31st JSSX Annual Meeting (Matsumoto, 2016)

血液組織関門における塩基性薬物吸排制御機構に関する研究

Impact of Carrier-Mediated Transport of Cationic Drug and Nutrients at the Blood-Tissue Barrier

久保　義行

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University of Toyama

Acknowledgements

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Daisuke Ando, Ph.D	Hiroaki Miura	Ayaka Tomise
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Yusuke Kusagawa	Yoshimi Shimizu	Diasuke Yoneyama
Ryo Matsuyama	Tatsuya Soutome	Chihiro Yuzurihara
Satoshi Miki	Yuma Tega	Nobuyuki Zakoji

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Masanori Tachikawa, Ph.D
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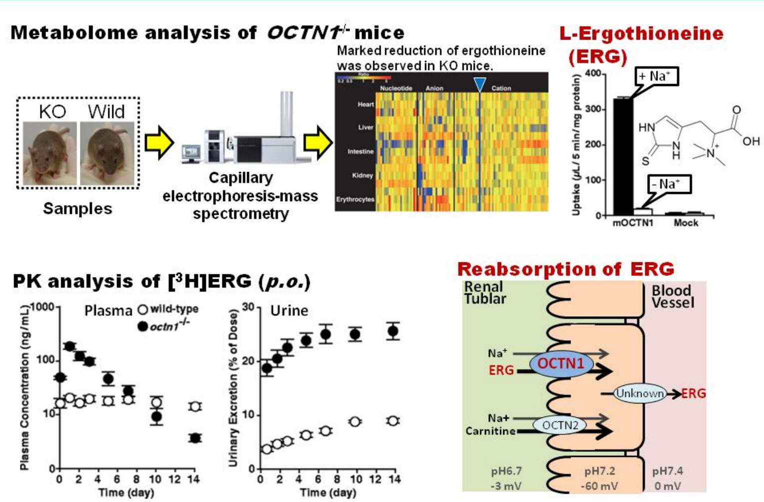
Masatoshi Tomi, Ph.D
Tomohiro Nishimura, Ph.D

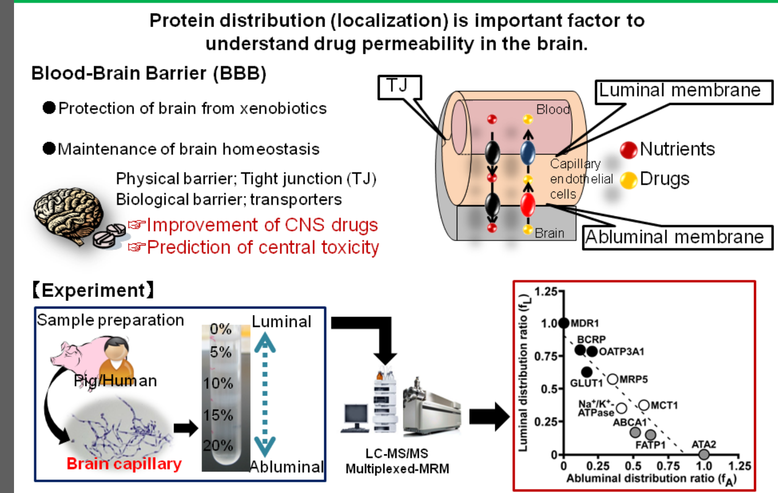
Acknowledgements

Study of organic cation transporter, OCTN1/SLC22A4



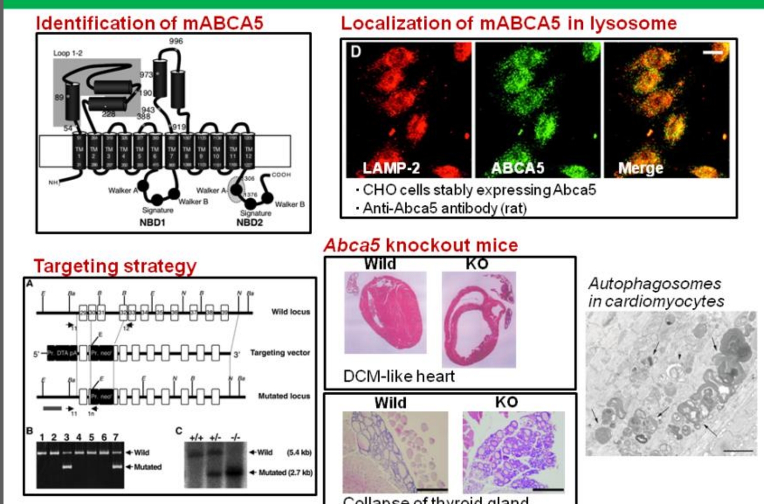
Kato et al. Pharm Res. 27:832-40 (2010)

Quantitative determination of membrane distributions of transporters



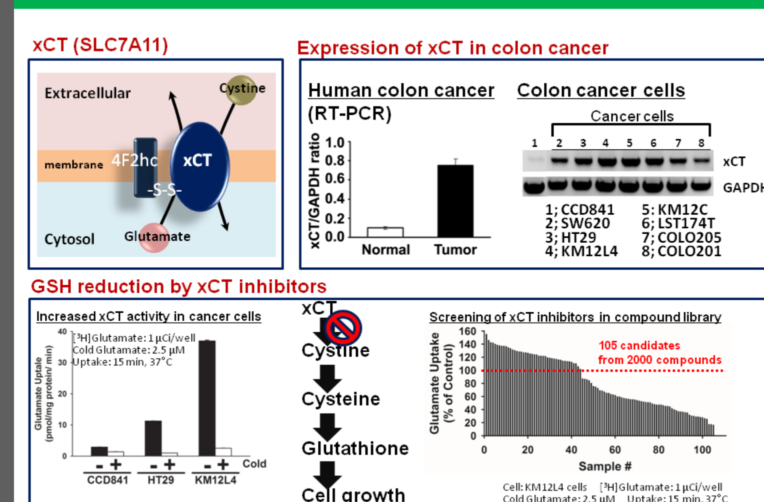
Kubo et al. J Pharm Sci. 104:3060-8 (2015)

Identification & KO mice analysis of mABCA5



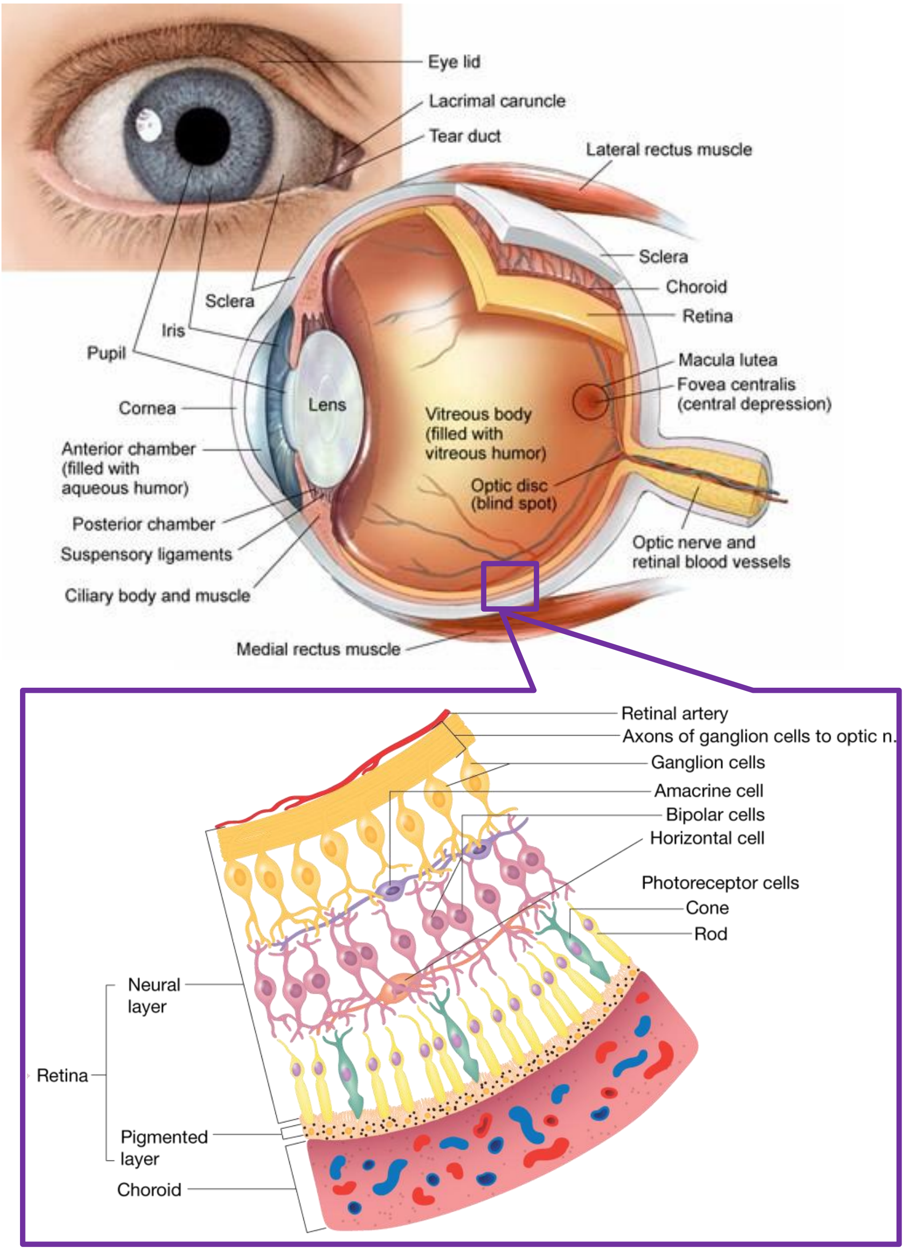
Kubo et al, Mol Cell Biol. 25(10):4138-49 (2005)

SLC transporters as therapeutic target for cancer



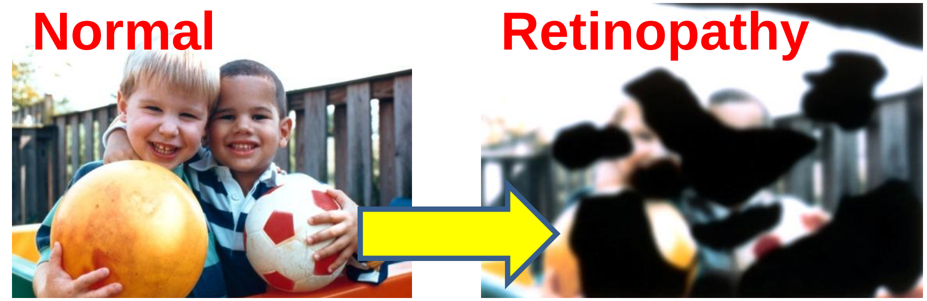
Kubo Y et al. American Association for Cancer Research (AARC) 100th Annual Meeting (Denver, U.S.A.)

Retina is essential for visual sense



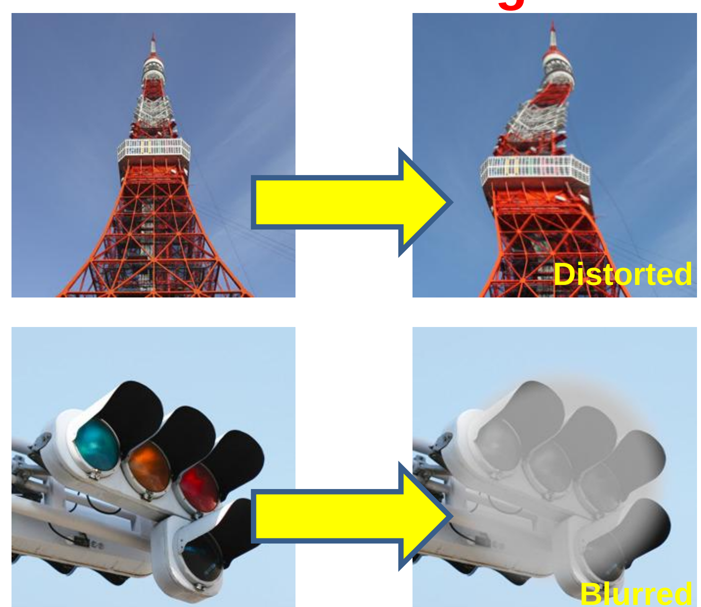
Illustrate; Specialty Eye Care Medical Center

Diabetic retinopathy



Macular degeneration

Normal | Degenerated

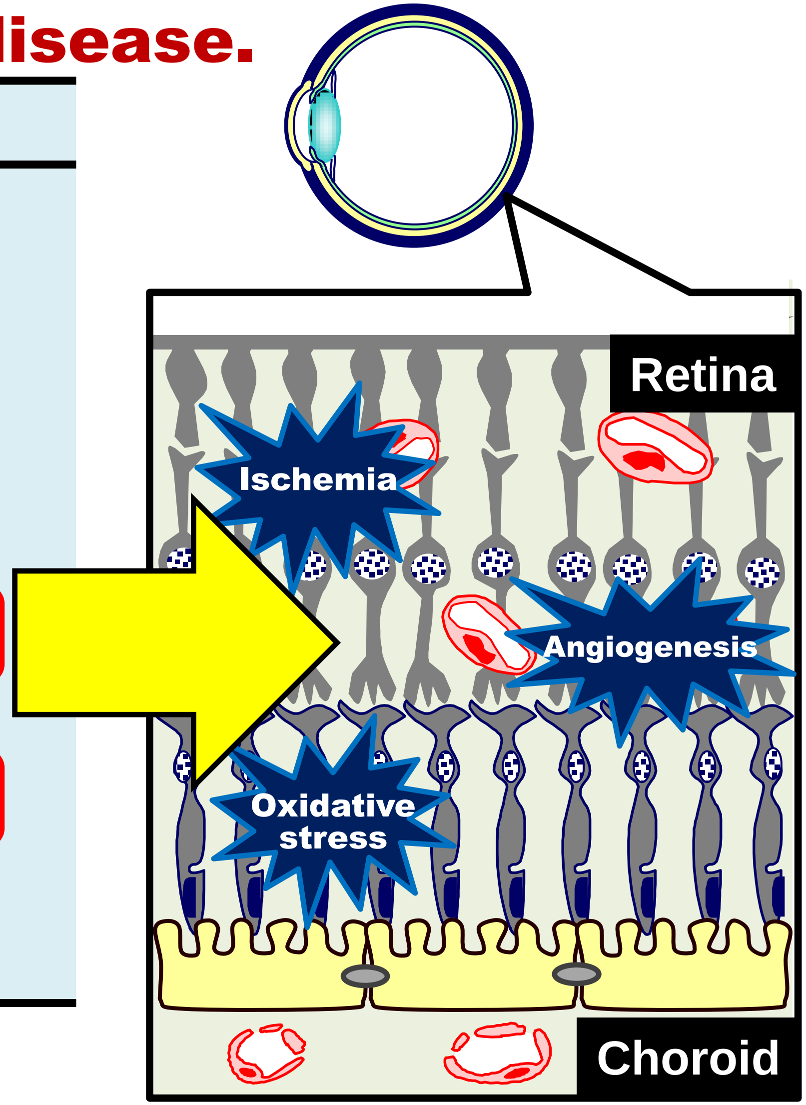


http://www.kareiouhan.com/
Japanese ophthalmological Society & Wikipedia

Cationic neuroprotectants for retinal disease

Some cationic drugs express neuroprotective effect, and their utilities are expected in the treatment of retinal disease.

Drugs	Effect
Imipramine	Neuroprotective
Desipramine	Neuroprotective
Memantine	Neuroprotective
Nipradilol	Neuroprotective
Timolol	Neuroprotective
Clonidine	Neuroprotective
Brimonidine	Neuroprotective
Propranolol	Anti-angiogenic
Pazopanib	Anti-angiogenic
Sorafenib	Anti-angiogenic



Mizuno et al. Invest Ophthalmol Vis Sci. 42:688–94. 2001
Arthur and Cantor. Exp Eye Res. 93:271–83. 2011
Lauterbach et al. J Neuropsychiatry Clin Neurosci. 22:8–18. 2010
Ristori et al. Invest Ophthalmol Vis Sci. 52:155–70. 2011

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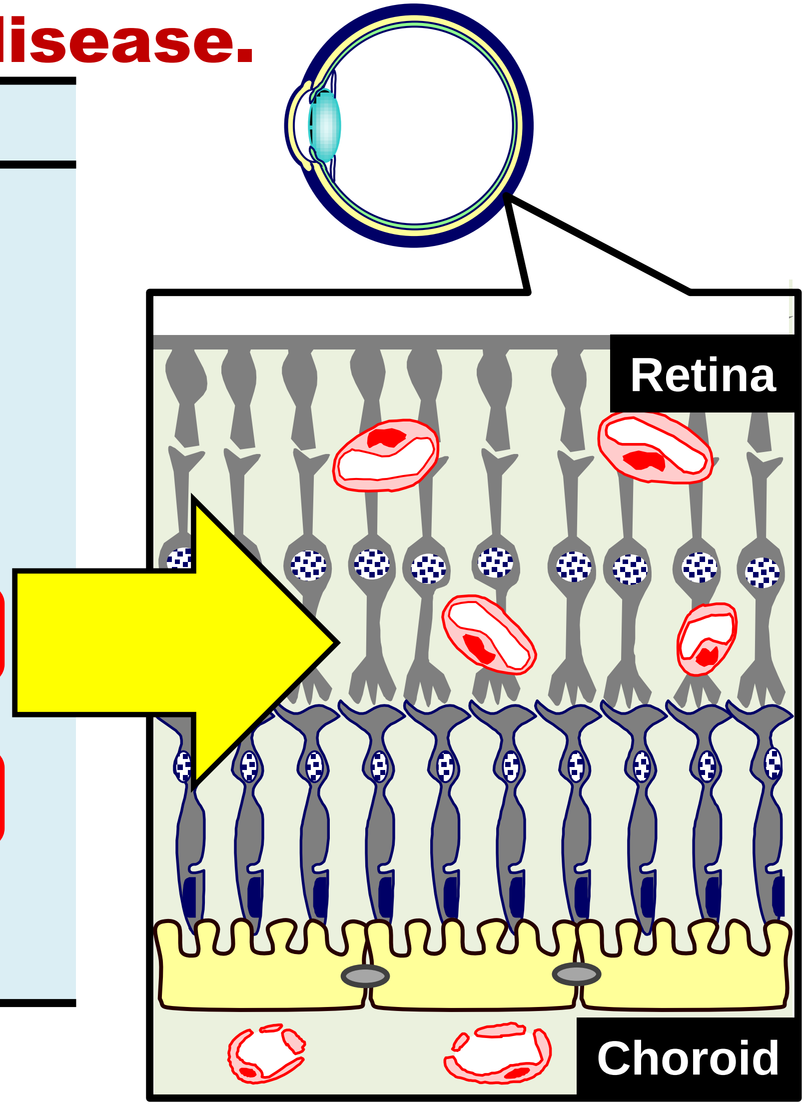
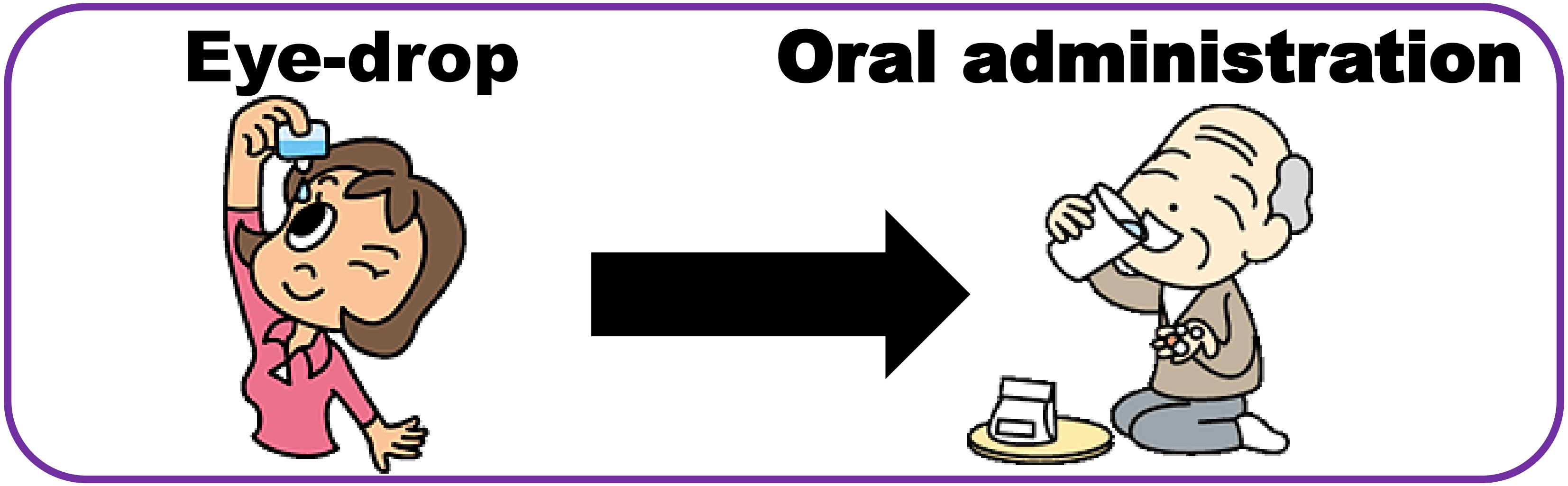
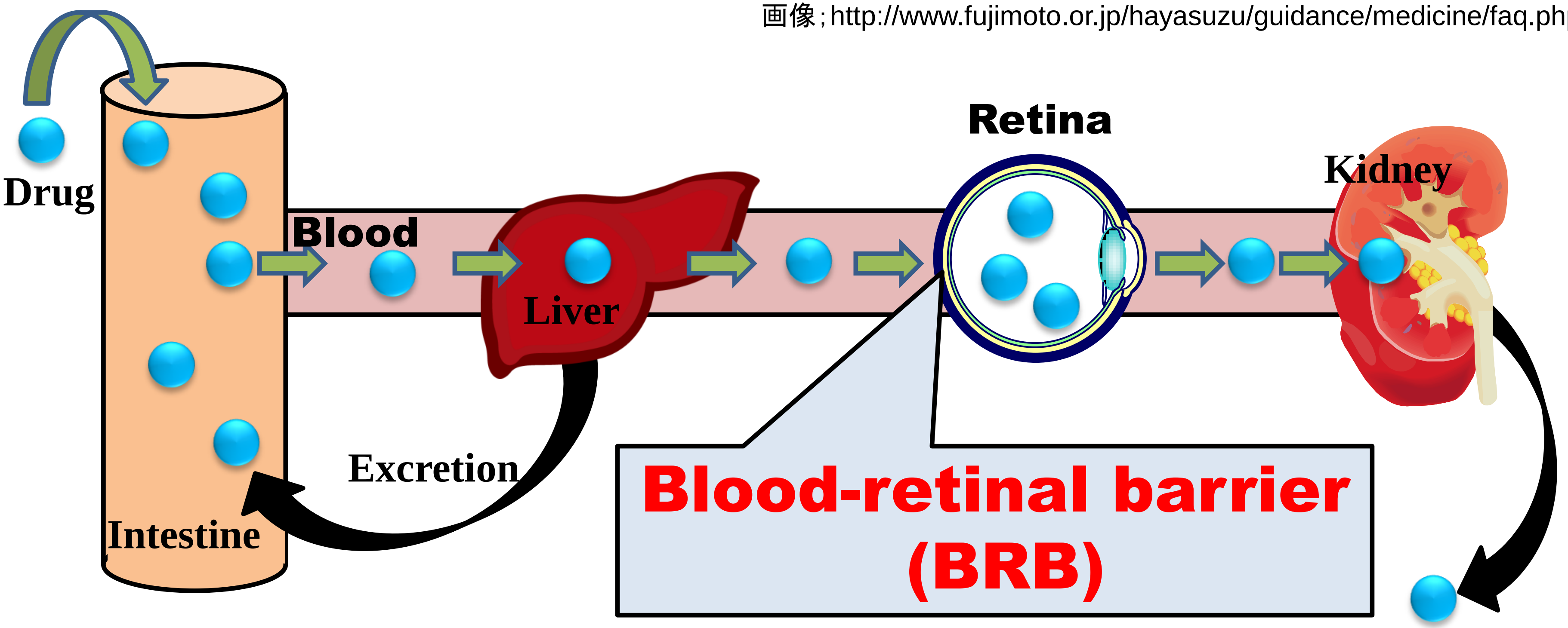


Mizuno et al. Invest Ophthalmol Vis Sci. 42:688–94. 2001
Arthur and Cantor. Exp Eye Res. 93:271–83. 2011
Lauterbach et al. J Neuropsychiatry Clin Neurosci. 22:8–18. 2010
Ristori et al. Invest Ophthalmol Vis Sci. 52:155–70. 2011

Systemic drug delivery to retina



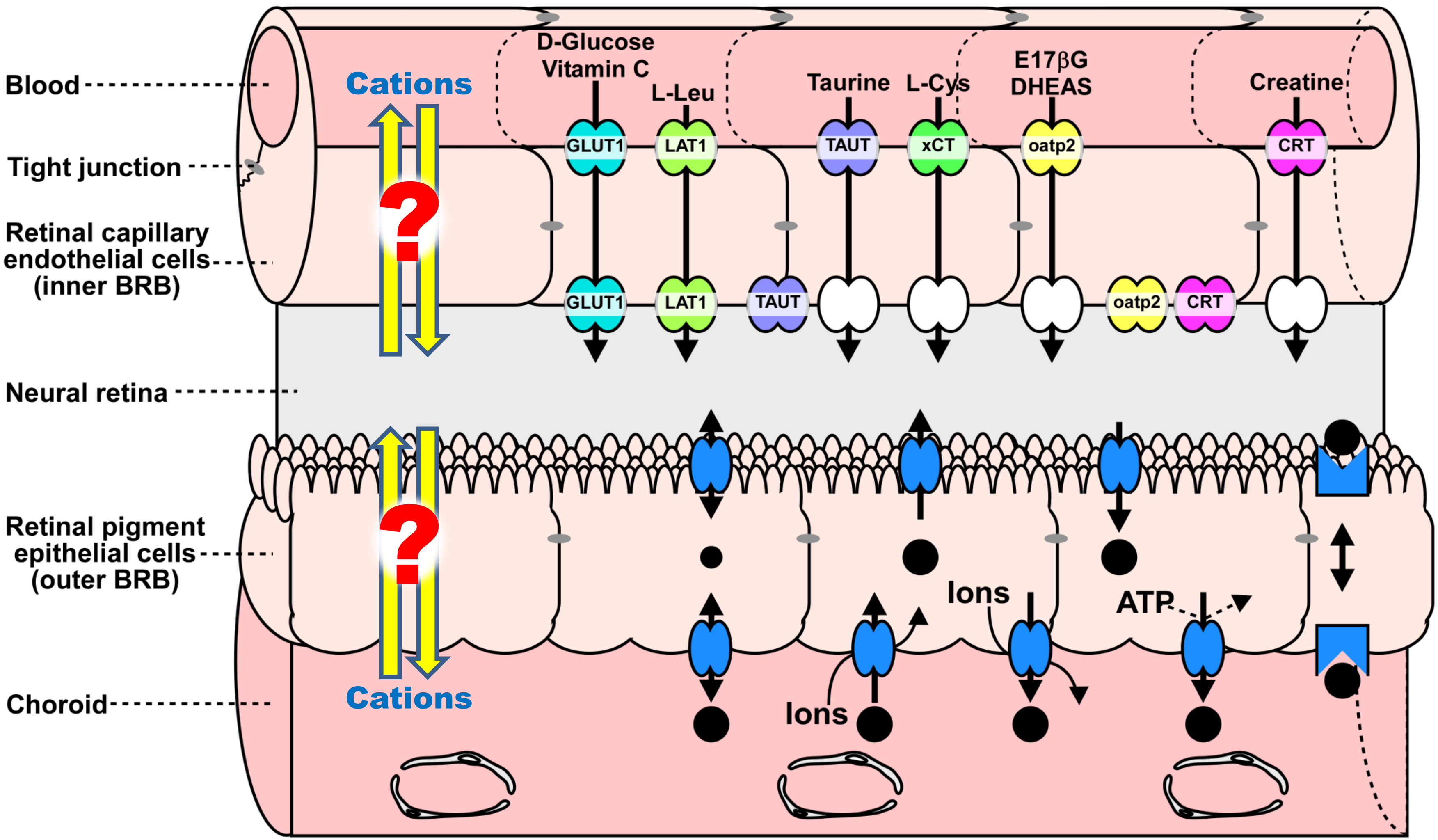
画像;http://www.fujimoto.or.jp/hayasuzu/guidance/medicine/faq.php



Kubo and Hosoya. Drug Delivery System. 27: 361-9 (2012)

Blood-retinal barrier (BRB)

The carrier-mediated transport of nutrients has been studied at the inner BRB. However, little is known about the transport of cations at the BRB.



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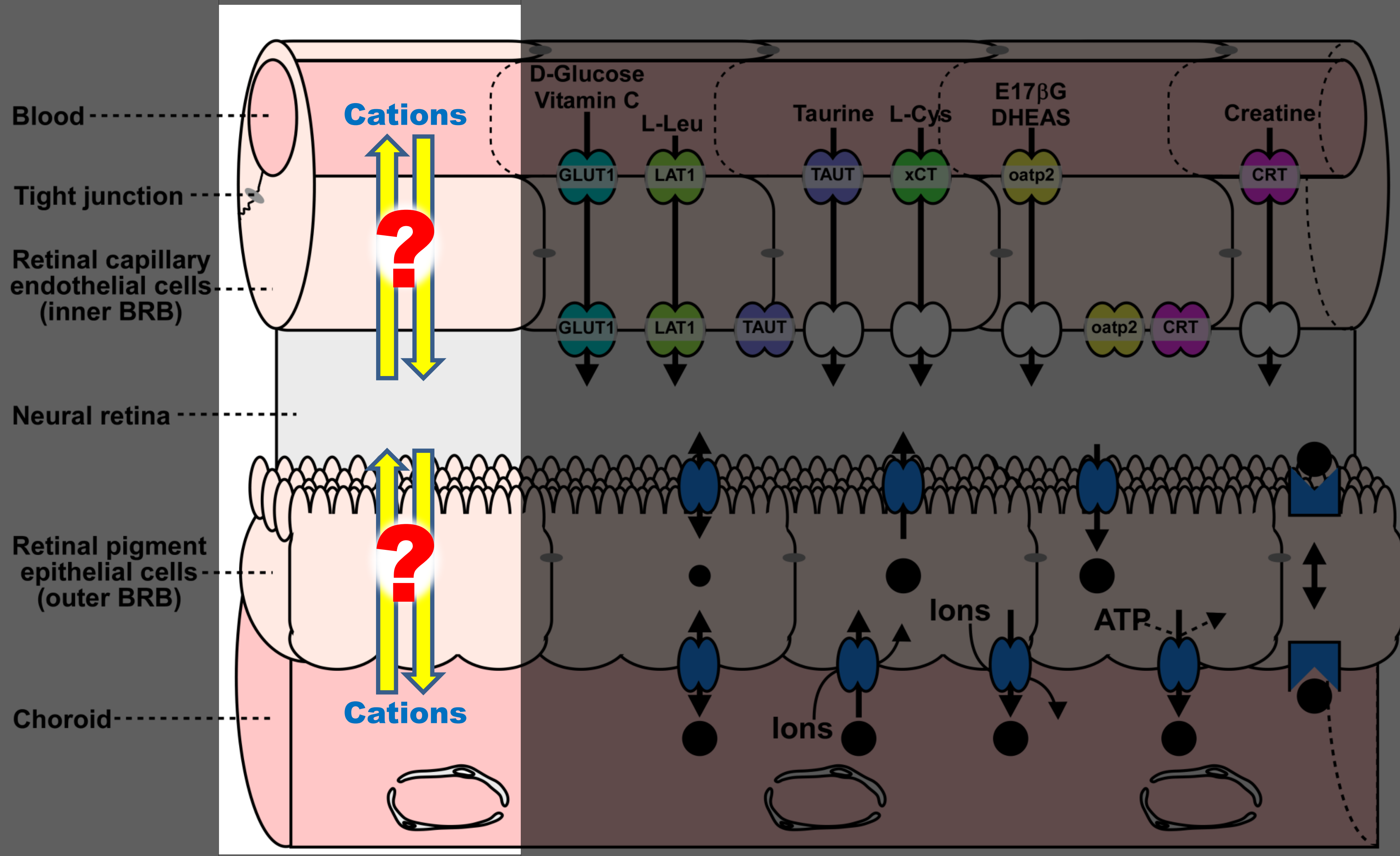
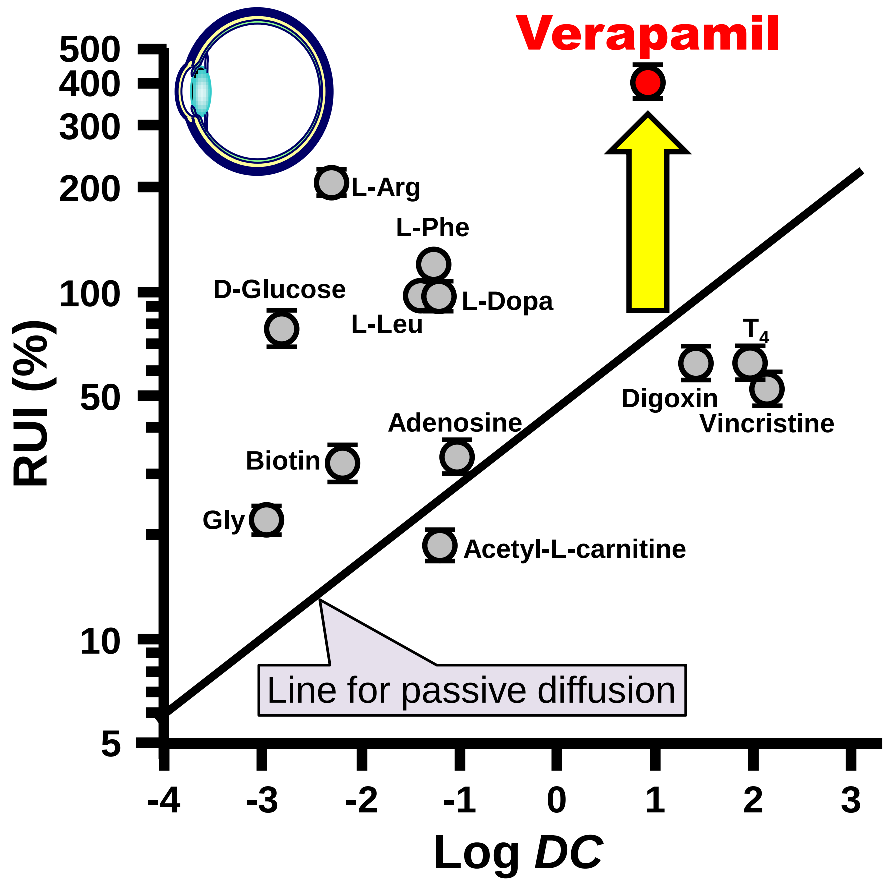


Kubo and Hosoya. Drug Delivery System. 27: 361-9 (2012)

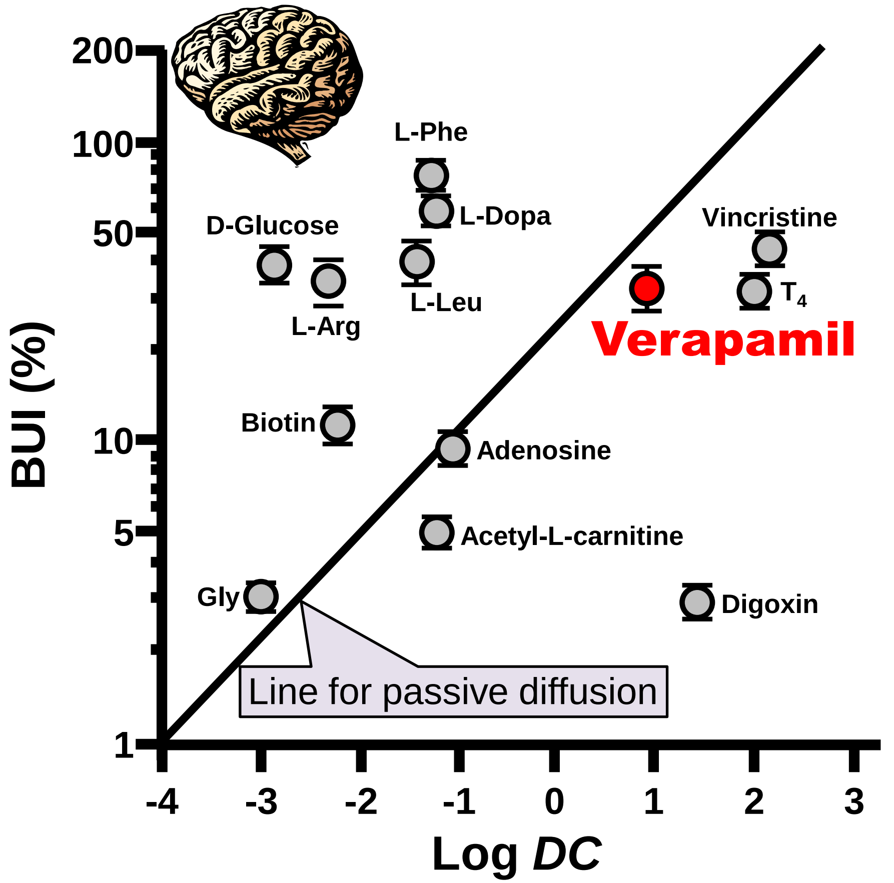
Cationic drug transport systems at the BRB

Retinal uptake index (RUI)



Comparison of the retinal (RUI) and brain uptake index (BUI) relationship in transporters and the lipophilicity trend line. The lipophilicity trend line indicates the correlation between the RUI and BUI and the log DC of the thirteen compounds. Blue and red circles are substrates for SLC transporters and P-gp, respectively. Each point represents the mean ± SEM (n = 3-6). L-Arg, L-arginine; L-Phe, L-phenylalanine; L-Leu, L-leucine; Gly, Glycine; T_4, thyroxine.

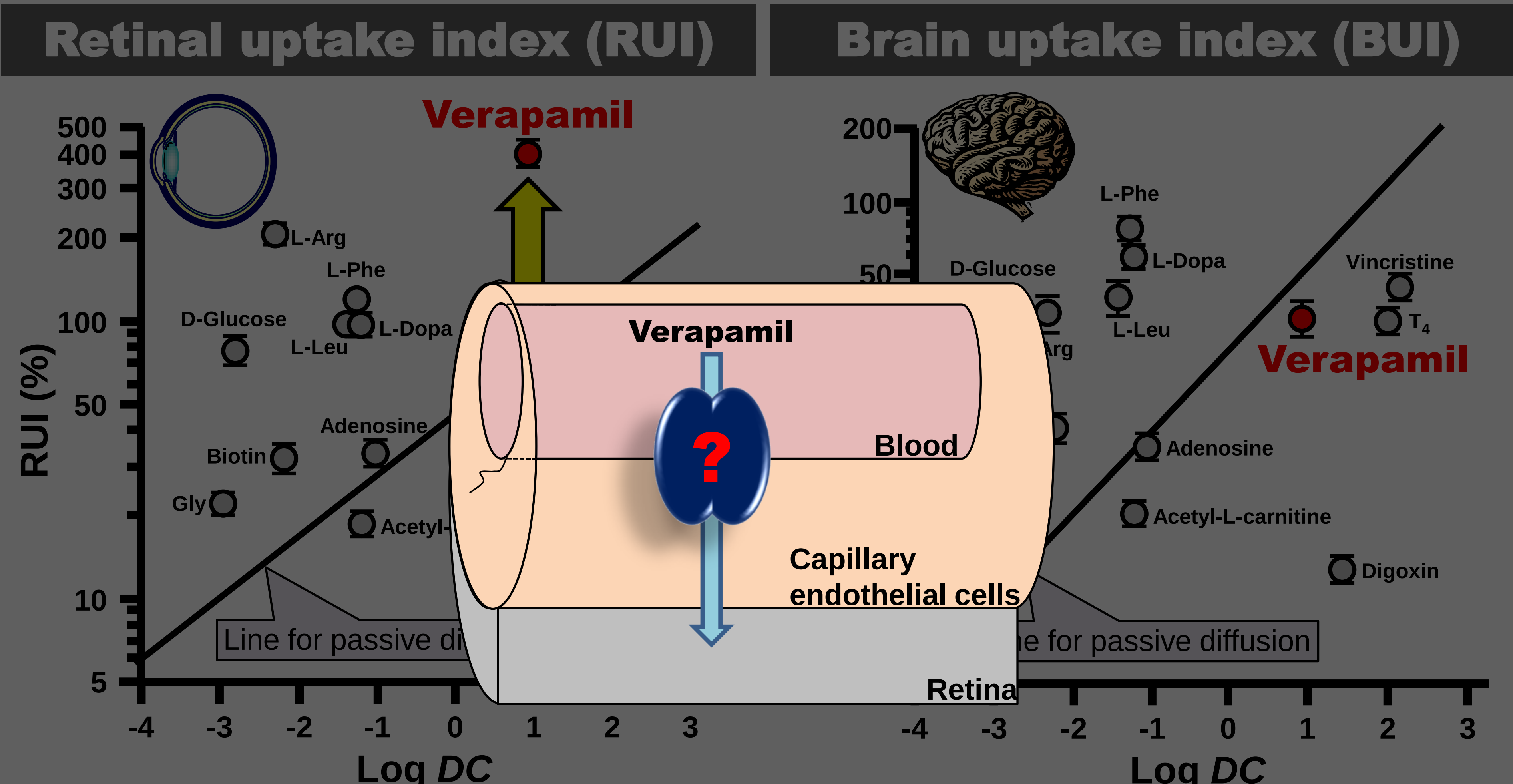
Brain uptake index (BUI)



Comparison of the initial uptake rate (V) and the *n*-octanol/Ringer distribution coefficient (DC) of compounds that cross the BRB by carrier-mediated transport. Uptake study was performed with an *in vitro* model cell line of inner BRB (TR-iBRB2 cells).

Kubo et al. J Pharm Sci. 102:3332-42 (2013)
Hosoya et al. Pharm Res. 27:2715-24 (2010)

Cationic drug transport systems at the BRB



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Kubo et al. J Pharm Sci. 102:3332-42 (2013)
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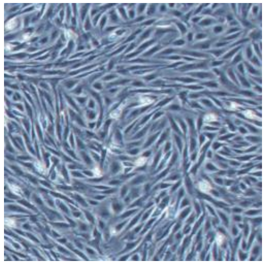
Carrier-mediated transport of [³H]verapamil



TR-iBRB2 cells

Conditionally immortalized cell line of retinal capillary endothelial cells

Hosoya et al. Exp Eye Res. 72:163-72. 2001

Concentration-dependence

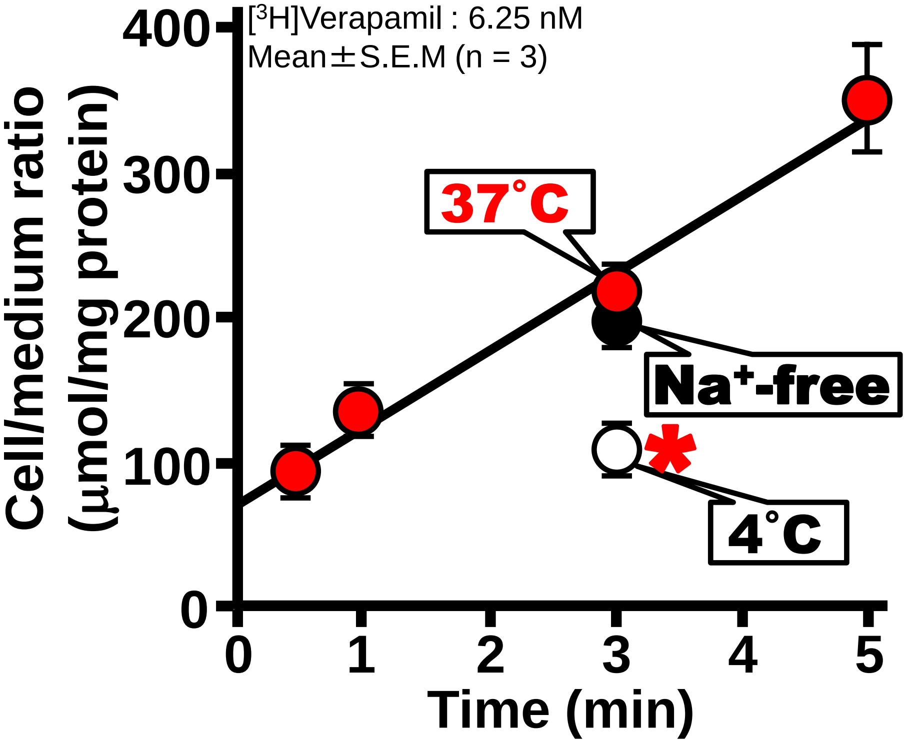
$$V = \frac{V_{max} \times S}{K_m + S} + K_d \times S$$

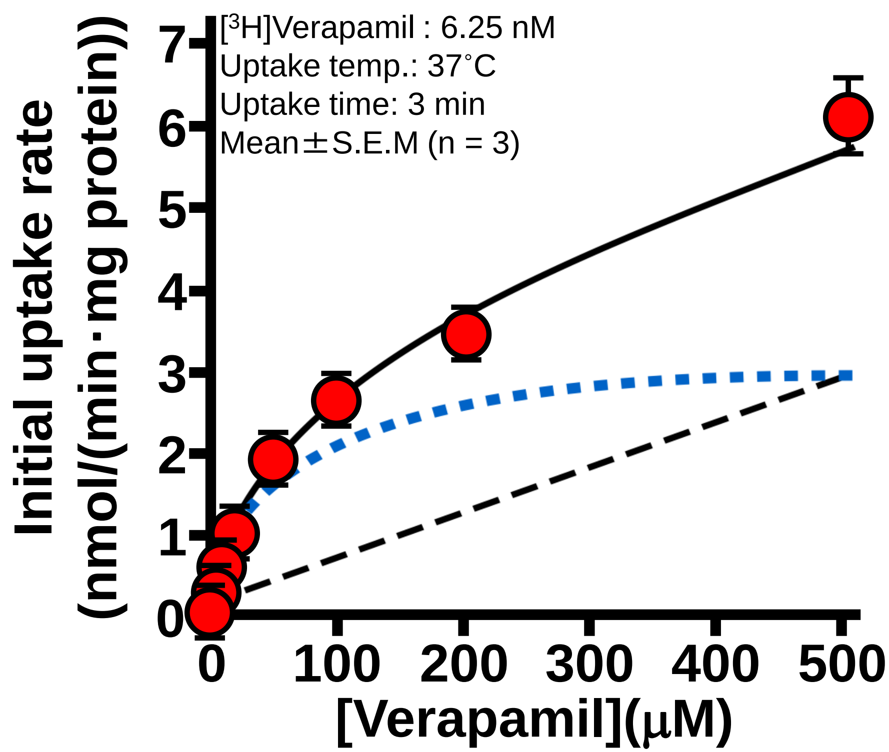
K_m; **61.9 ± 3.9** μM

V_{max}; **3.31 ± 0.21** nmol/(min·mg protein)

K_d; **5.93 ± 0.95** μL/(min·mg protein)

Temperature-dependence



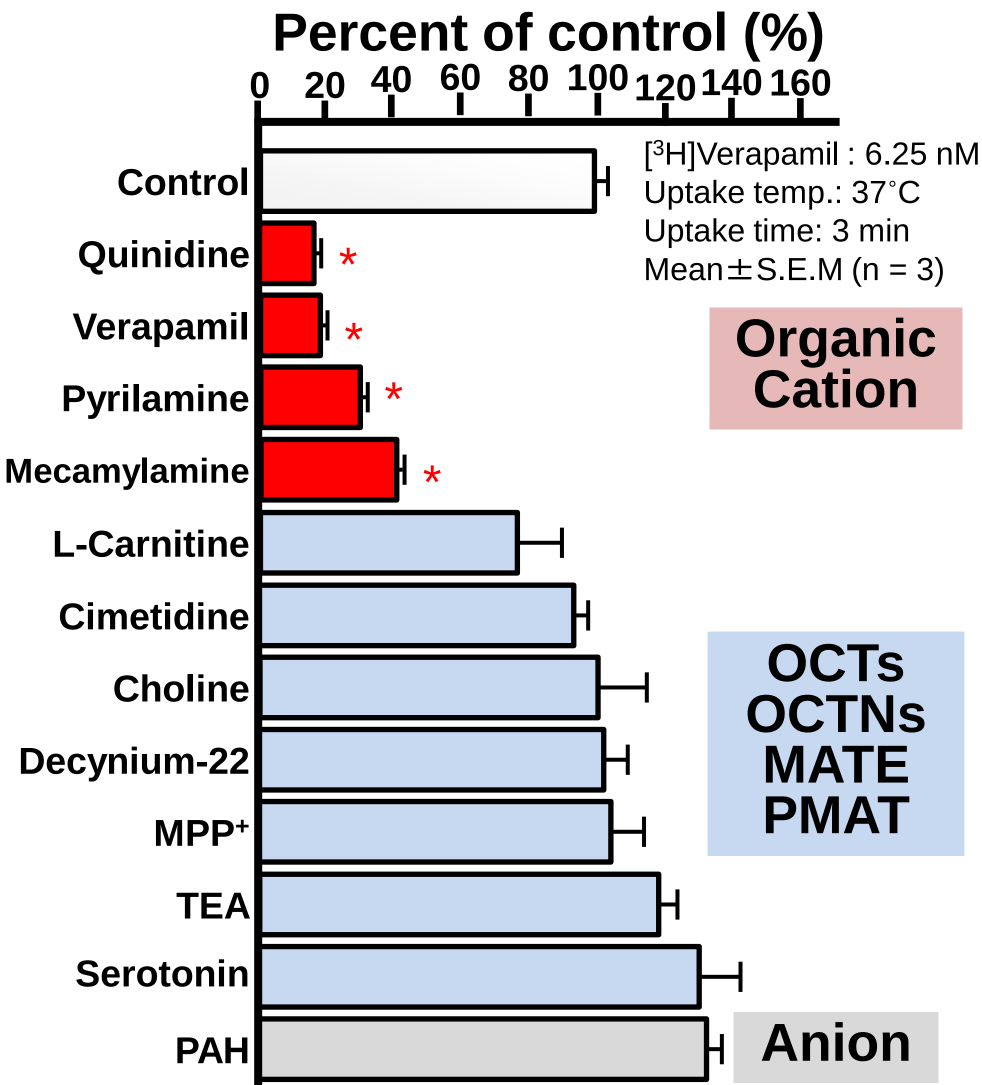


**p* < 0.01, significantly different from the control.

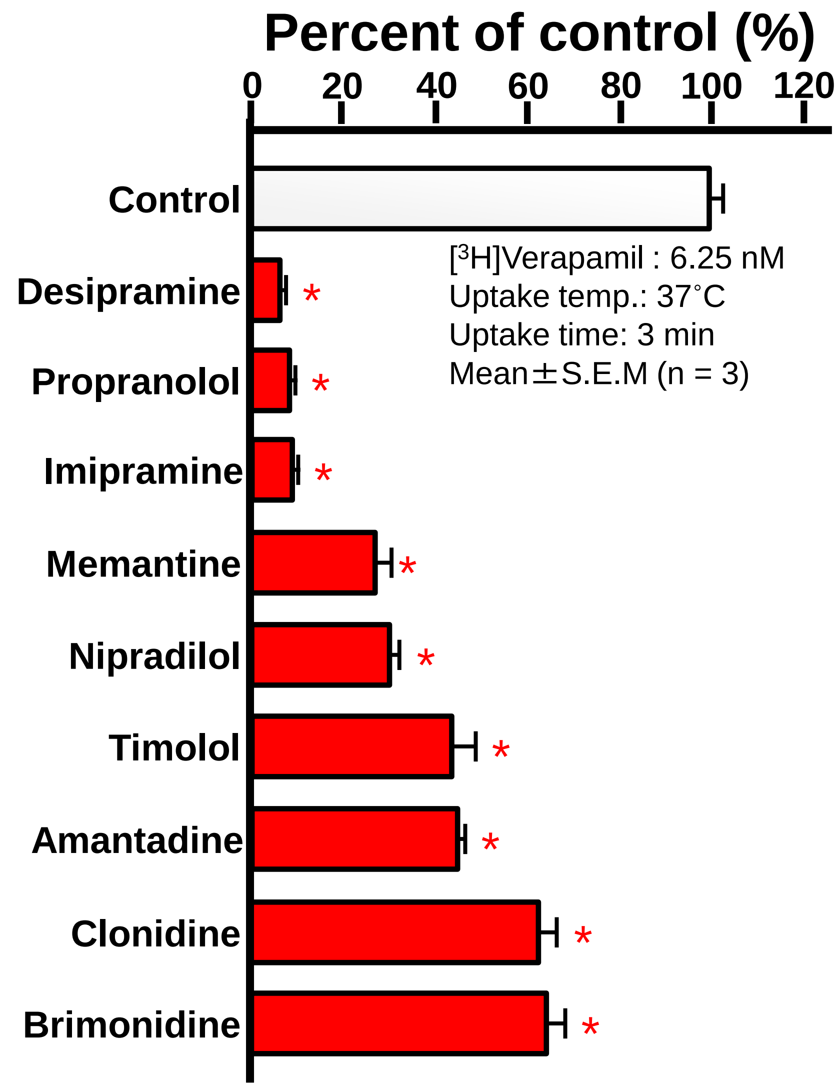
Kubo et al. Pharm Res. 30:847-56 (2013)

Inhibition study of [^{3}H]verapamil uptake

Cations and anions



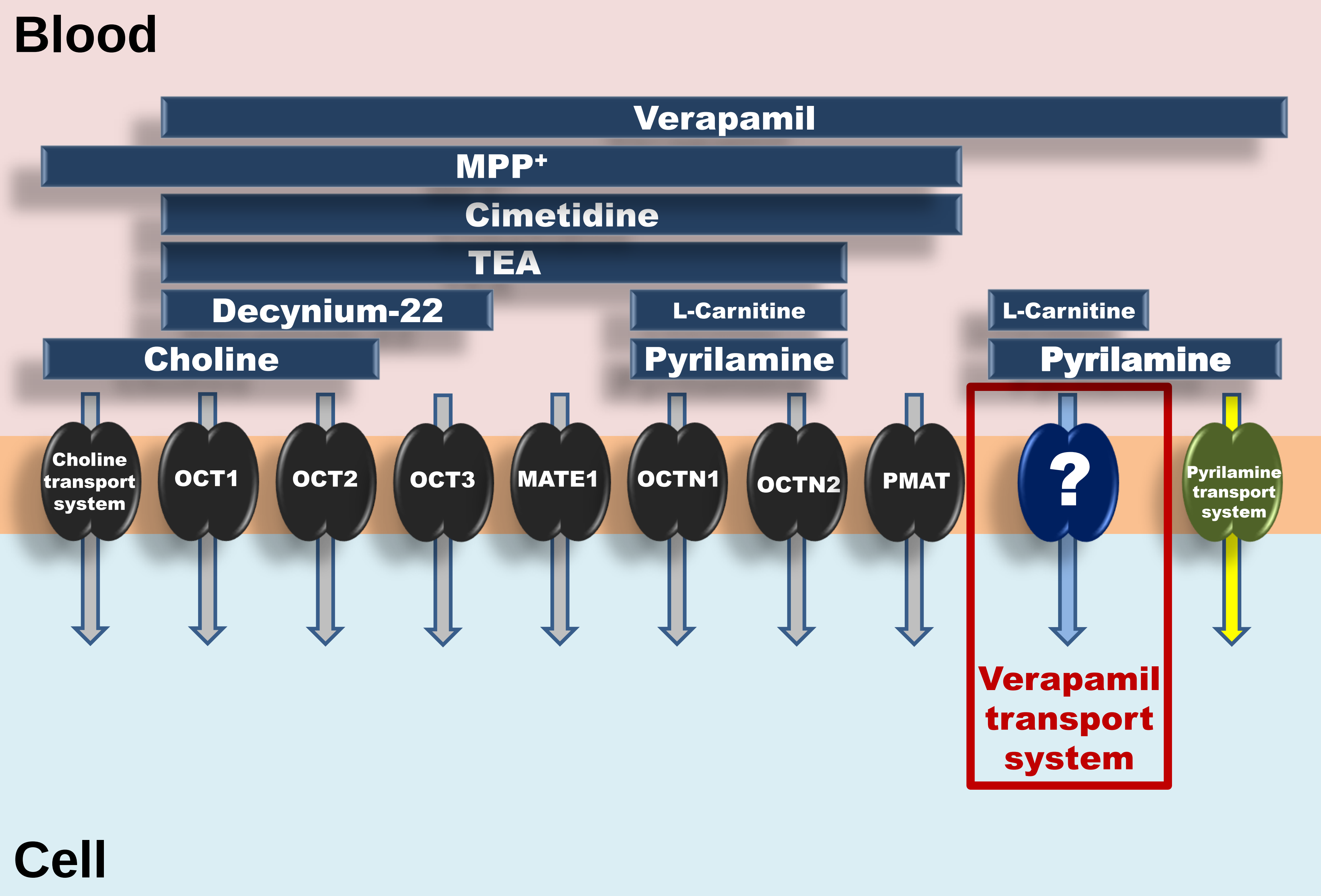
Neuroprotectants



In the inhibition study, compounds were used at a concentration of 1 mM. MPP^+, 1-methyl-4-phenylpyridinium; TEA, tetraethylammonium; PAH, *p*-aminohippuric acid. **p* < 0.01, significantly different from the control.

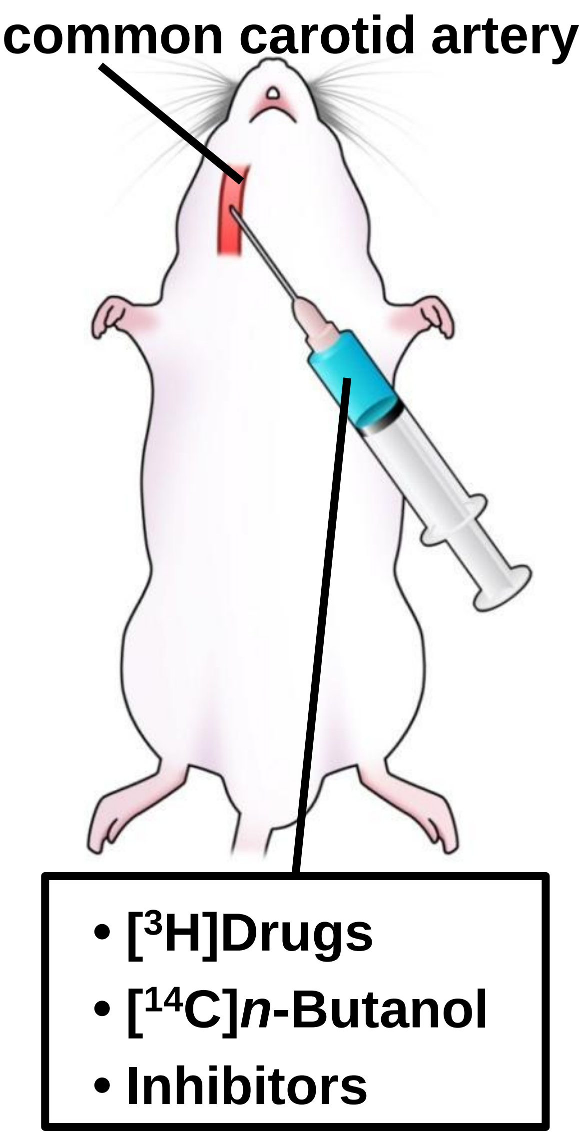
Kubo et al. Pharm Res. 30:847-56 (2013)

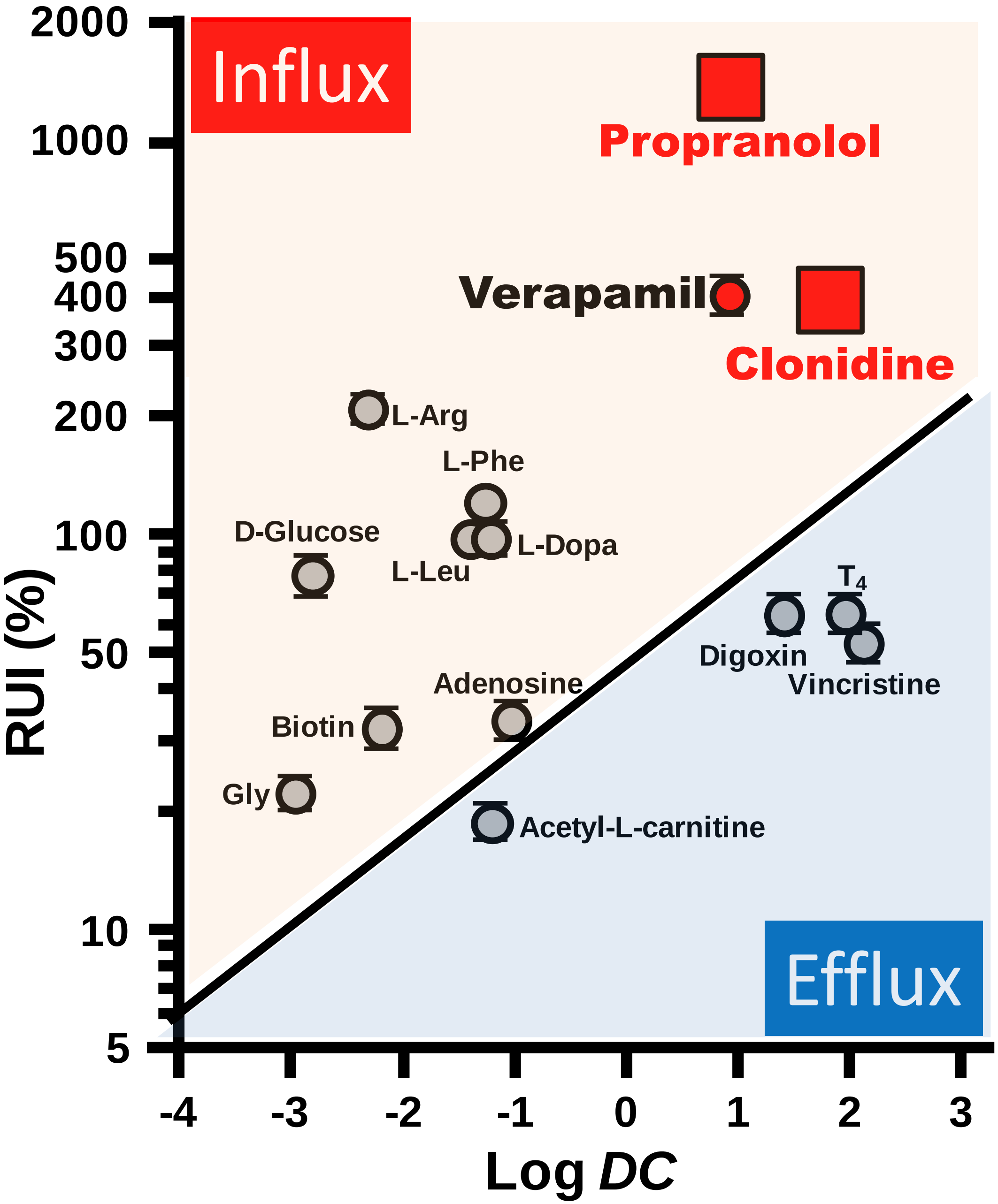
Novel organic cation transporter is responsible



Influx transport of cationic neuroprotectants

Retinal uptake index (RUI)





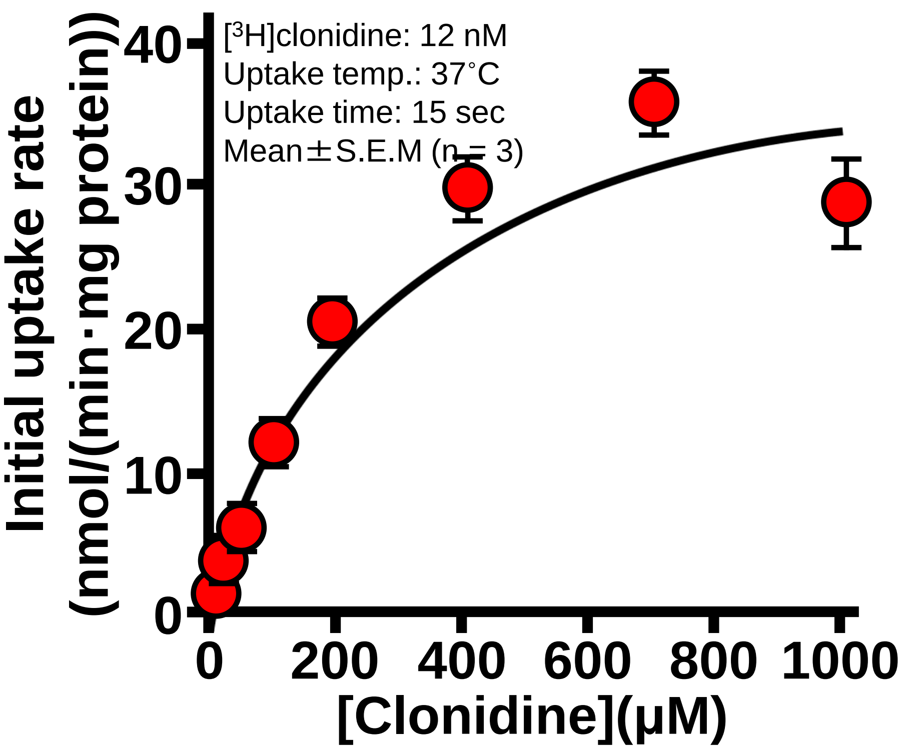
Carrier-mediated transport of [^{3}H]clonidine

[^{3}H]Clonidine uptake by TR-iBRB2 cells

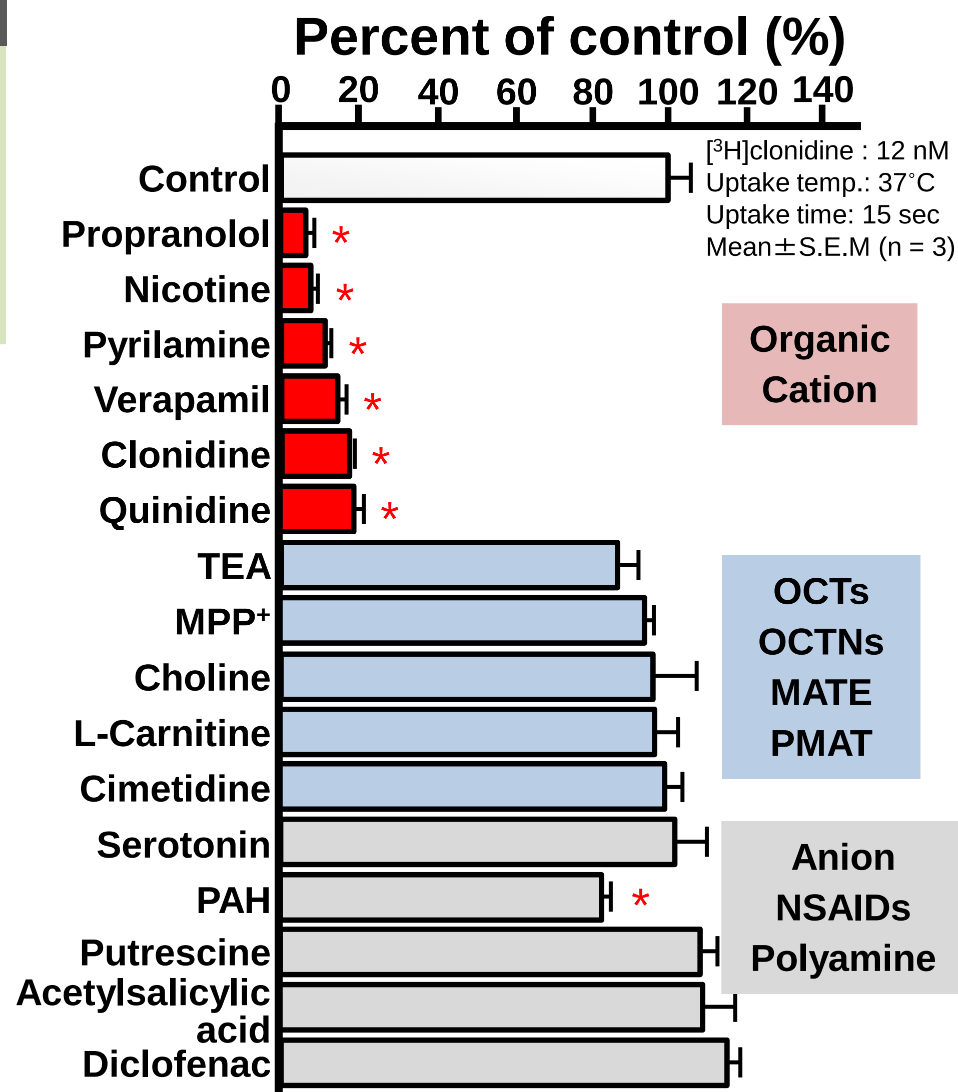
$$V = \frac{V_{max} \times S}{K_m + S}$$

K_m; **286 ± 44** μM

V_{max}; **43.7 ± 4.7** nmol/(min·mg protein)



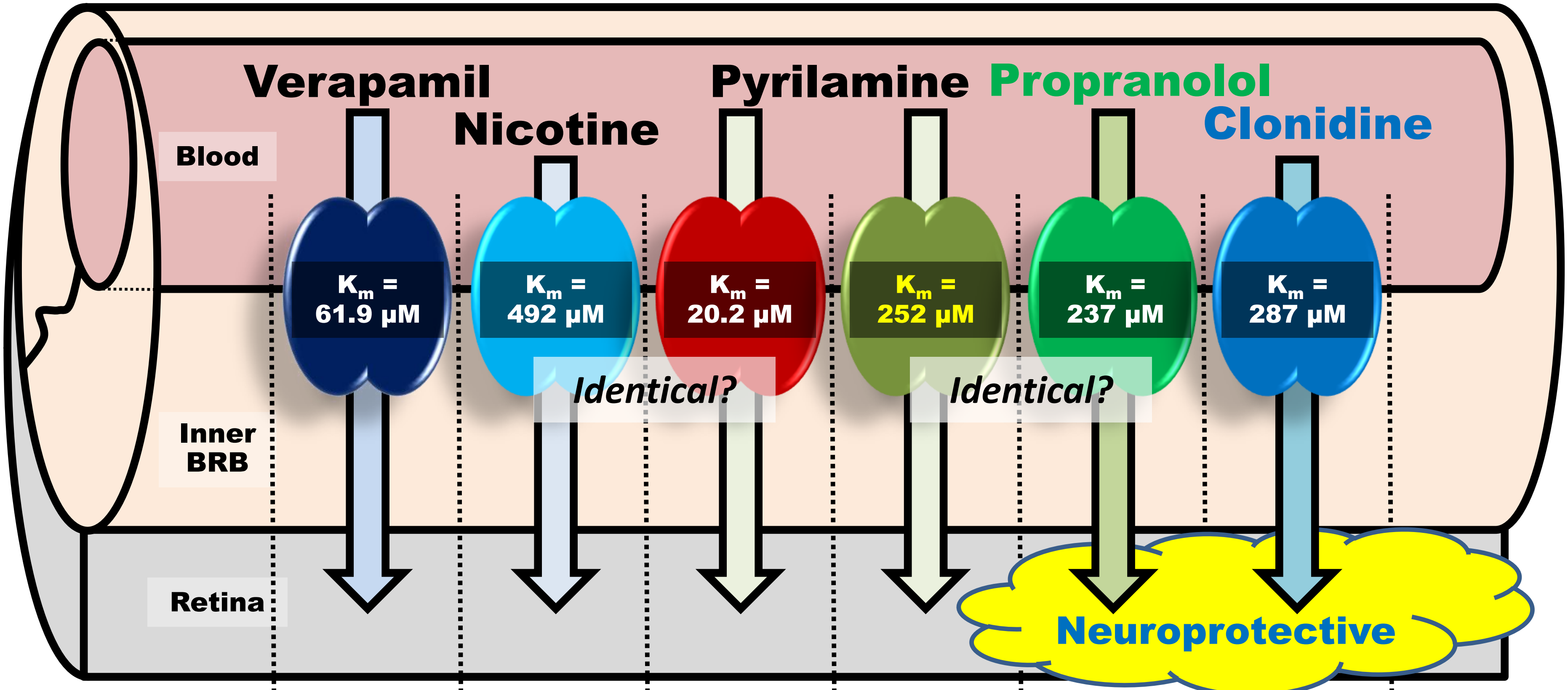
In vitro inhibition study



*$p < 0.01$, significantly different from the control.

Kubo et al. Mol Pharm. 11:3747-53 (2014)

Organic cation transport systems at the inner BRB

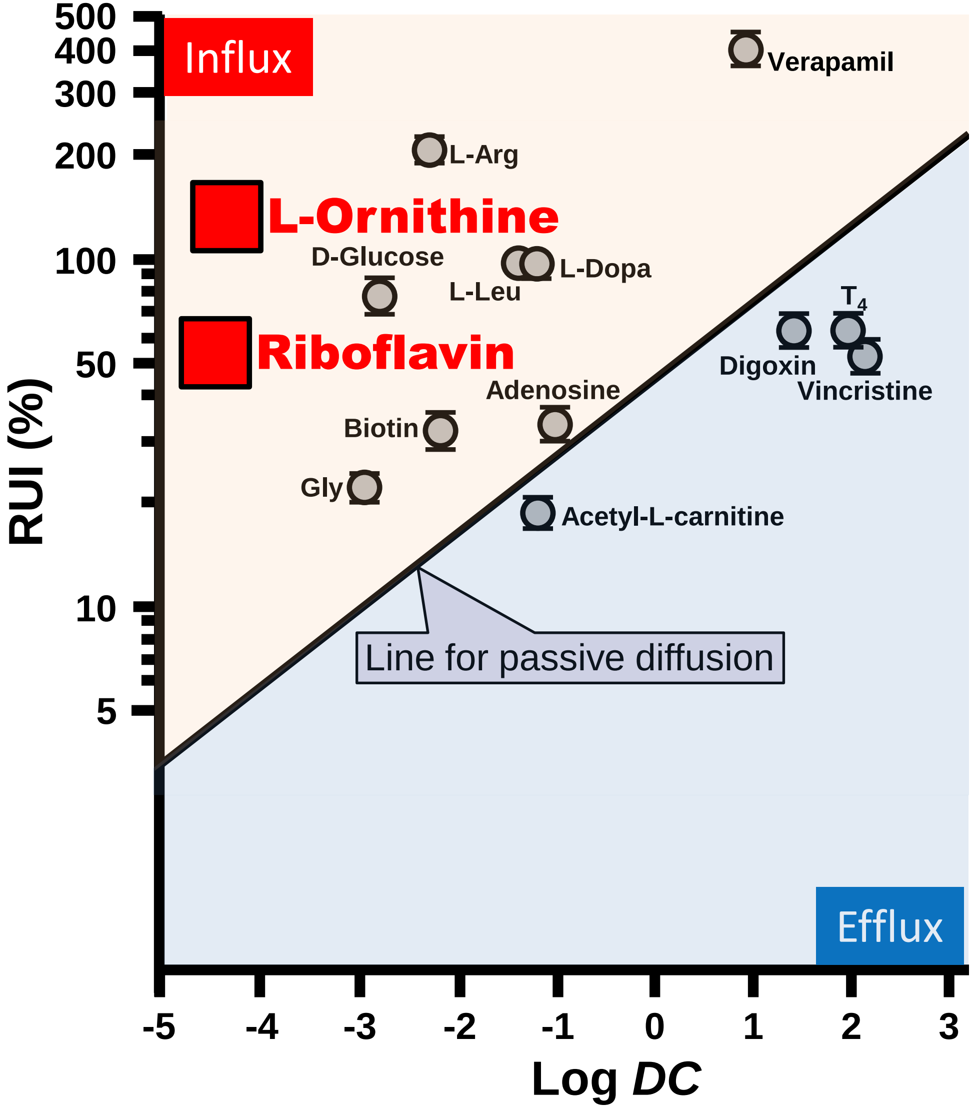


K_i (µM)	82.0 (pyrilamine)	67.7 (pyrilamine)	N.D.	N.D.	356 (pyrilamine)	90.4 (verapamil)
pH-sensitive	No	Yes	Yes	Yes	Yes	Yes
Na^+-sensitive	No	N.D.	No	No	No	No

Tega et al. J Pharm Sci. 104(3069-75 (2015), Kubo et al. J Pharm Sci. 102:3332-42 (2013), Kubo et al. Pharm Res. 30:847-56 (2013), Kubo et al. Mol Pharm. 11:3747-53 (2014)

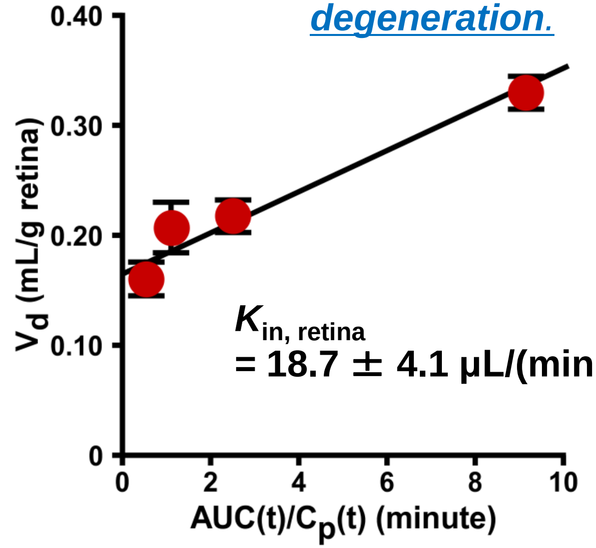
Influx transport of cationic nutrients

Retinal uptake index (RUI)



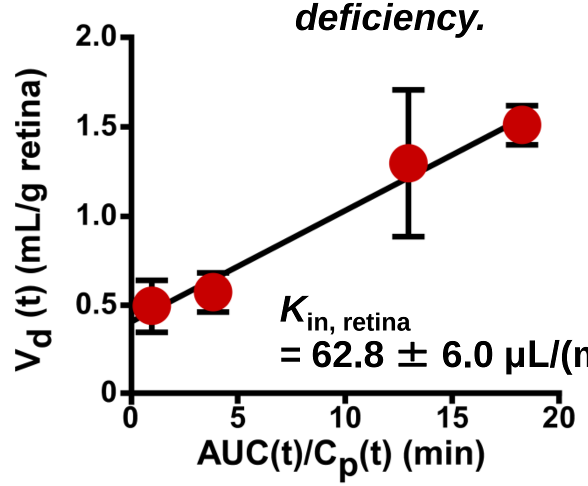
L-Ornithine

Hyperornithinemia causes the progressive retinal degeneration.



Riboflavin

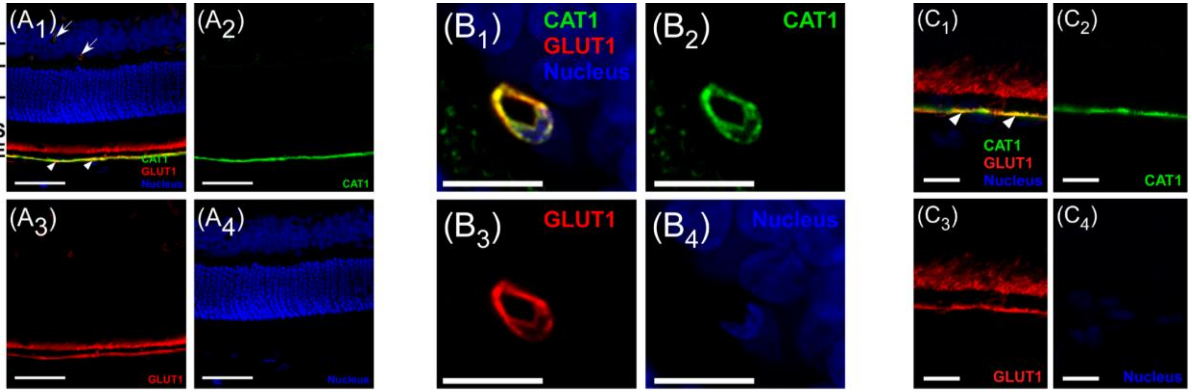
The degeneration of retinal ganglion cells in riboflavin deficiency.



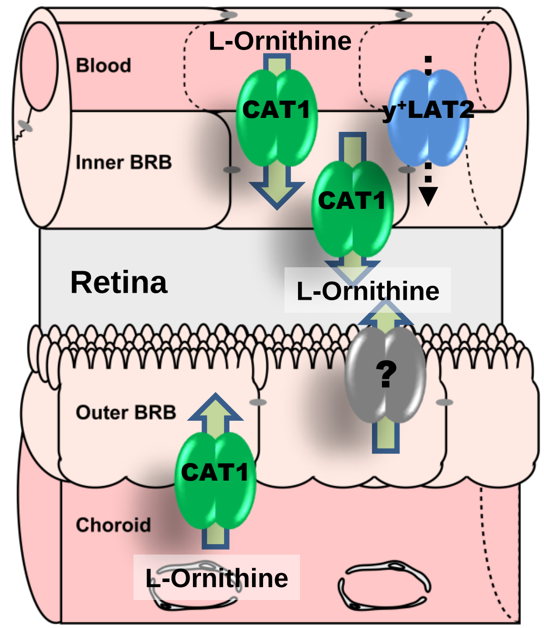
Kubo et al. Invest Ophthalmol Vis Sci. 56:5925-32 (2015), Kubo et al. Drug Metab Pharmacokinet. in press.

Influx transport of cationic nutrients

Immunohistochemical analysis of CAT1

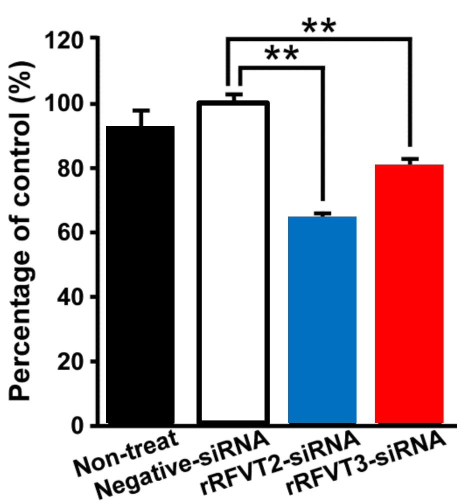


The rat retina was stained with anti-CAT1 antibodies and anti-GLUT1 antibodies (A). The colocalization of CAT1 and GLUT1 proteins was observed in the retinal capillary endothelial cells (B) and RPE cells (C). Scale bars, 50 μm (A), 10 μm (B) and 10 μm (C).

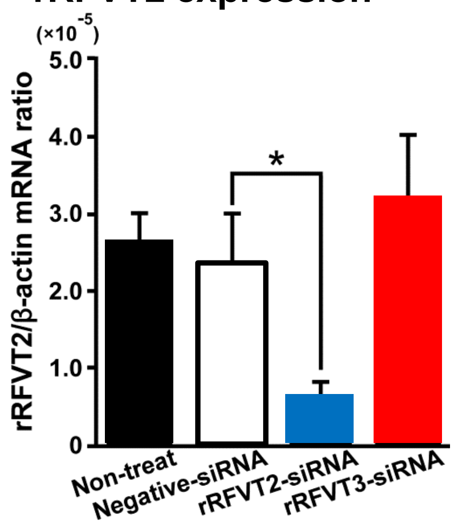


Knockdown analysis of RFVTs

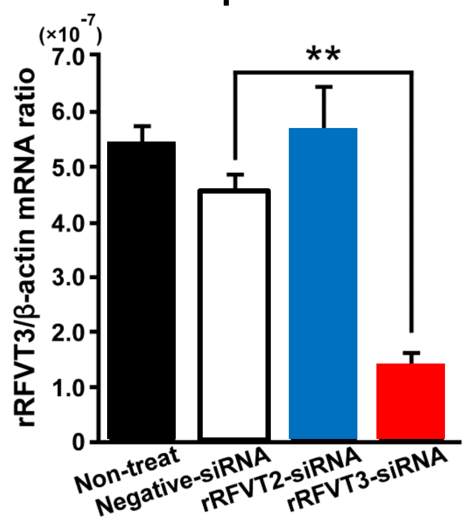
Uptake activity

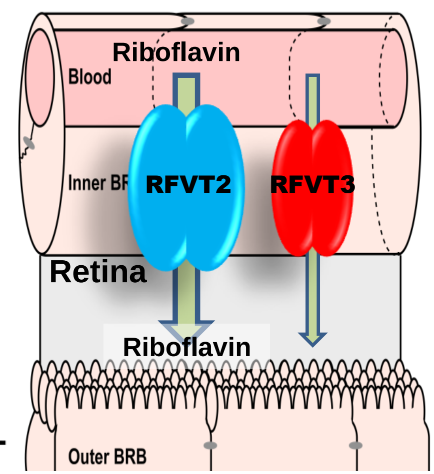


rRFVT2 expression



rRFVT3 expression

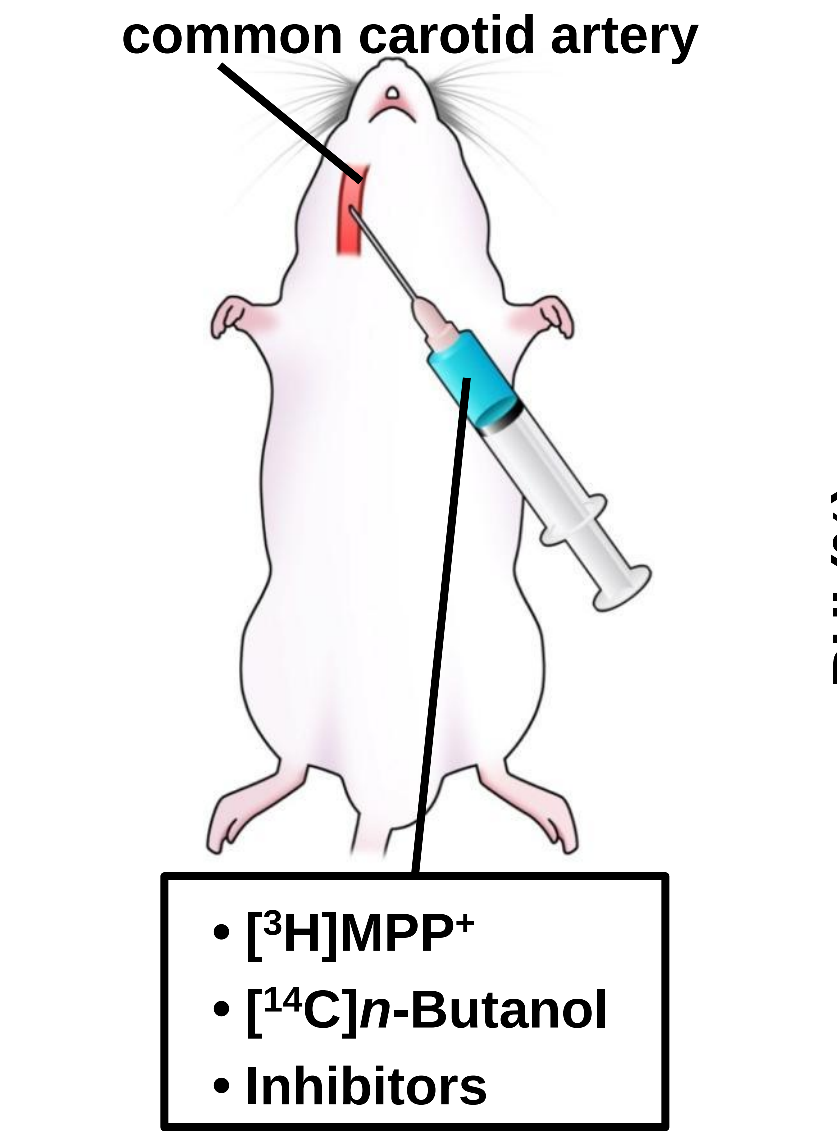


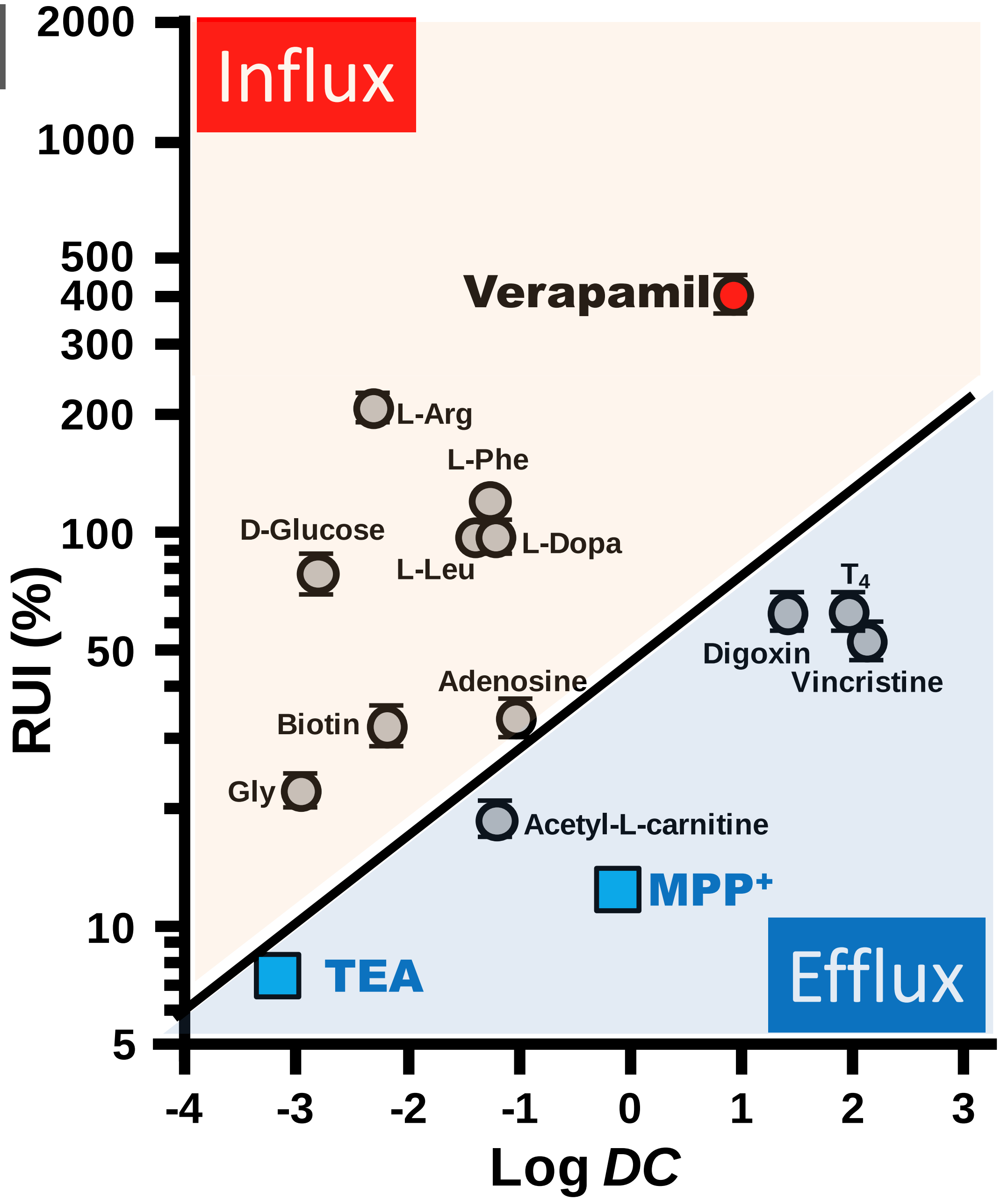


Kubo et al. Invest Ophthalmol Vis Sci. 56:5925-32 (2015), Kubo et al. Drug Metab Pharmacokinet. in press.

Efflux of 1-methyl-4-phenylpyridinium (MPP^+)

Retinal uptake index (RUI)

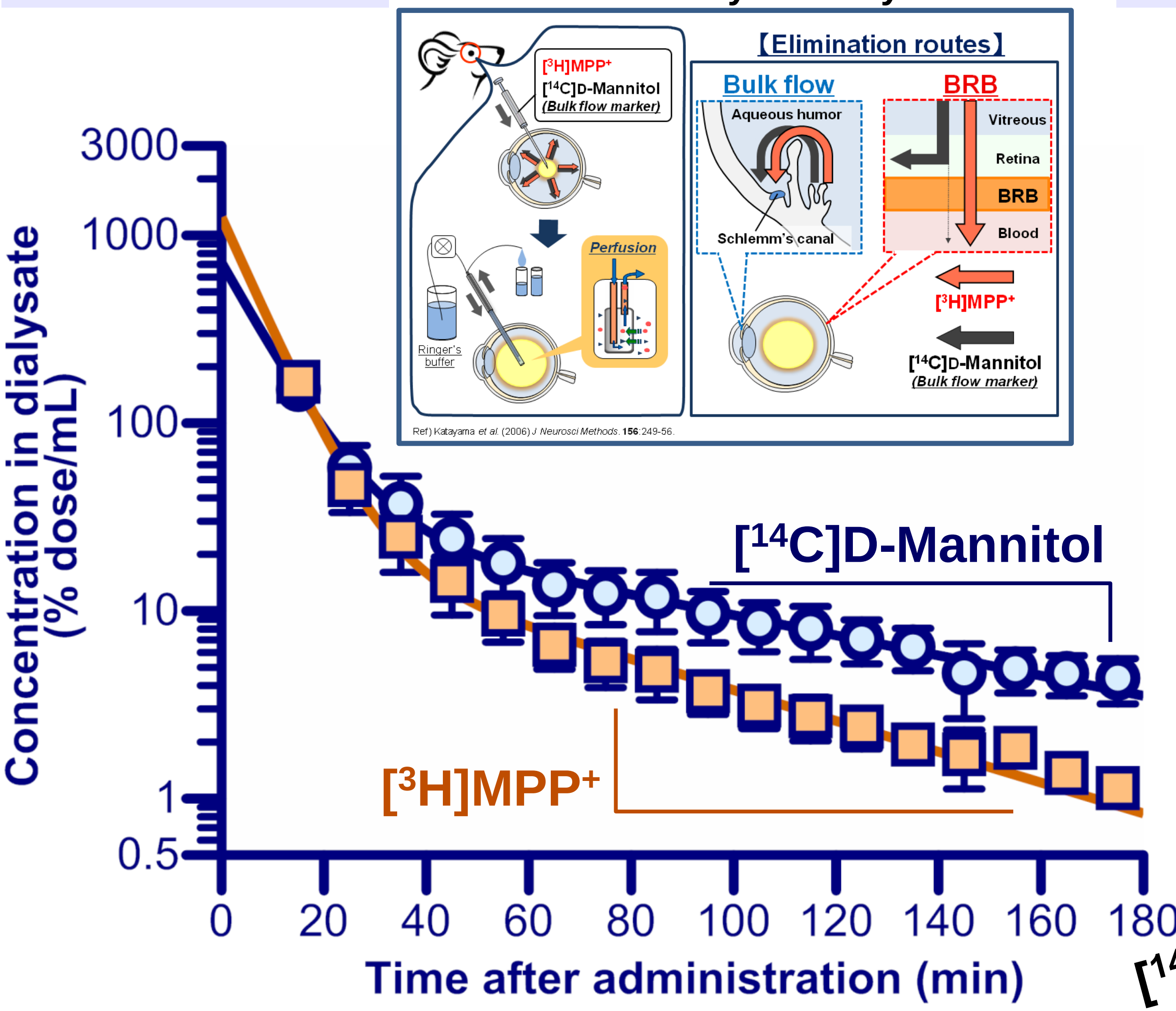




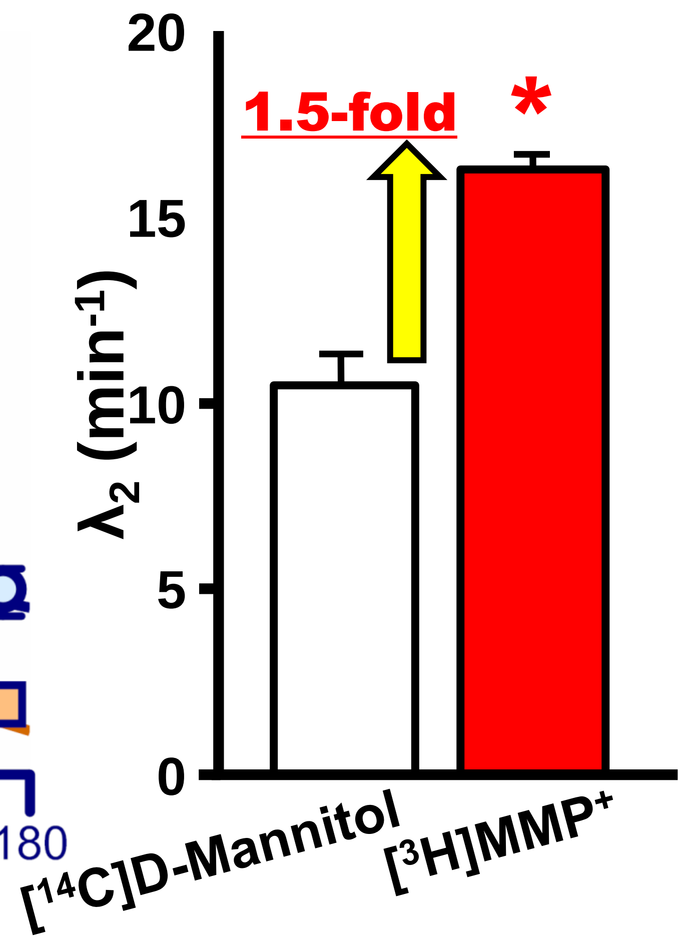
Elimination of [^{3}H]MPP$^+$ across the BRB

Time-course

Microdialysis study



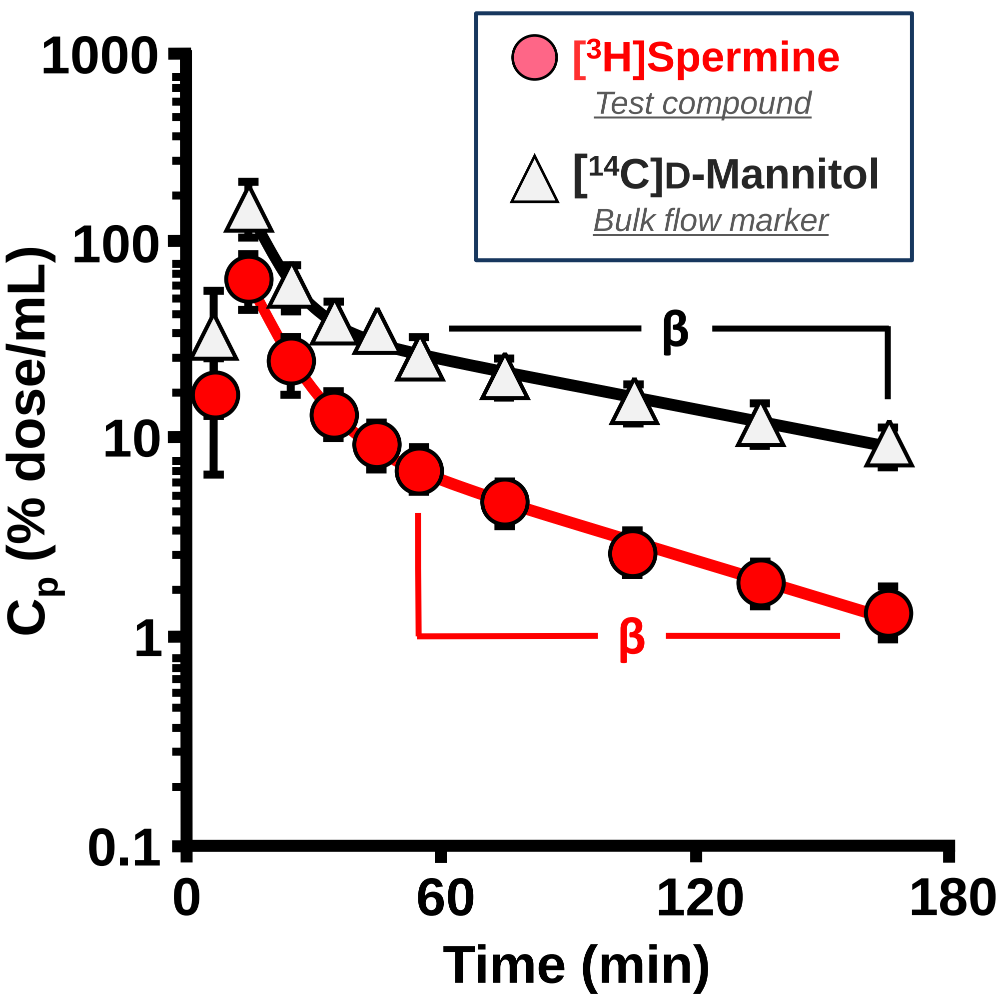
Difference of λ_2 values



Each value represents the mean ± SEM (n = 3). *p<0.01, significantly difference from the value of [^{14}C]D-mannitol.

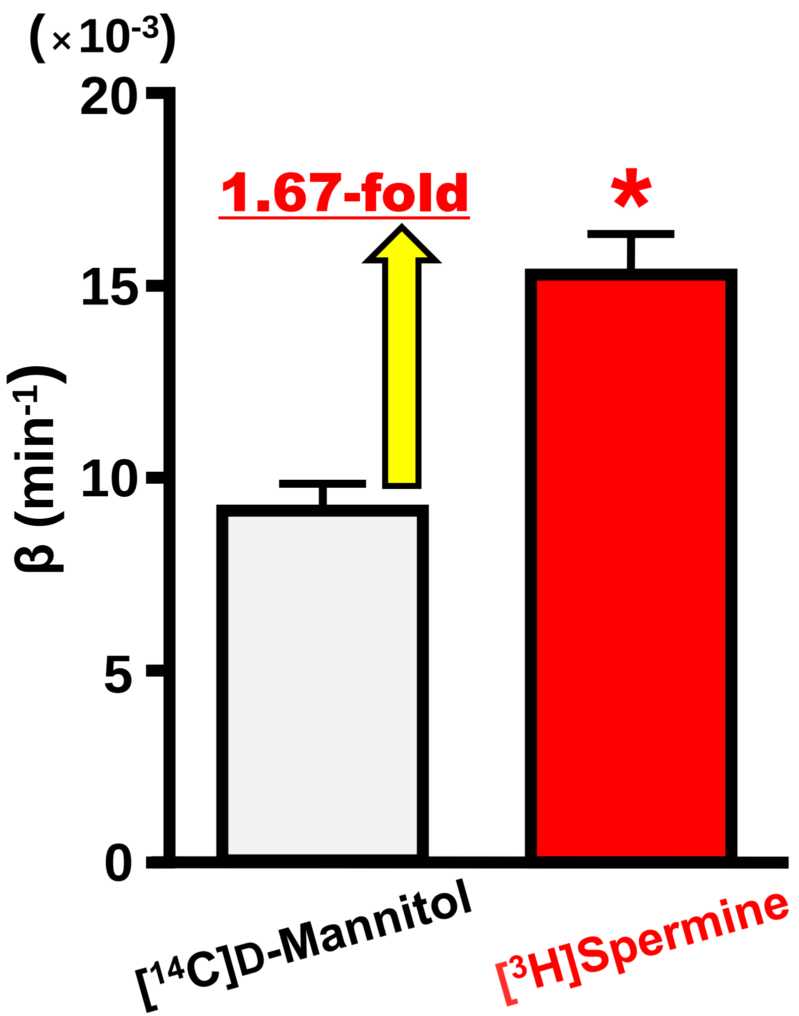
Elimination of [³H]spermine across the BRB

Time-course



Each point represents the mean ± S.E.M. (n = 5).

Difference of λ_2 values

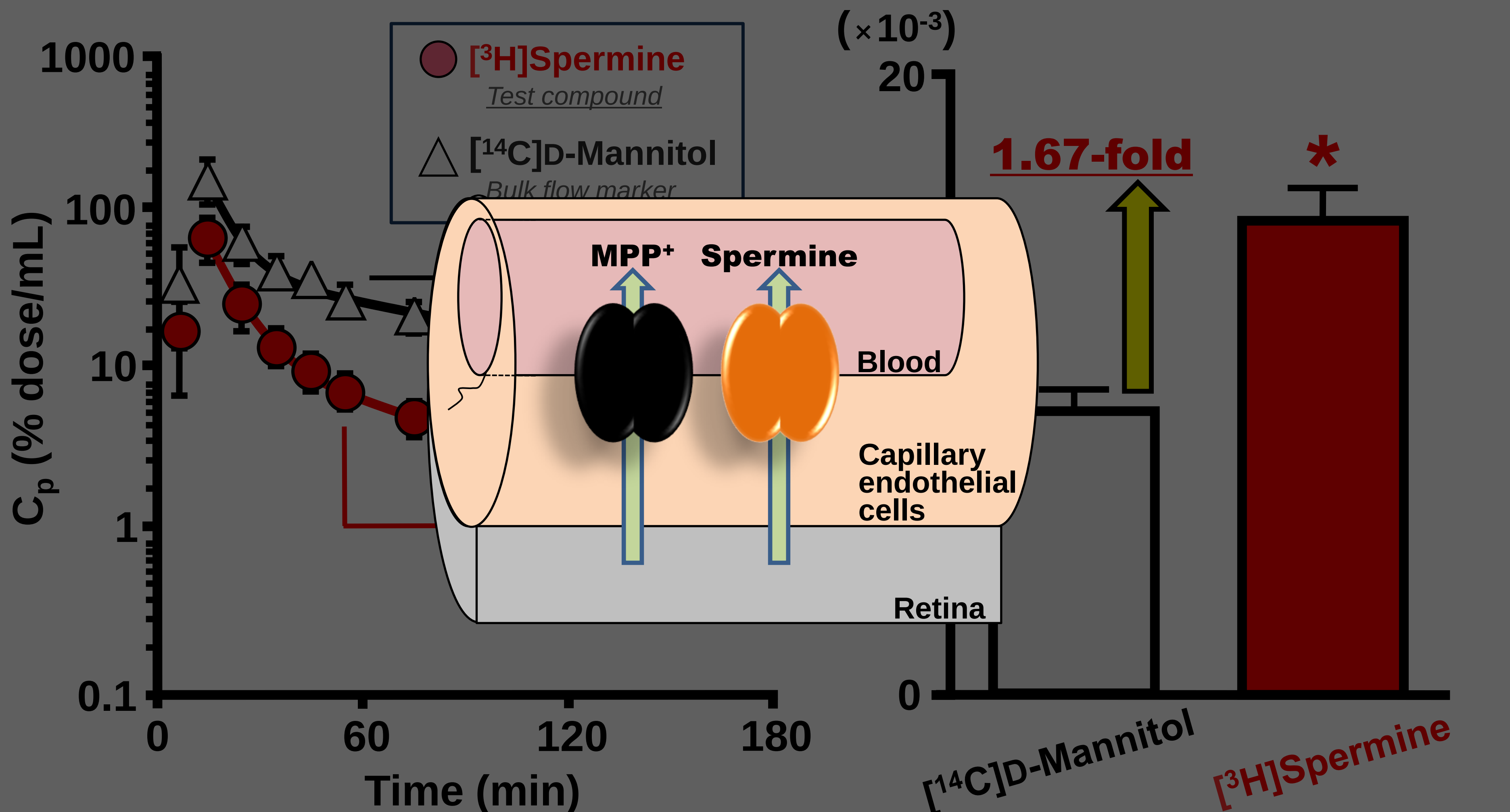


Each column represents the mean ± S.E.M. (n = 5).
*p < 0.01, significantly different from [¹⁴C]D-mannitol.

Kubo et al. Exp Eye Res. 124:17-23 (2014)

Elimination of [³H]spermine across the BRB

Time-course | **Difference of λ_2 values**



Each point represents the mean ± S.E.M. (n = 5).

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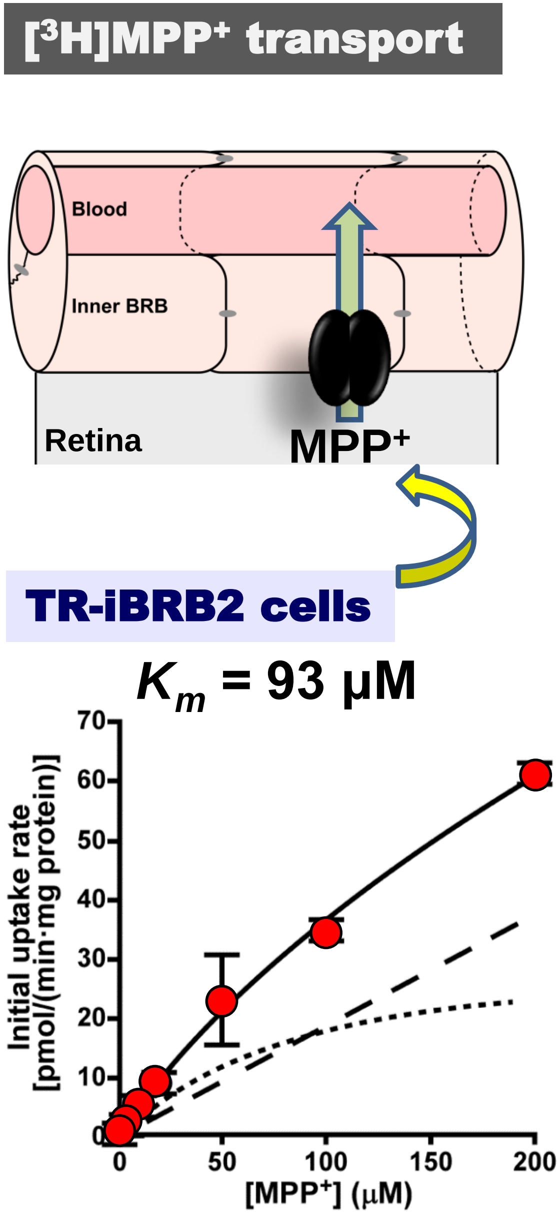
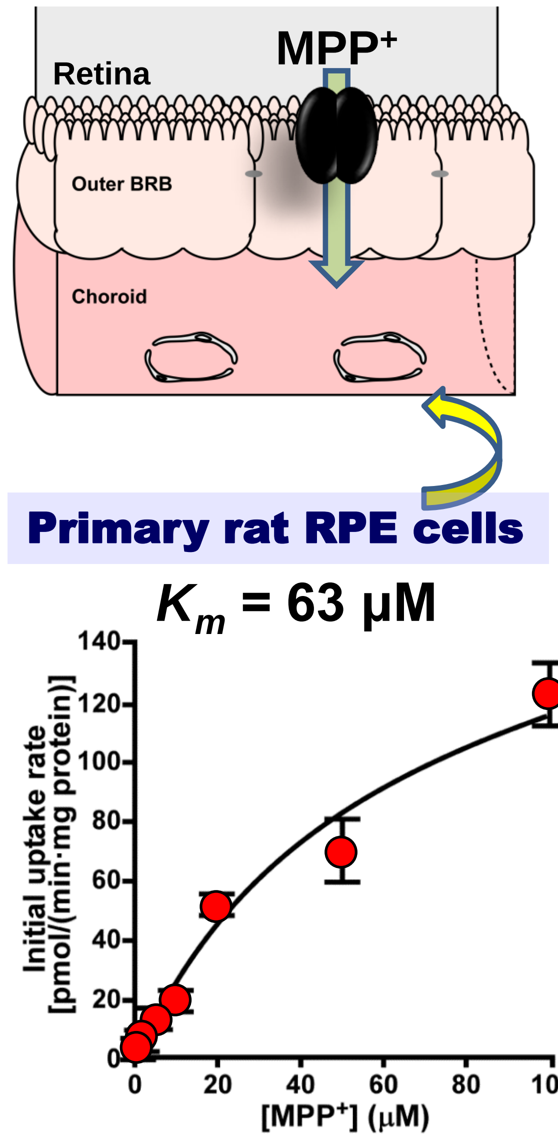
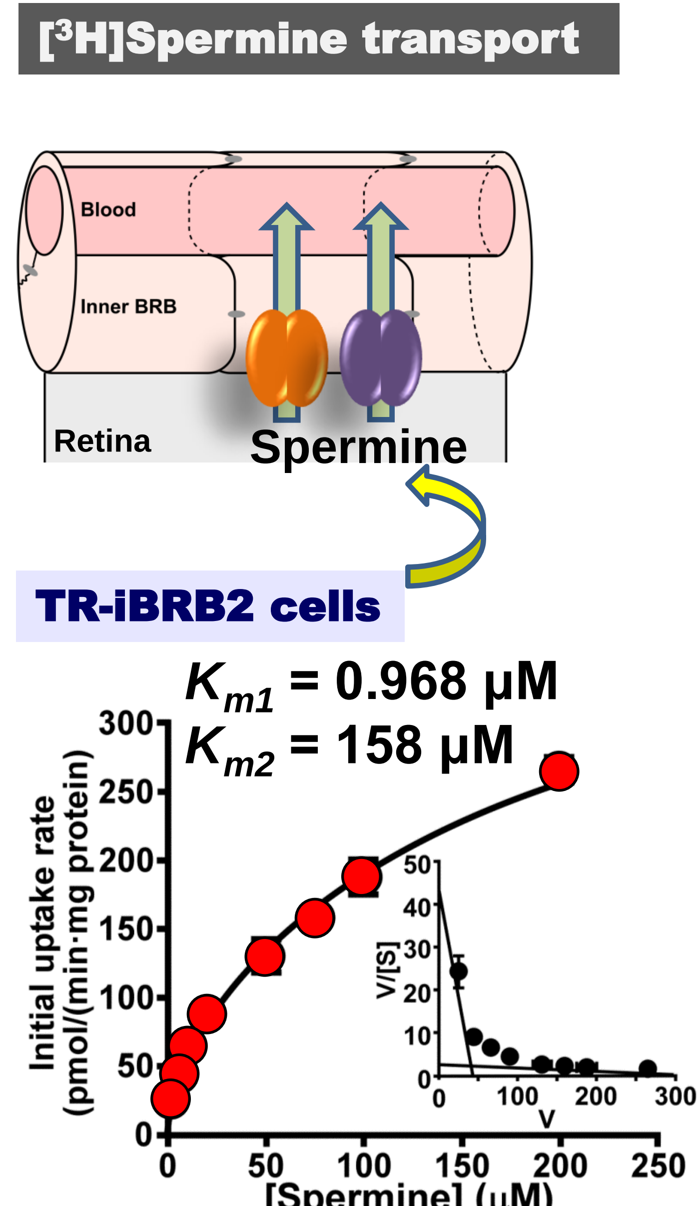
Kubo et al. Exp Eye Res. 124:17-23 (2014)

Carrier-mediated efflux of [^{3}H]MPP$^+$ and [^{3}H]spermine

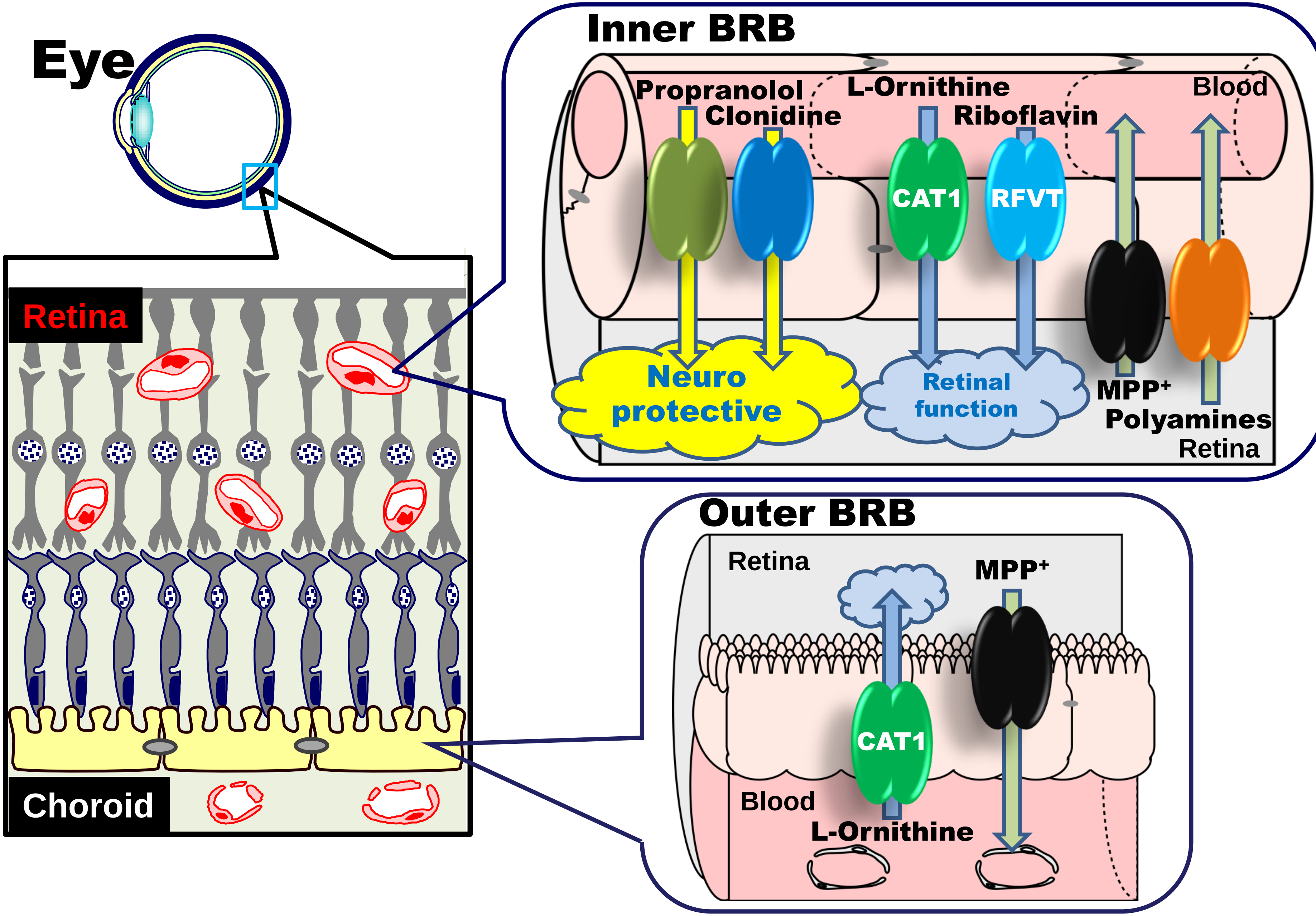






Kubo et al. Exp Eye Res. 124:17-23 (2014)

Summary


Eye
Retina
Choroid
Inner BRB
Propranolol
Clonidine
L-Ornithine
Riboflavin
Blood
CAT1
RFVT
Neuro protective
Retinal function
MPP+
Polyamines
Retina
Outer BRB
Retina
MPP+
CAT1
Blood
L-Ornithine

Snow wall in Tateyama

Thank you for your attention.