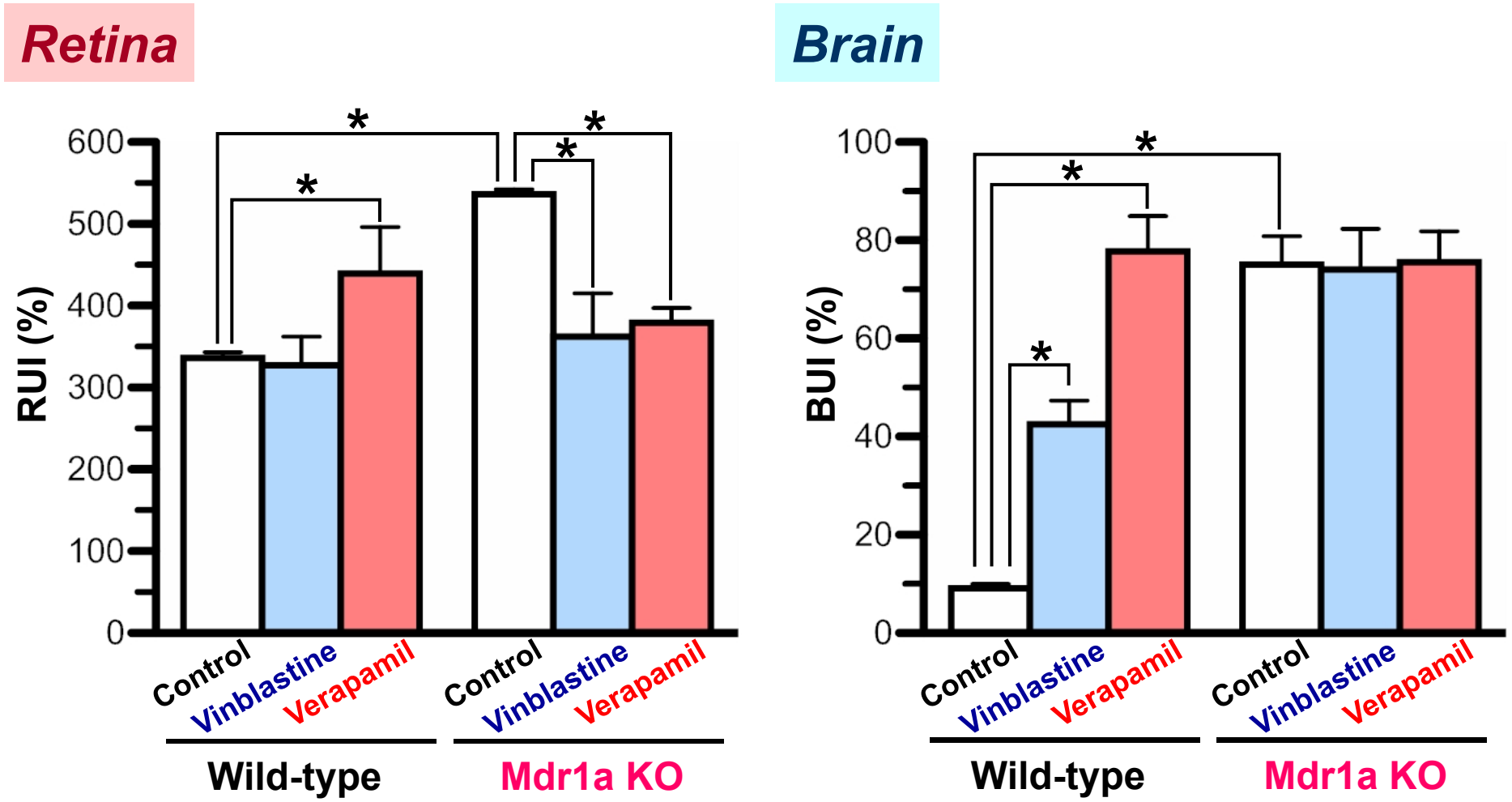
Inner BRB (ILM - OPL)



Outer BRB (ONL - RPE)

Kubo et al. ***Pharm Res*** 35:93 (2018).

Retinal uptake index of [^{3}H]verapamil in P-gp-deficient rats



Each column represents the mean ± SEM (n = 3-6). *p<0.05, significant difference.

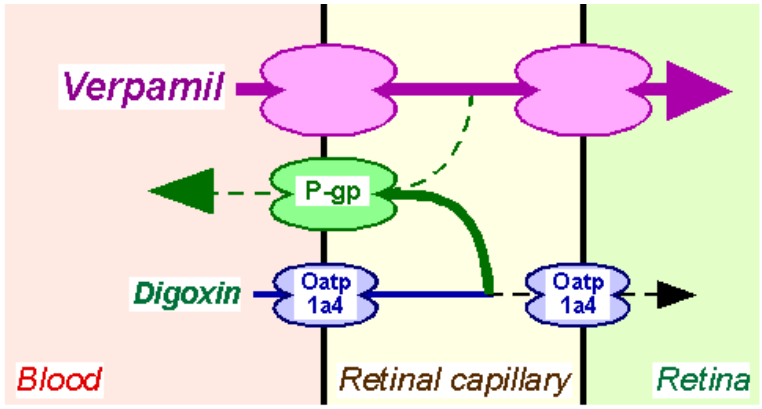
Fujii et al. ***Invest Ophthalmol Vis Sci*** 55:4650-4658 (2014).

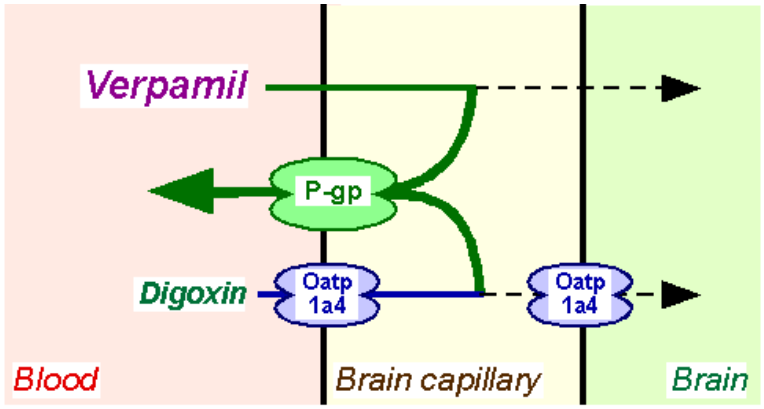
Verapamil transport at the BRB

Blood-retinal barrier

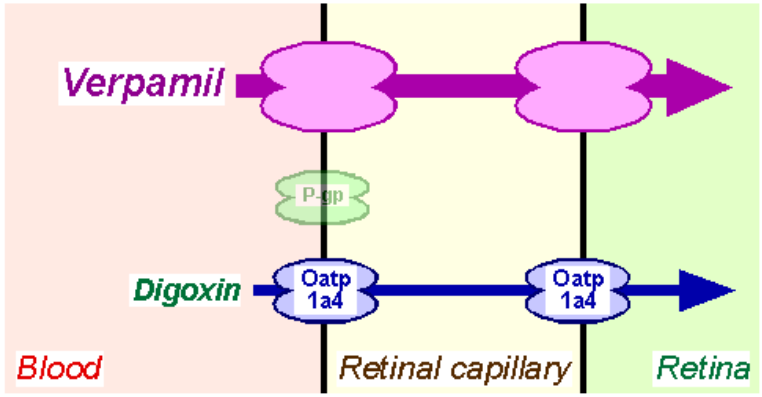
Blood-brain barrier

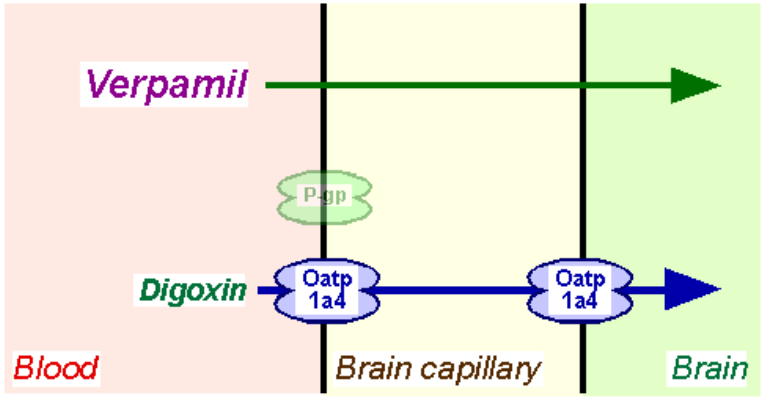
Wild-type



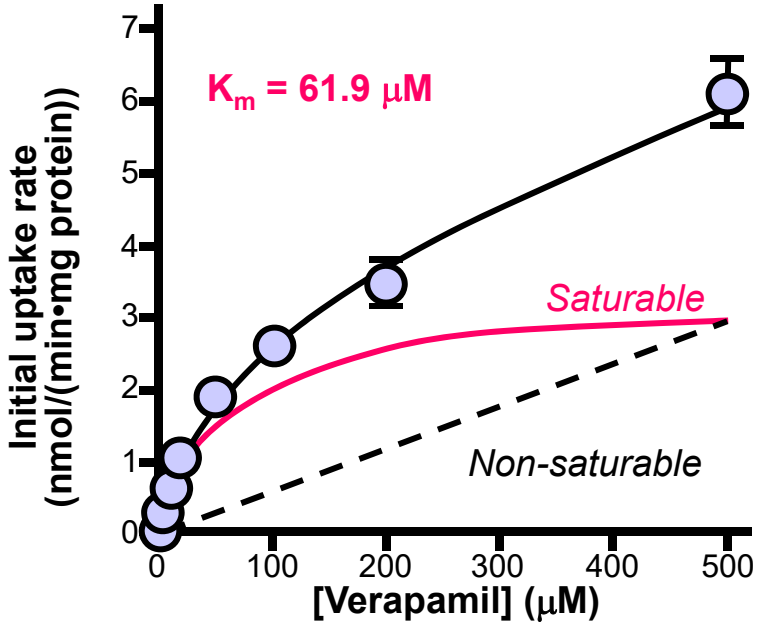


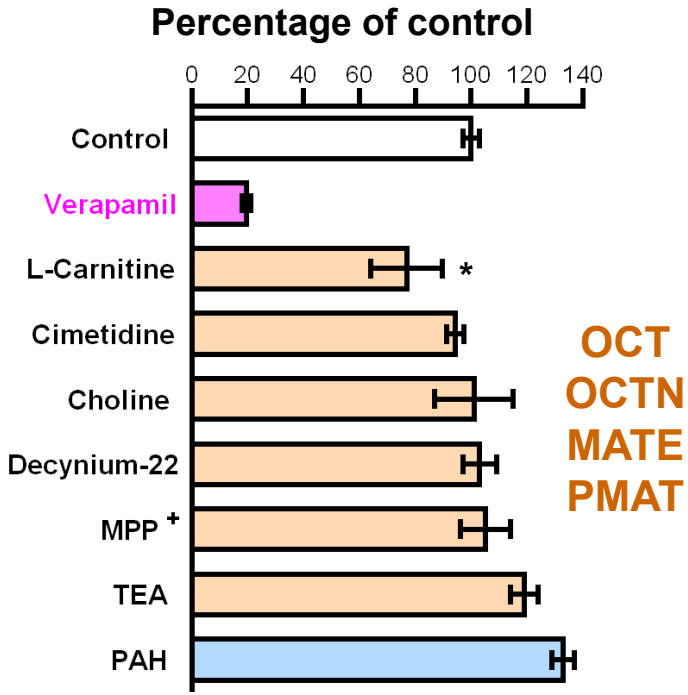
Mdr1a knockout



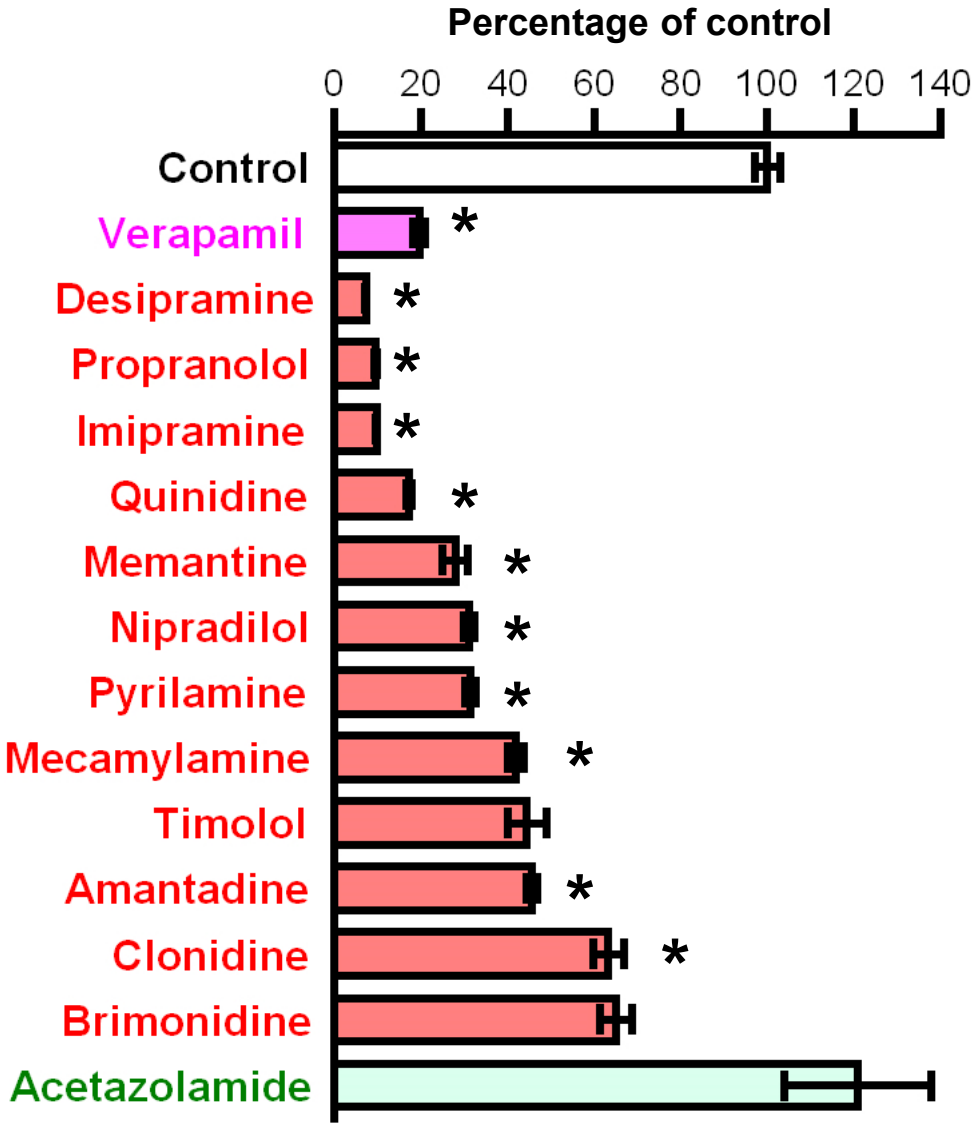


In vitro [^{3}H]verapamil transport in TR-iBRB2 cells





Effect of drugs (500 μM)

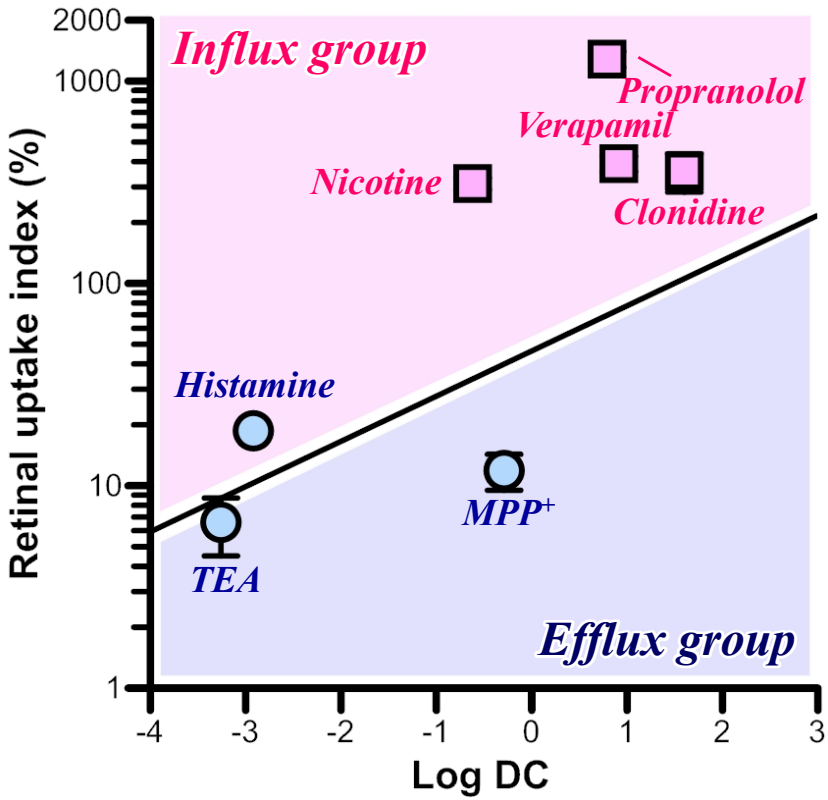


*p<0.01, significantly different from the control (n = 3-16).

Kubo et al. ***Pharm Res*** 30:847-856 (2013).

Cationic drug transport systems at the BRB

Cationic drugs/compounds



Substrate

- Verapamil
- Propranolol
- Clonidine

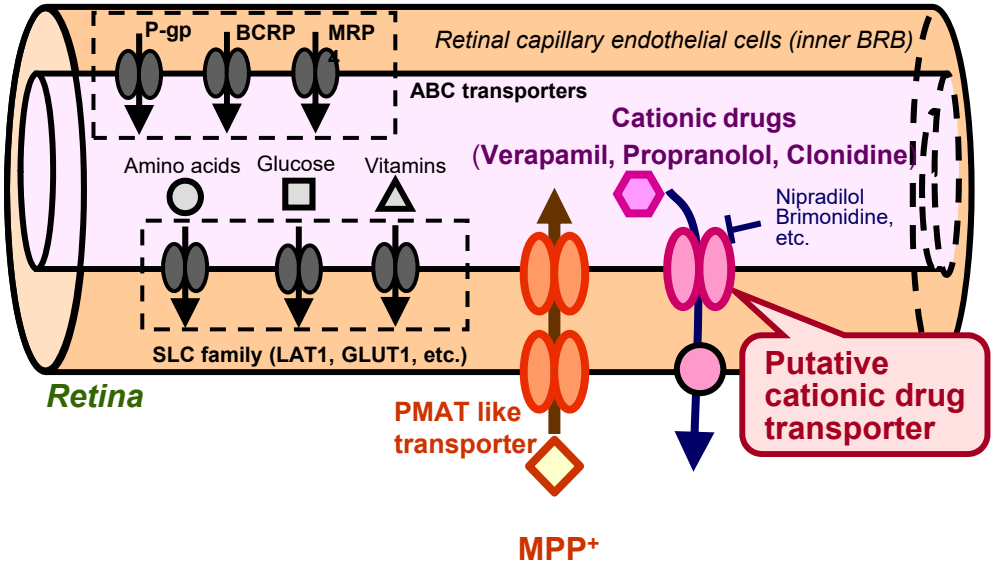
+

Recognition

- Anti-angiogenesis drugs
- Neuroprotectant

Drug delivery to treat…

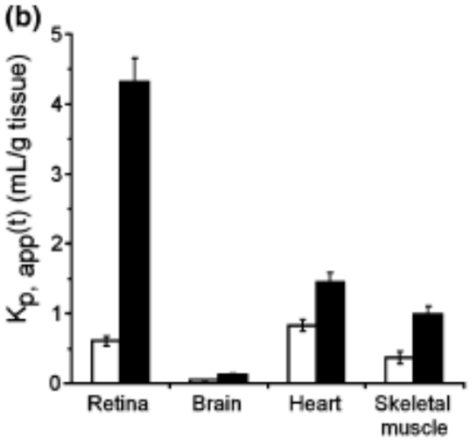
- Diabetic retinopathy
- Glaucoma



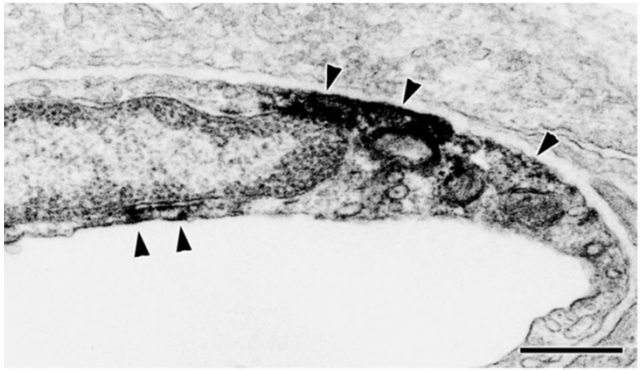
Kubo et al. ***J Pharm Sci*** 101:2596-2605 (2012); Kubo et al. ***Pharm Res*** 30:847-856 (2013); Kubo et al. ***Mol Pharm*** 11:3747-3753 (2014).

Creatine (Cr) transport across the BRB

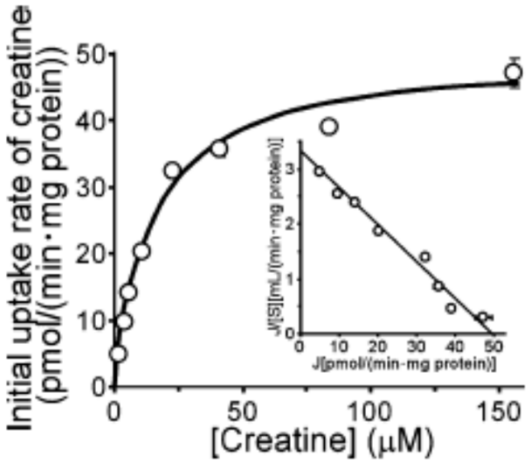
Kp of creatine



CRT expression at the inner BRB

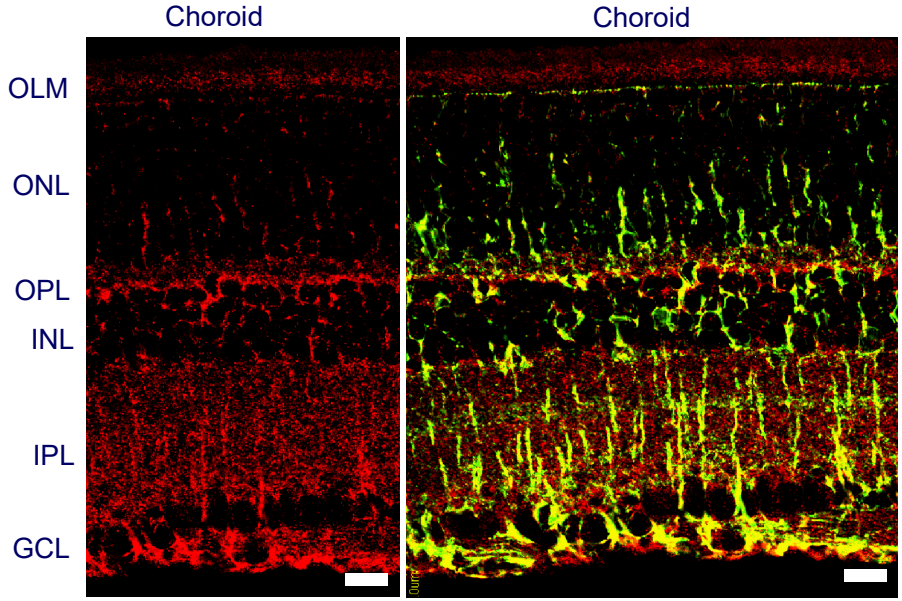


Creatine uptake by TR-iBRB cells



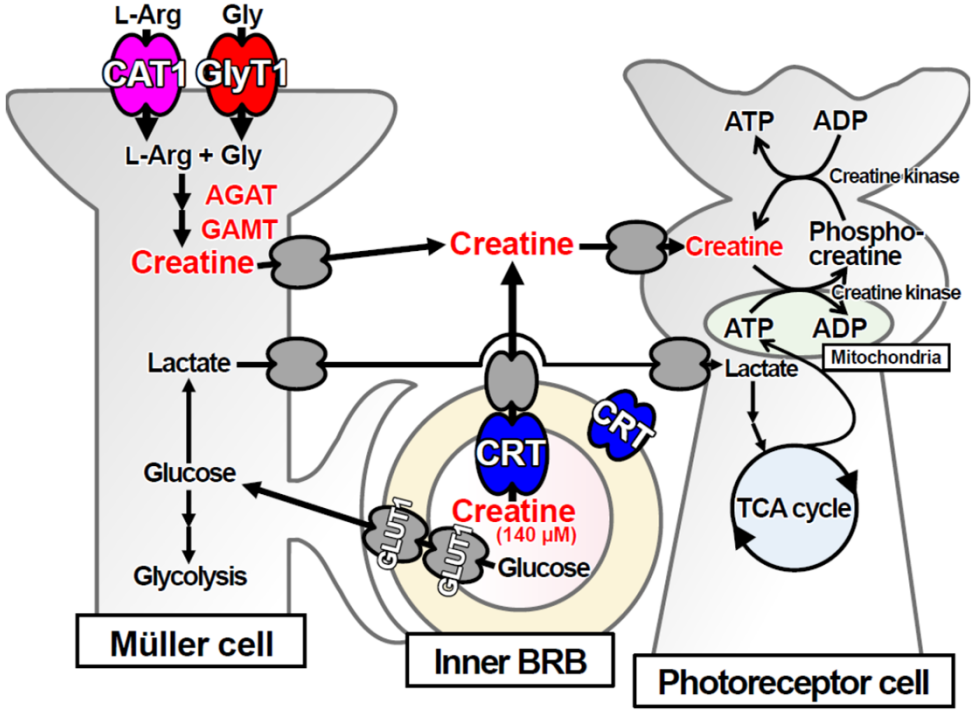
Nakashima et al. ***J Neurochem*** 89:1454-1461 (2004).

GAMT localization in the retina

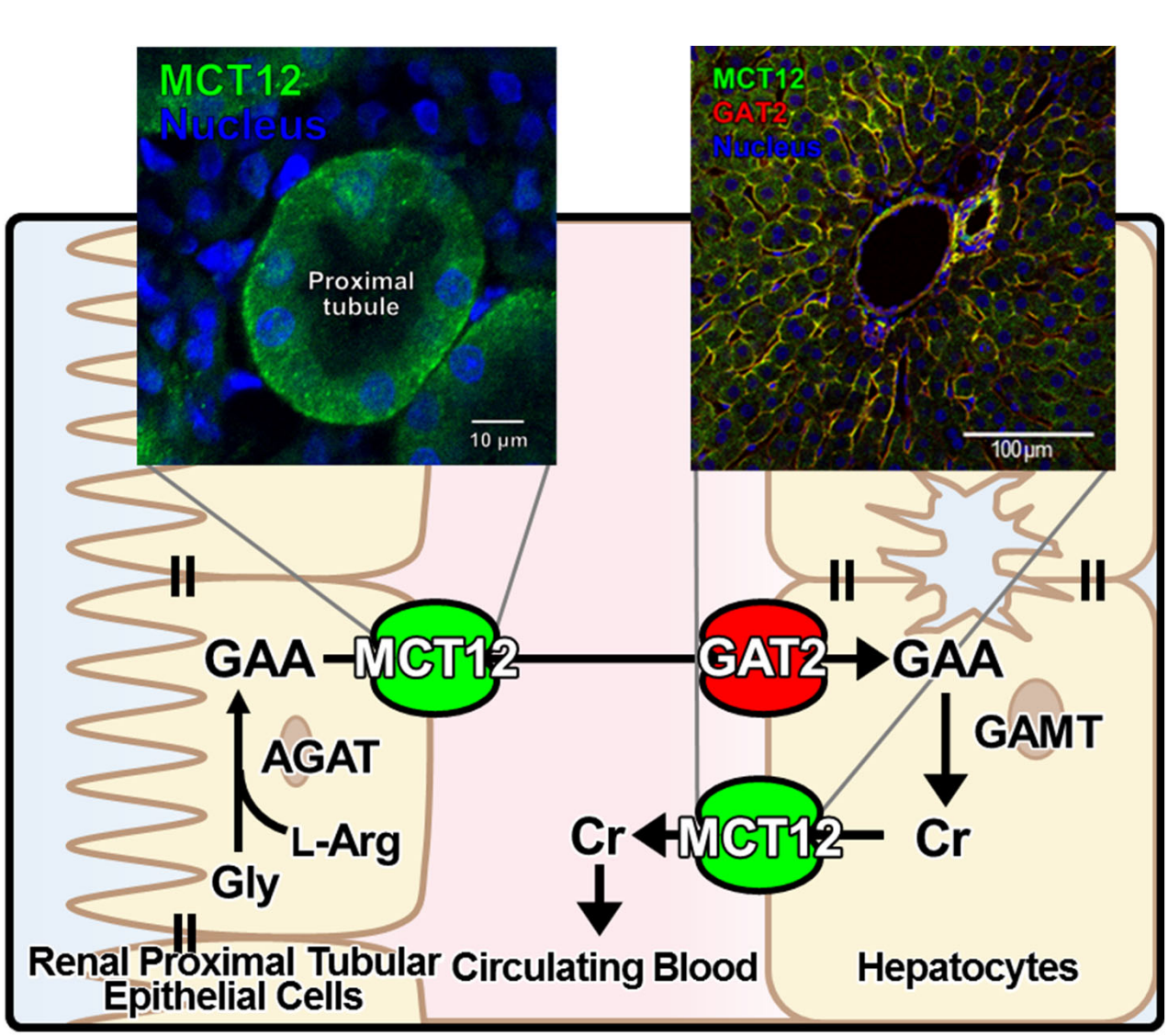


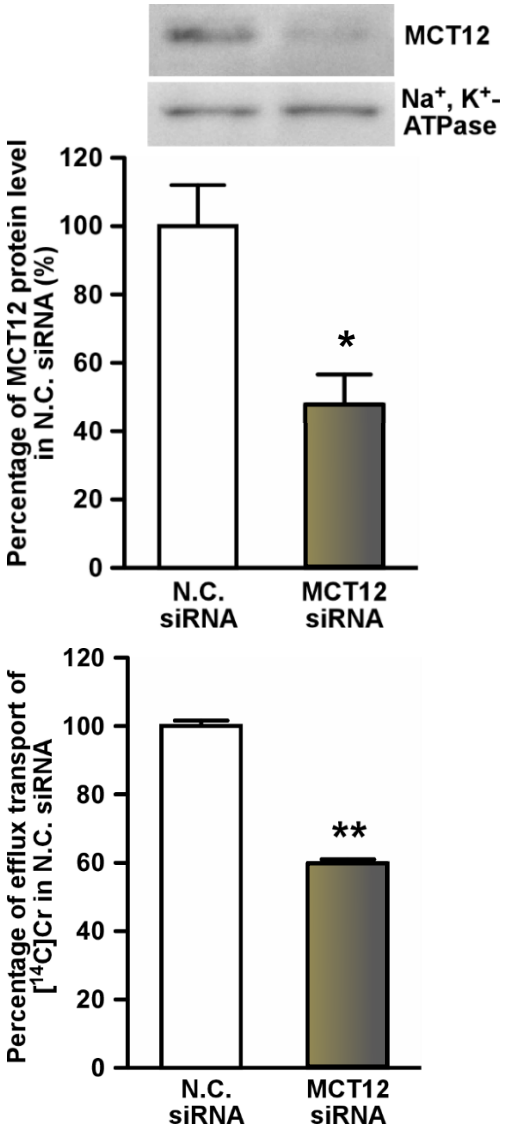
Red: GAMT Green: Glutamine synthetase

Nakashima et al. ***Glia*** *52*:47-52 (2005).



Involvement of GAT2/SLC6A13 and MCT12 in the process of Cr biosynthesis





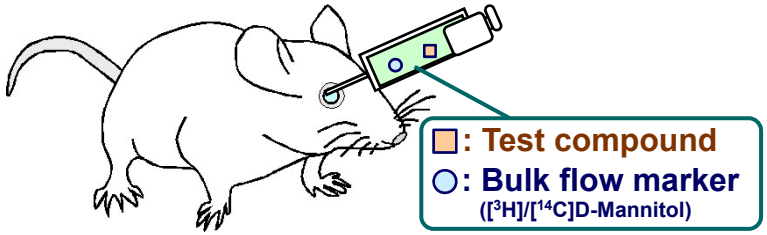
*p < 0.05, **p < 0.01 significantly different from the condition of negative control (N.C.) siRNA transfection.

Tachikawa et al. ***PLoS One*** 7: e32557 (2012); Jomura et al. ***Biochim Bipphys Act Biomembr*** 862:183434 (2020);
Jomura et al. ***Am J Physiol Gastrointest Liver Physiol*** 321:G113-G122 (2021).

In vivo microdialysis after intravitreous injection

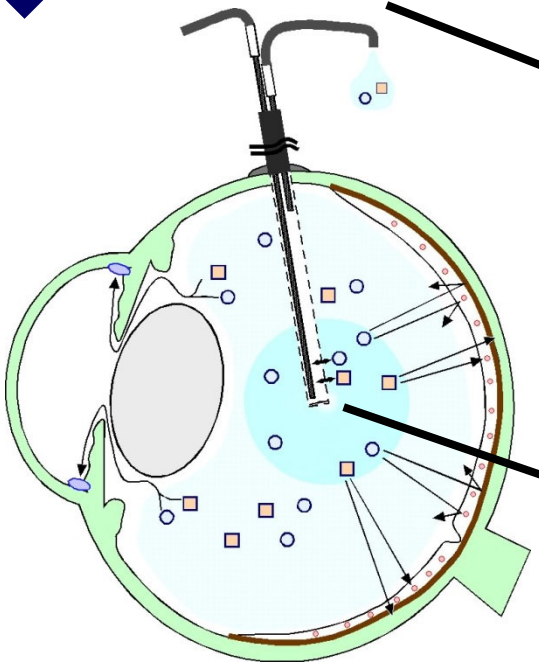
Experimental procedure

Male Wistar rat (~250 g)

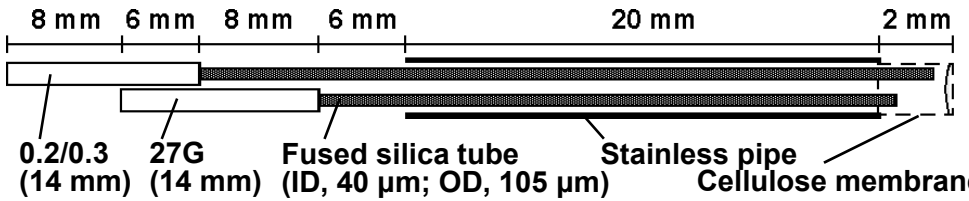


Intravitreous administration (1 μL)

Implantation of microdialysis probe into the vitreous chamber



Customized dialysis probe

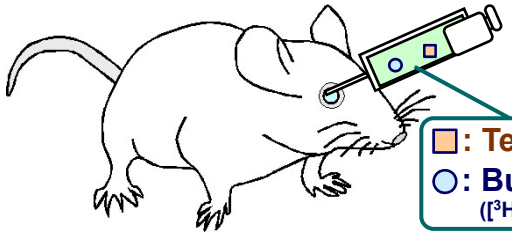


Katayama *et al.* ***J Neurosci Method*** 156:249-56 (2006)

In vivo microdialysis after intravitreous injection

Experimental procedure

Male Wistar rat (~250 g)



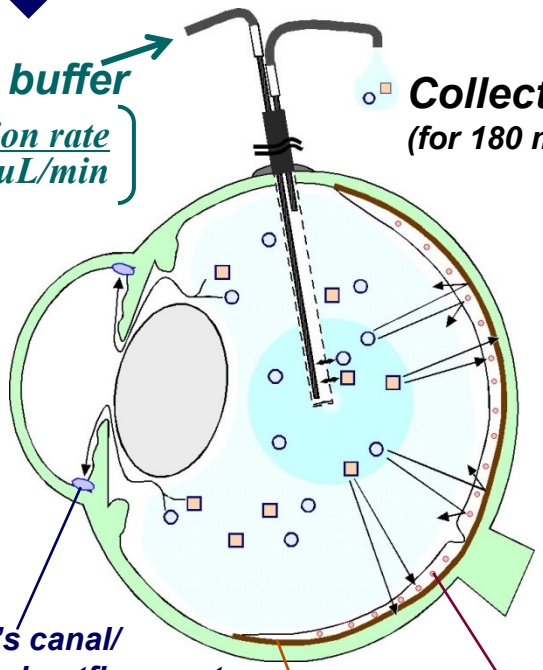
Intravitreous administration (1 μL)

Implantation of microdialysis probe into the vitreous chamber

Ringer buffer

Perfusion rate = 2.2 μL/min

Collection (for 180 min)



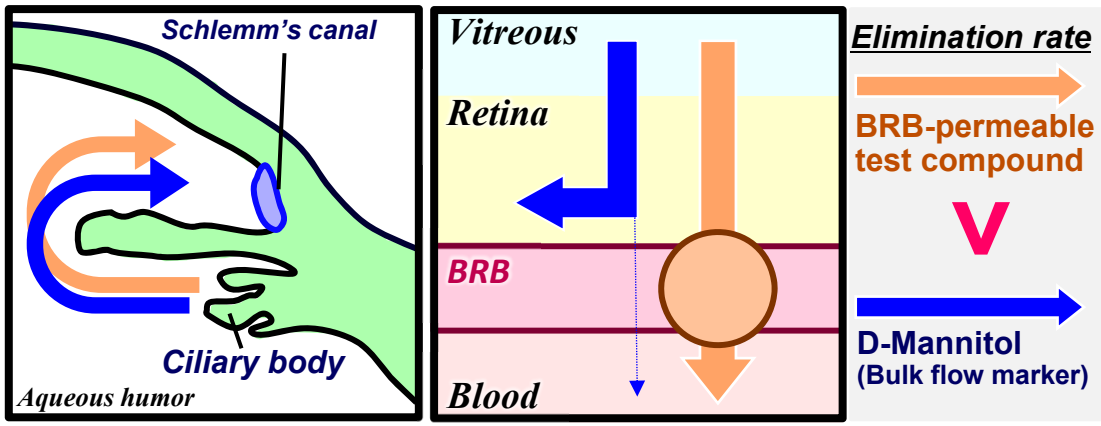
Schlemm's canal/ uveoscleral outflow route

Bulk flow ***Outer BRB*** ***Inner BRB***

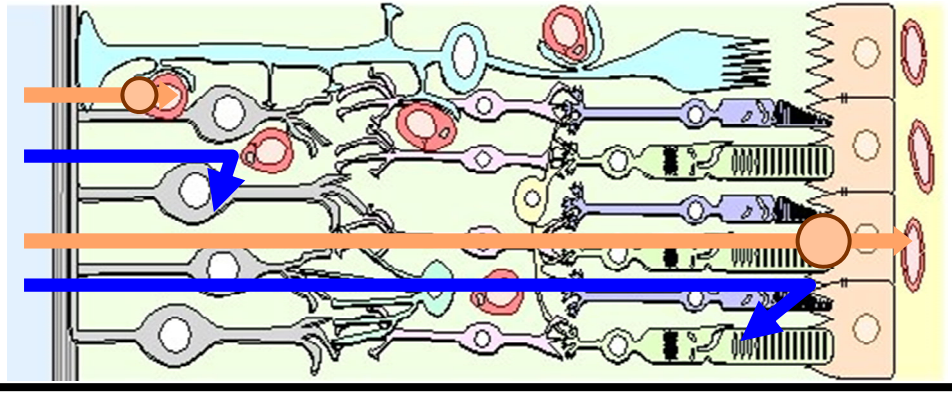
Katayama *et al.* ***J Neurosci Method*** 156:249-56 (2006)

Elimination route from vitreous humor

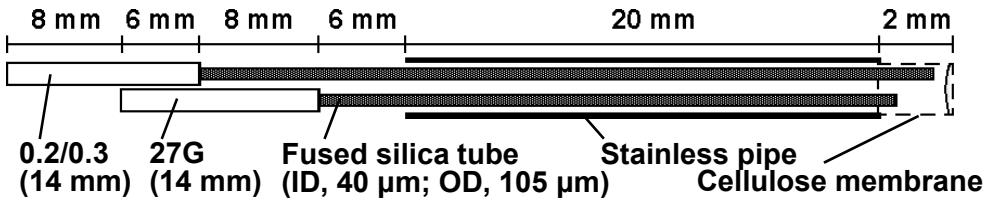
1. Bulk flow ***2. Elimination across the BRB***



Inner BRB **Outer BRB**

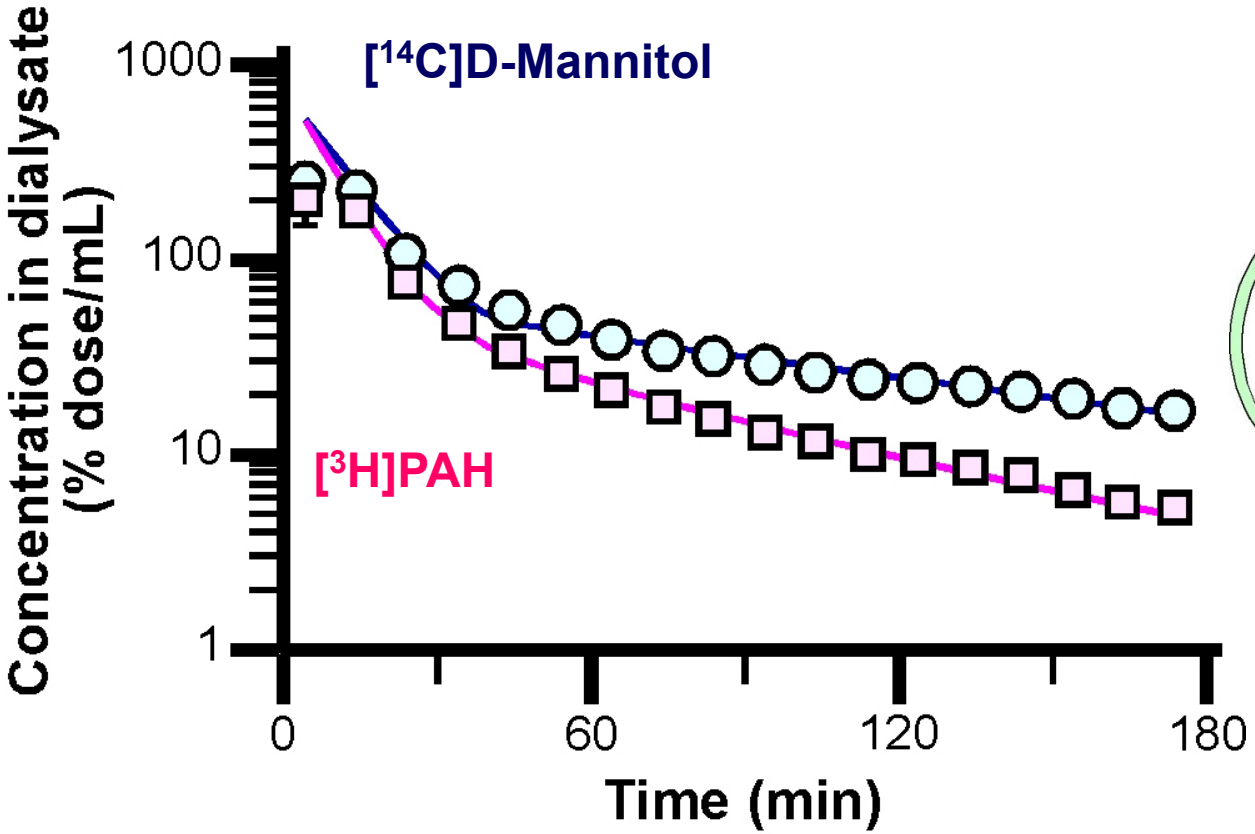


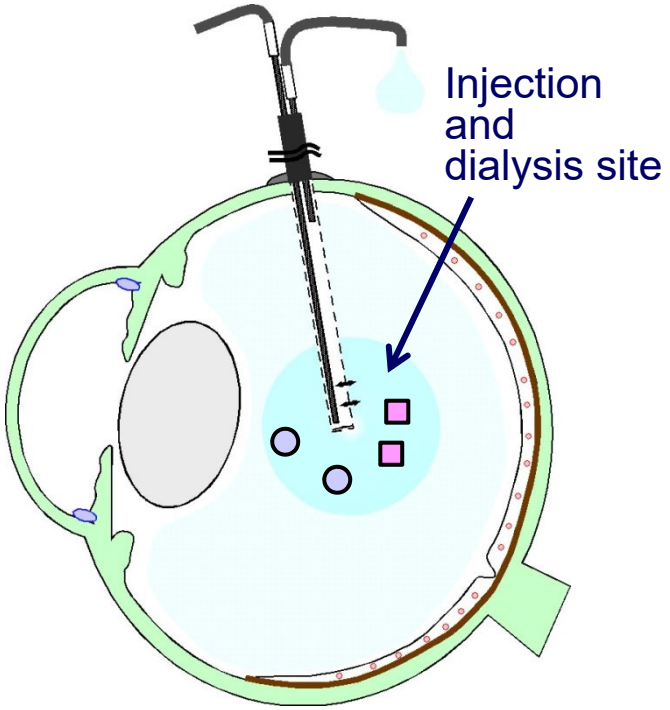
Customized dialysis probe



Elimination of [^{3}H]PAH from rat vitreous humor

Time course





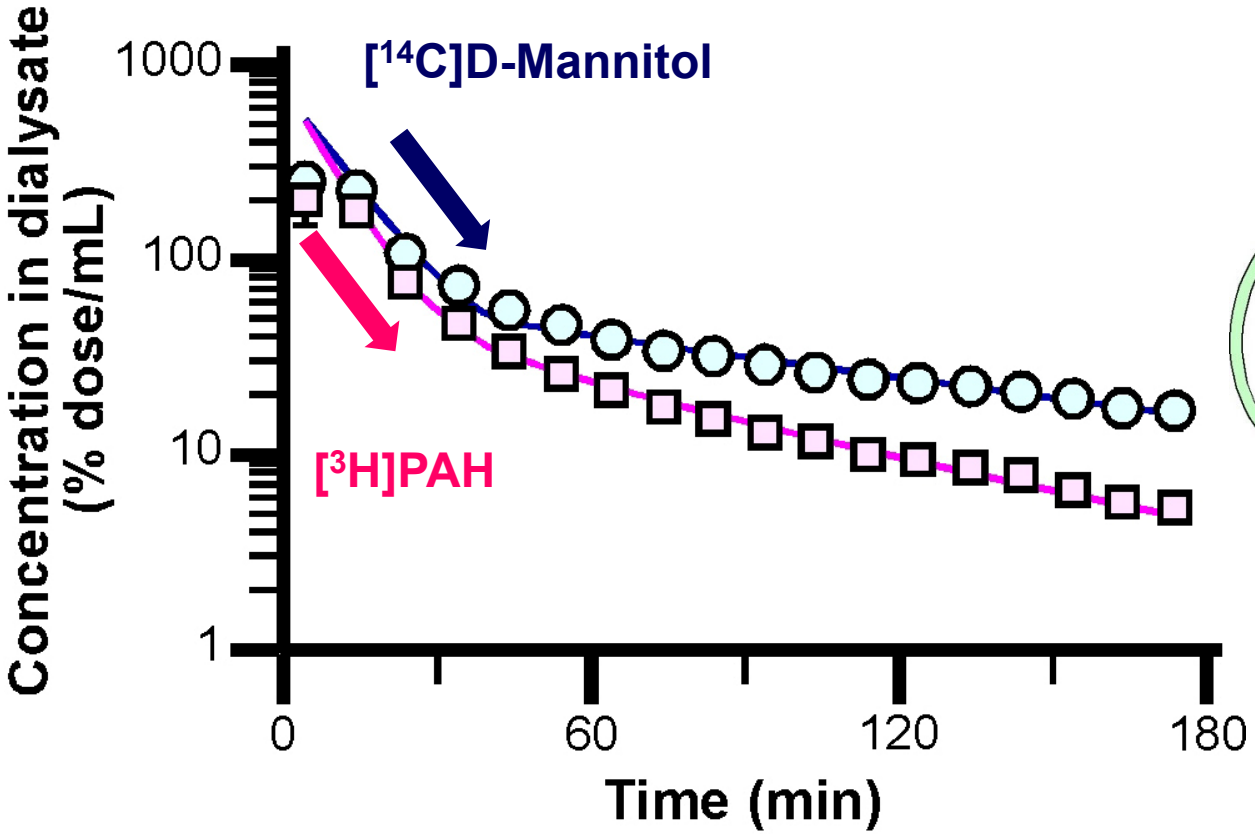
Equation

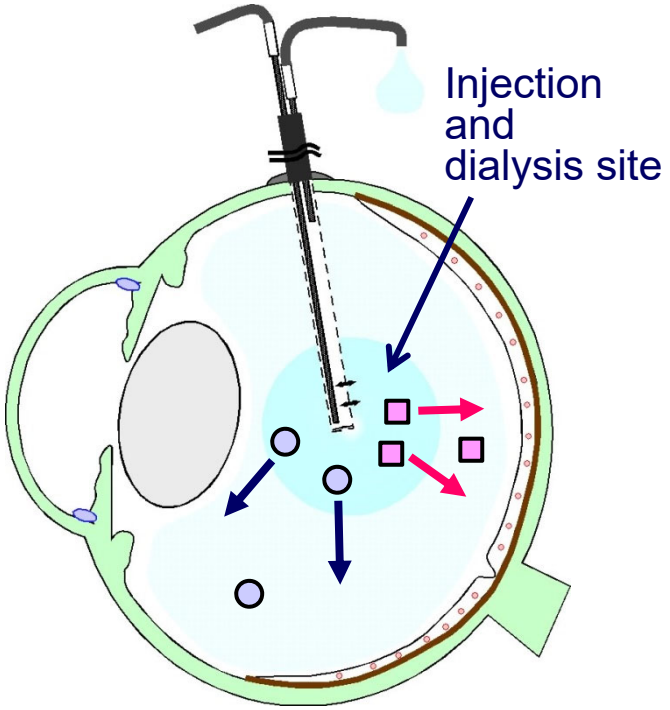
$$C_d = A \times exp\,(-\lambda_1 \times t\,) + B \times exp\,(-\lambda_2 \times t)$$

Each value represents the mean ± SEM (n = 5-16). **p<0.01, significantly different from the value of [^{14}C]D-mannitol.

Elimination of [^{3}H]PAH from rat vitreous humor

Time course





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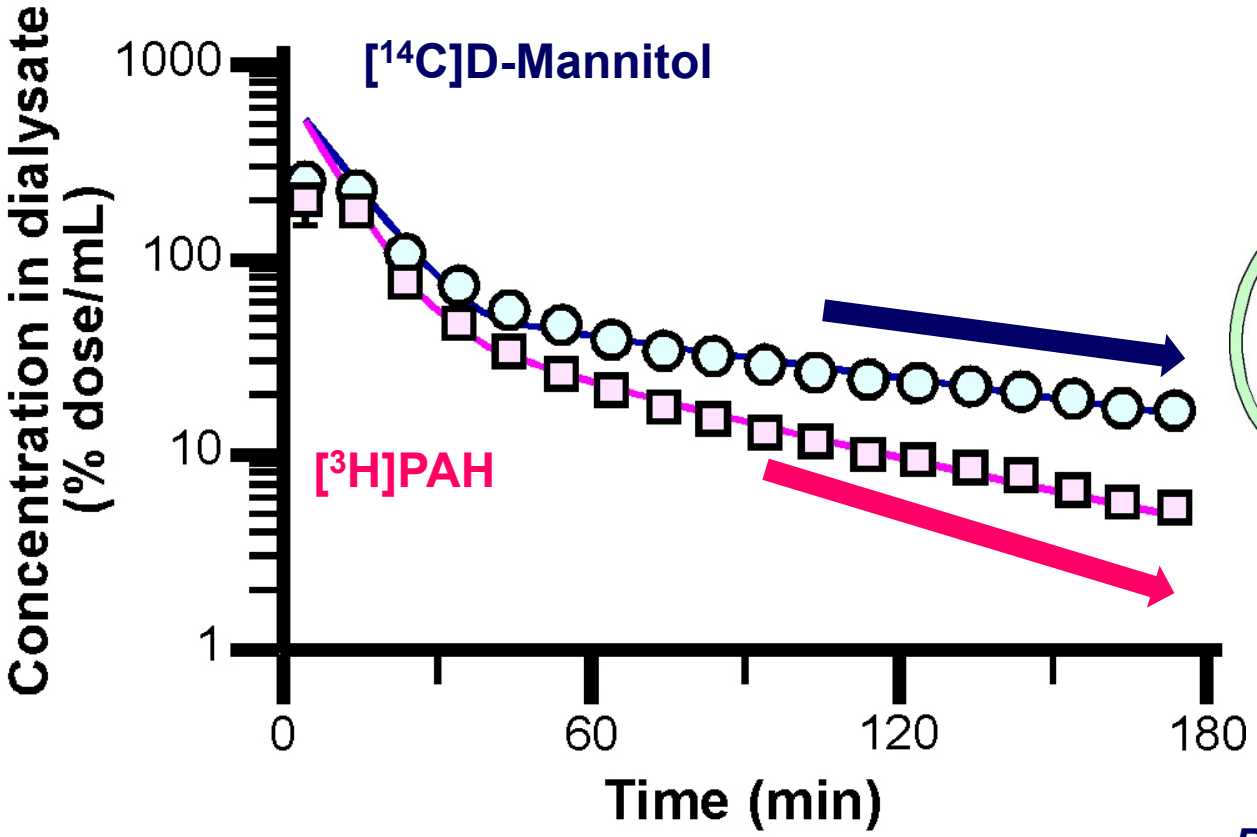
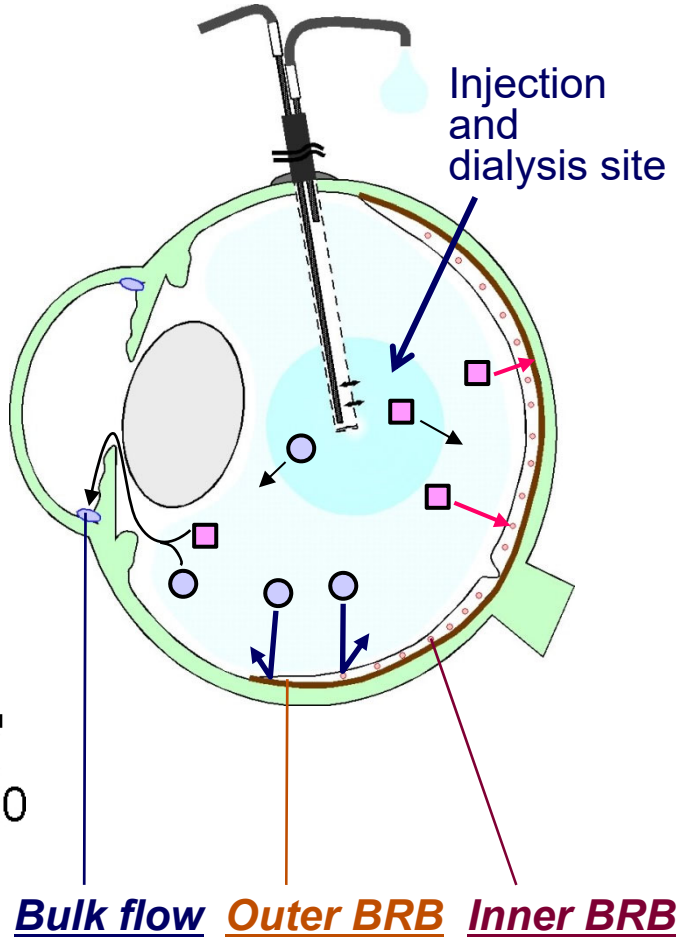
Elimination of [^{3}H]PAH from rat vitreous humor

Time course





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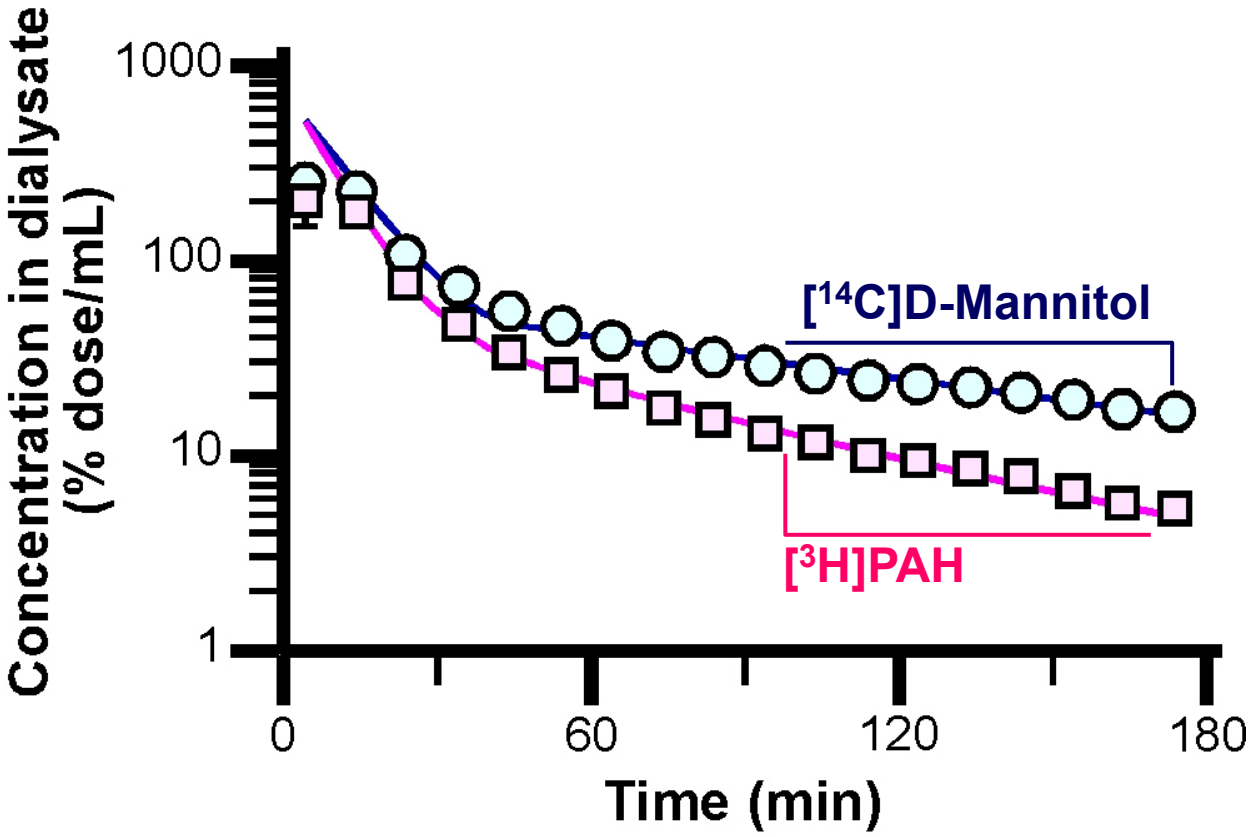
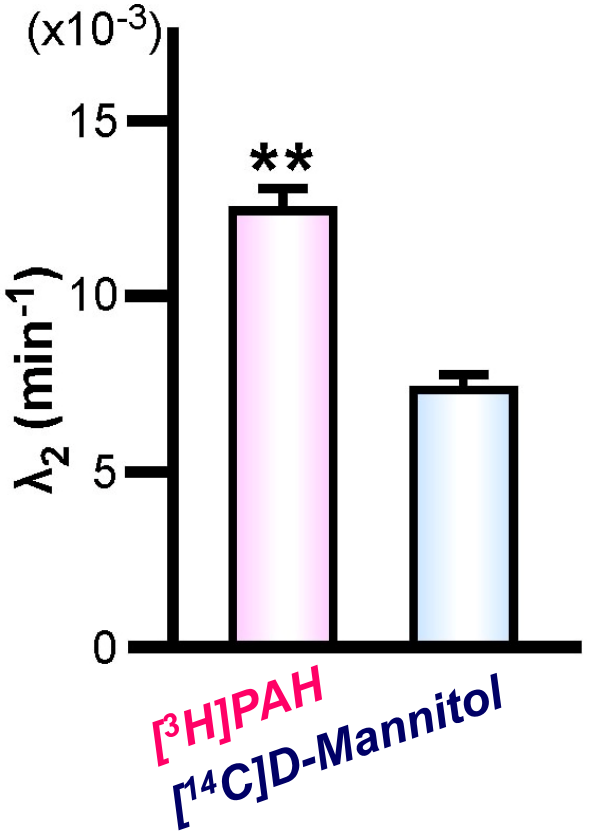
Elimination of [^{3}H]PAH from rat vitreous humor

Time course



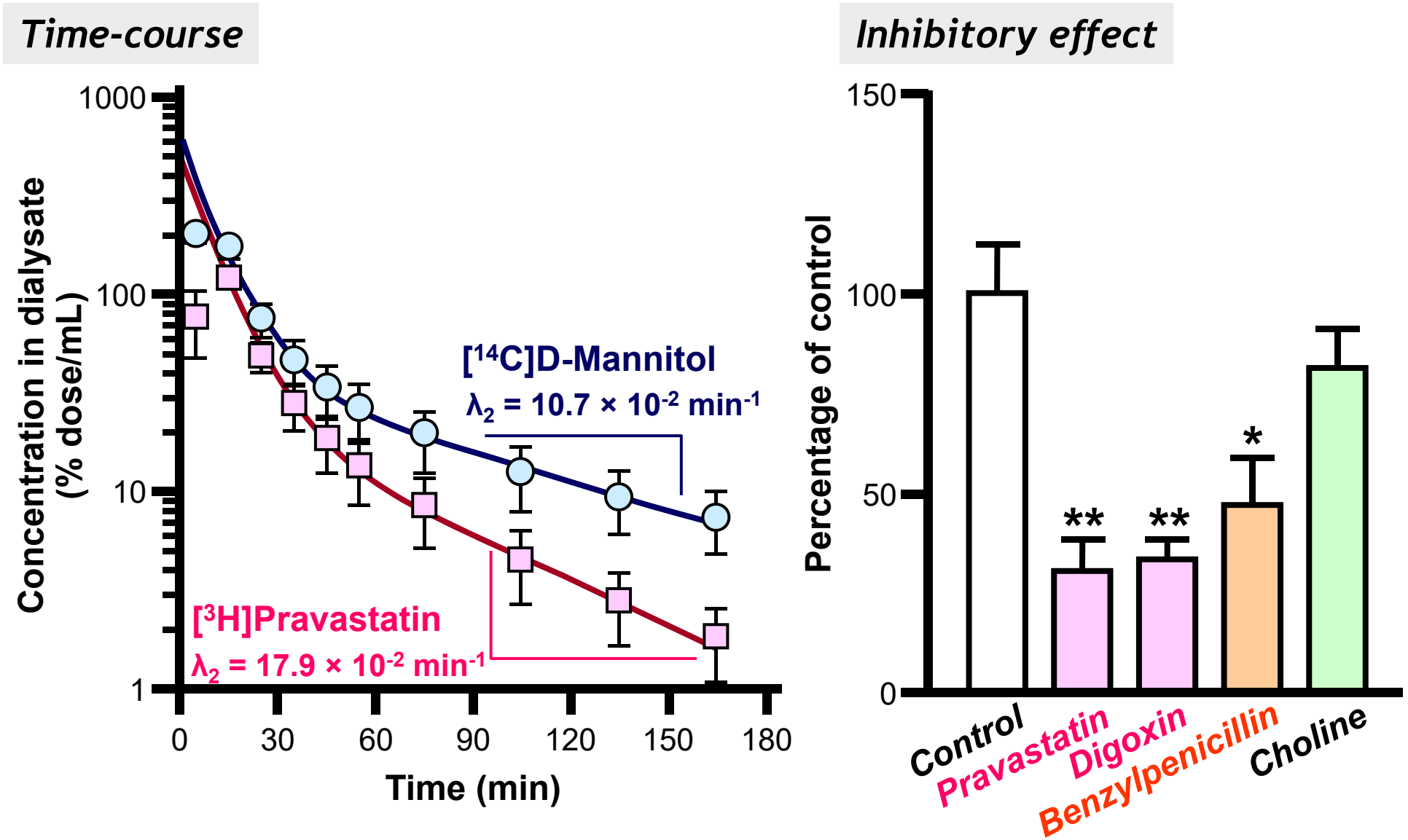
λ_2 values



Each value represents the mean ± SEM (n = 5-16). **p<0.01, significantly different from the value of [^{14}C]D-mannitol.

Hosoya et al. ***J Pharmacol Exp Ther*** 329:87-93 (2009)

Elimination of [^{3}H]pravastatin from rat vitreous humor

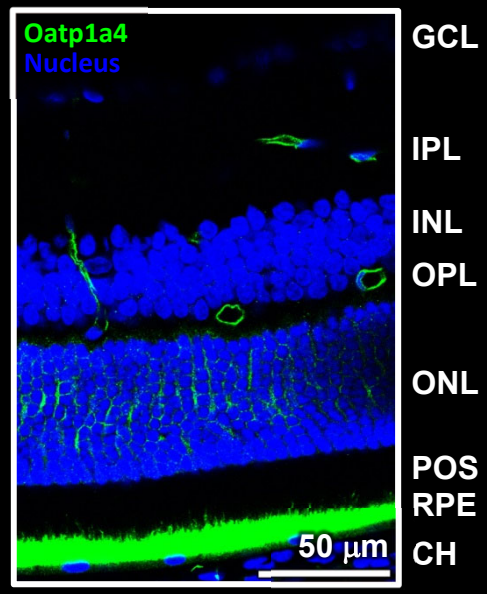


Each value represents the mean ± SEM (n = 3-4). *p<0.05, **p<0.01, significantly different from the control.

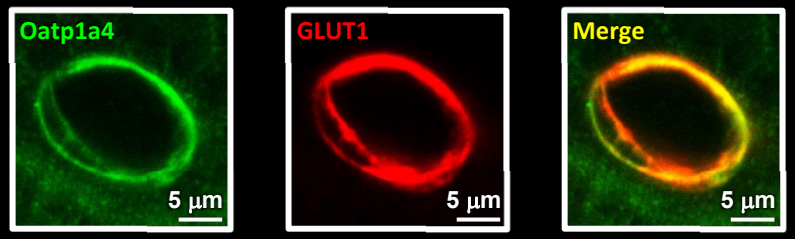
Fujii et al. ***Drug Metab Dispos*** 43:1956-1959 (2015)

Immunohistochemical analysis of the expression of Oatp1a4 and Oatp1c1 in the rat retina

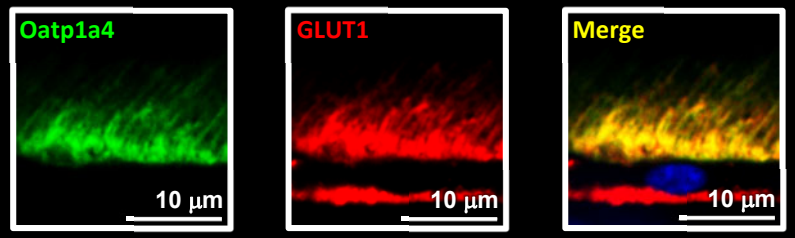
Oatp1a4



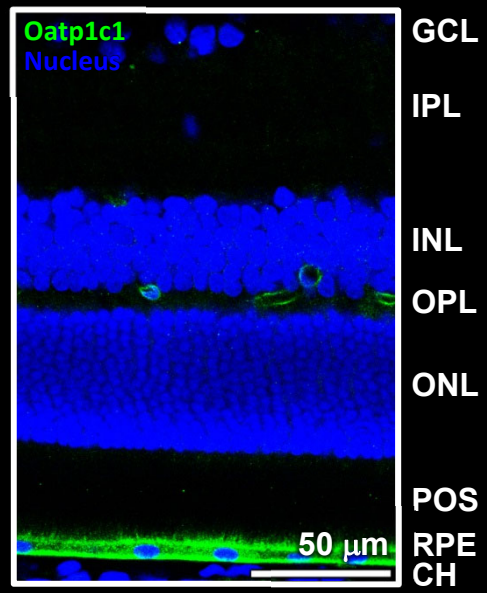
Inner blood-retinal barrier



Outer blood-retinal barrier



Oatp1c1



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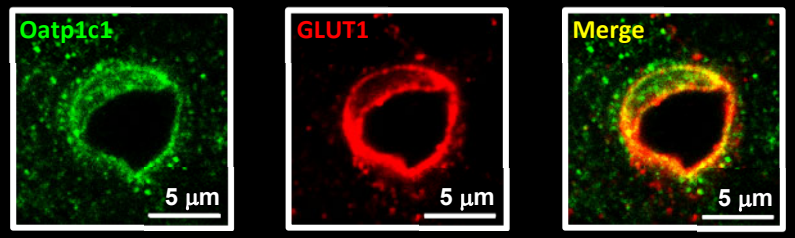
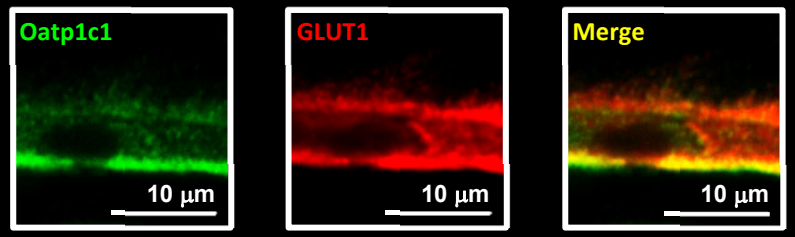
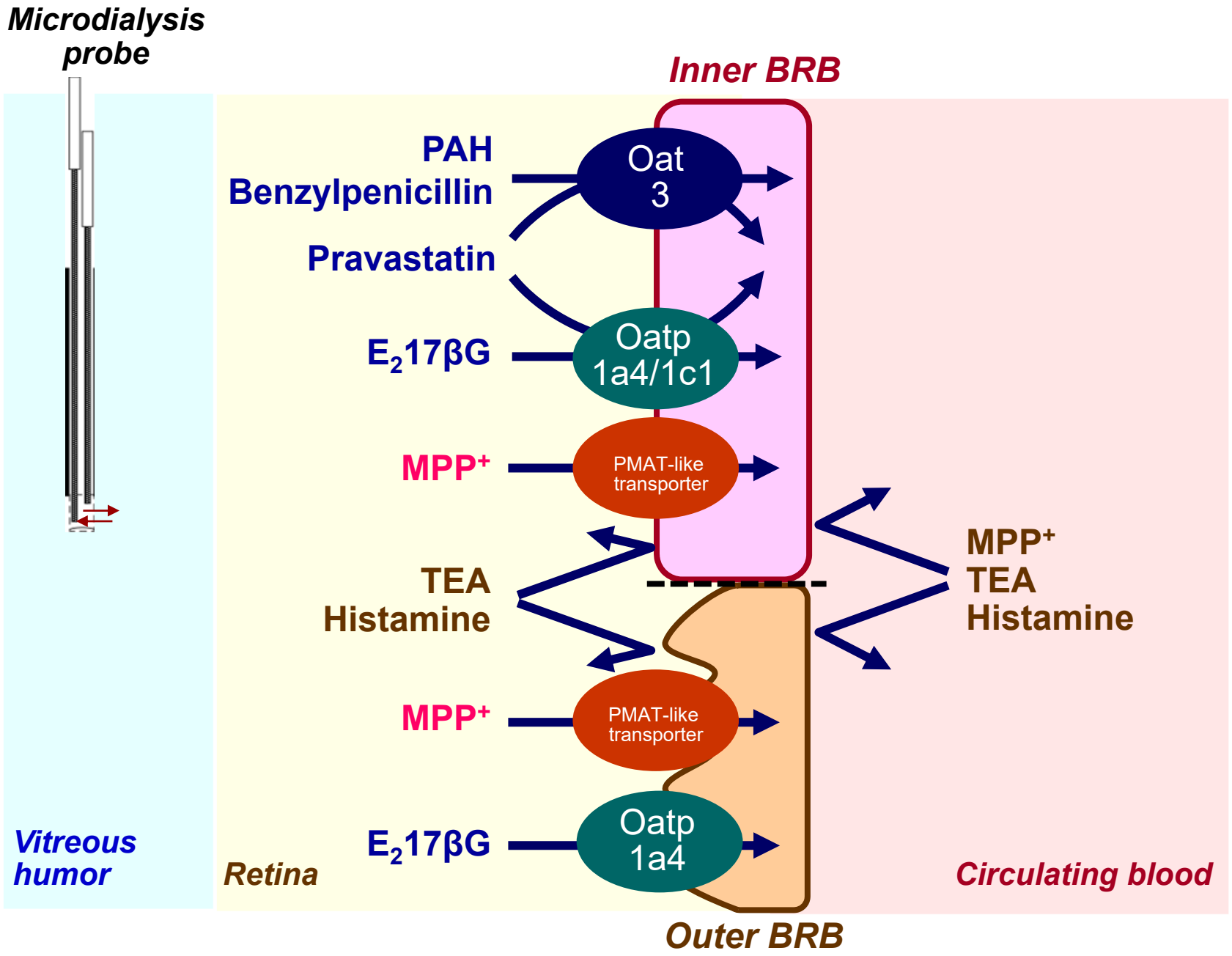


Outer blood-retinal barrier



Akanuma *et al.* ***Fluid Barrier CNS*** 10:29 (2013).

Conclusion | Organic anion/cation efflux at the BRB



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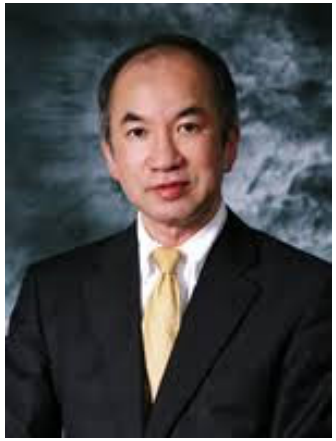

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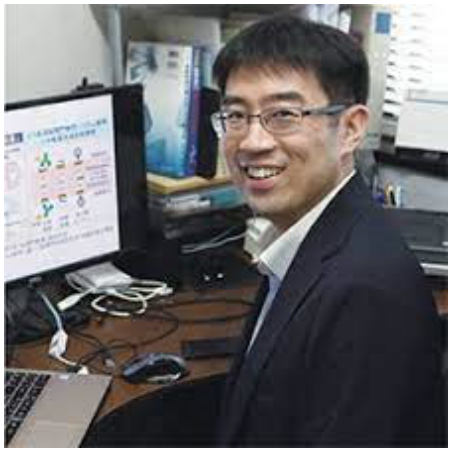

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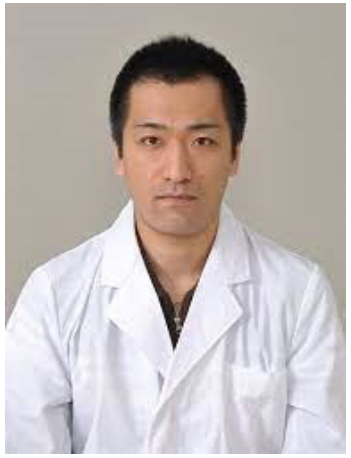

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