JSSX Young Investigator's Award Presentation
Tsukuba; Dec. 11 (2019)

Functional elucidation of membrane transporters at brain/retinal barriers for overcoming inflammation-related CNS diseases

炎症性中枢神経系疾患克服に指向した 脳・網膜関門における輸送分子機構の機能解明

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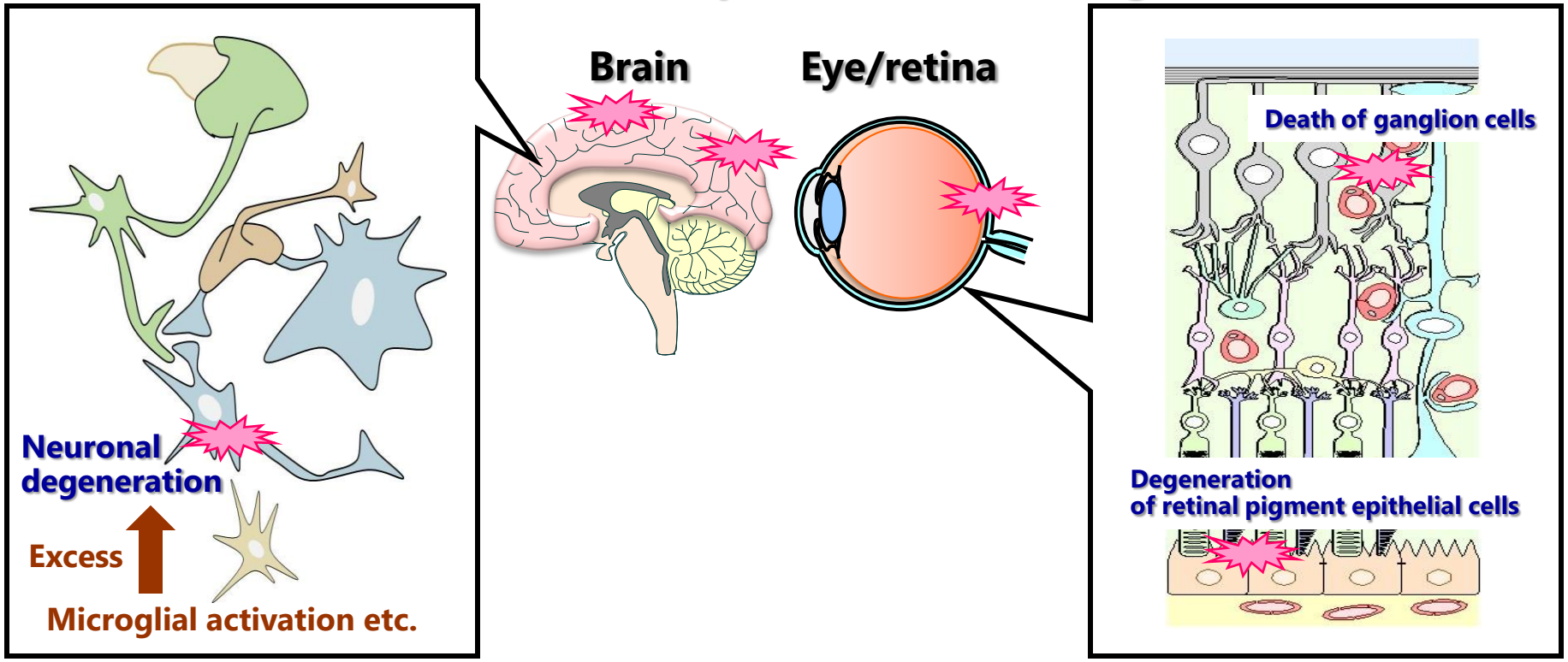
In the 1st grade of Ph.D. course of Tohoku University

Inflammation relates to neurodegenerative diseases

Inflammation

links to the onset and/or development of neuro-degenerative diseases.

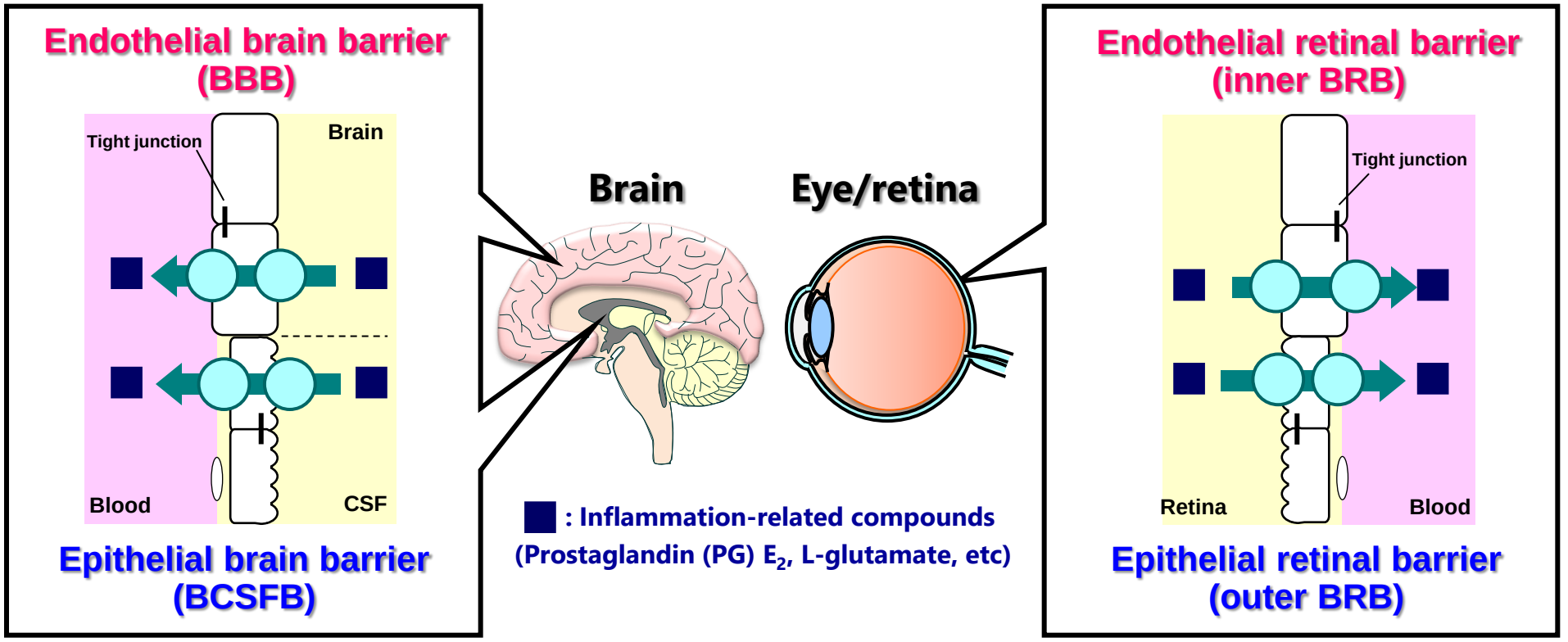


- Epilepsy
- Alzheimer's diseases, etc.

- Glaucoma
- Diabetic retinopathy, etc.

"Inflammation" is one of the master physiological responses for the refractory brain and retinal diseases.

The barriers at the brain and retina



It is hypothesized that…

the inflammation-related compounds are actively eliminated across the brain/retinal barriers.

⇨ **Contributing molecules for the compound elimination**

= Target to regulate the neuro-inflammatory responses.

Our recent outcomes about membrane transporters

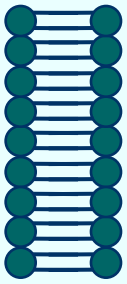
1. **Elimination of inflammation-related compounds at the BBB (PGE_2 elimination - glutamate receptor coupling)**

2. **BRB elimination of inflammation-related compounds (L-glutamate, hypoxanthine, etc.)**

3. **New transporters of drugs for the brain/retinal diseases at the barriers**

Neural prostaglandin E_2 (PGE_2) dynamics

Production



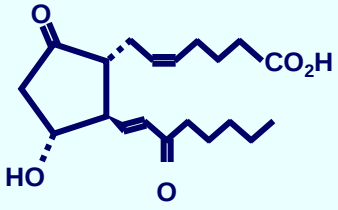
PLA_2

AA

Arachidonate cascade

COX-PGES

Prostaglandin E_2 (PGE_2)



Lipid bilayer

PGE_2 is associated with…

Pathological effect

Fever progression, Allodynia,

***Disease* (↑PGE_2 conc.)**

- **Epilepsy**
- **Alzheimer's diseases**

Inactivation

~~15-PGDH~~

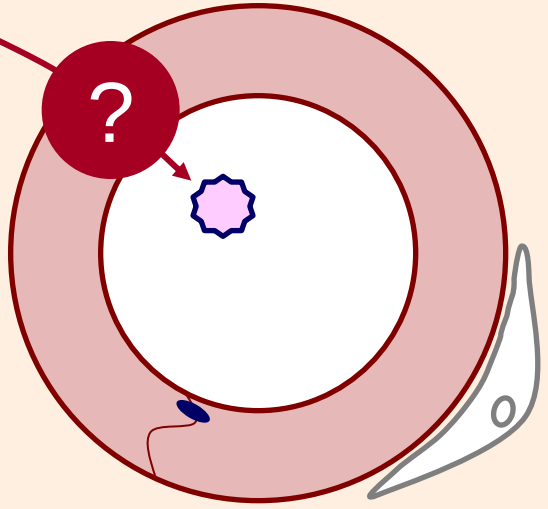
15-oxo-PGE_2

(Brain Res. **39**, 545-8 (1972))

Clearance

?

?



Blood-brain barrier (BBB)

Neurological effects

Ca^{2+} ↑
cAMP ↑or ↓

EPs

PGE_2

Caspase-dependent pathway activation

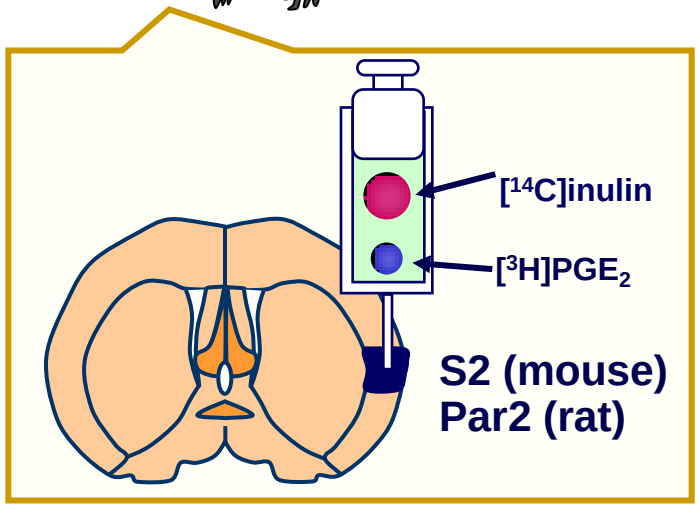
Neuronal cells apoptosis

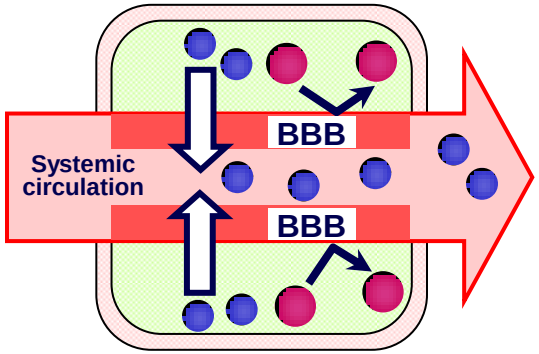
Neuron or Glial cells

AA, arachidonic acid; COX, cycloxygenase; EP, E-type prostanoid receptor; PG, prostaglandin; 15-PGDH, 15-prostaglandin dehydrogenase; PGES, prostaglandin E synthase; PLA_2, phospholipase A_2

Brain efflux index (BEI) method | *in vivo* BBB efflux evaluation

Wistar rats (150-200 g, male)
C57BL/6J mice (20-30 g, male)





● : Test compound

● : Reference compound
(limited BBB permeability)

Definition

$$BEI\ (\%) = \frac{PGE_2 \text{ undergoing efflux via BBB}}{PGE_2 \text{ injected into the brain}} \times 100$$

Determination

$$100 - BEI\ (\%) = \frac{[^3H]PGE_2 \text{ in the brain} / [^{14}C]\text{inulin in the brain}}{[^3H]PGE_2 \text{ in injectate} / [^{14}C]\text{inulin in injectate}} \times 100$$

Percentage of $[^3H]PGE_2$ remaining in the ipsilateral cerebrum

Constant of efflux rate from brain via BBB

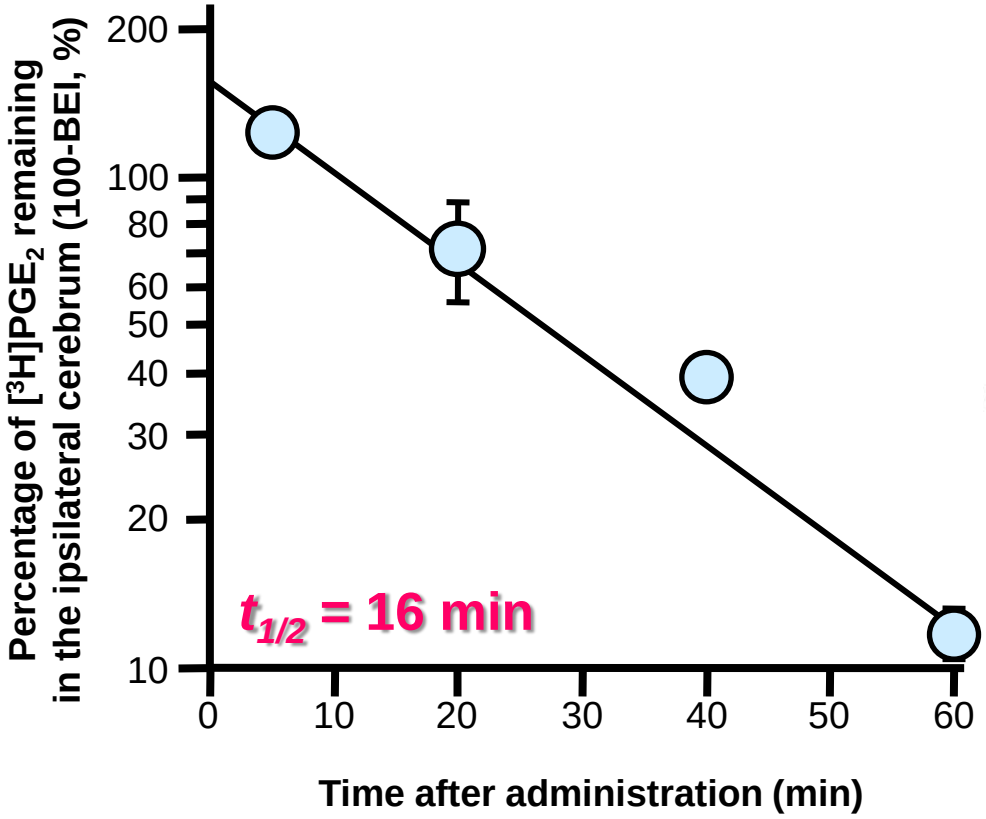
k_{eff} = -slope of log (100-BEI (%)) vs. time

$\Rightarrow t_{1/2} = \log_e 2 / k_{eff}$

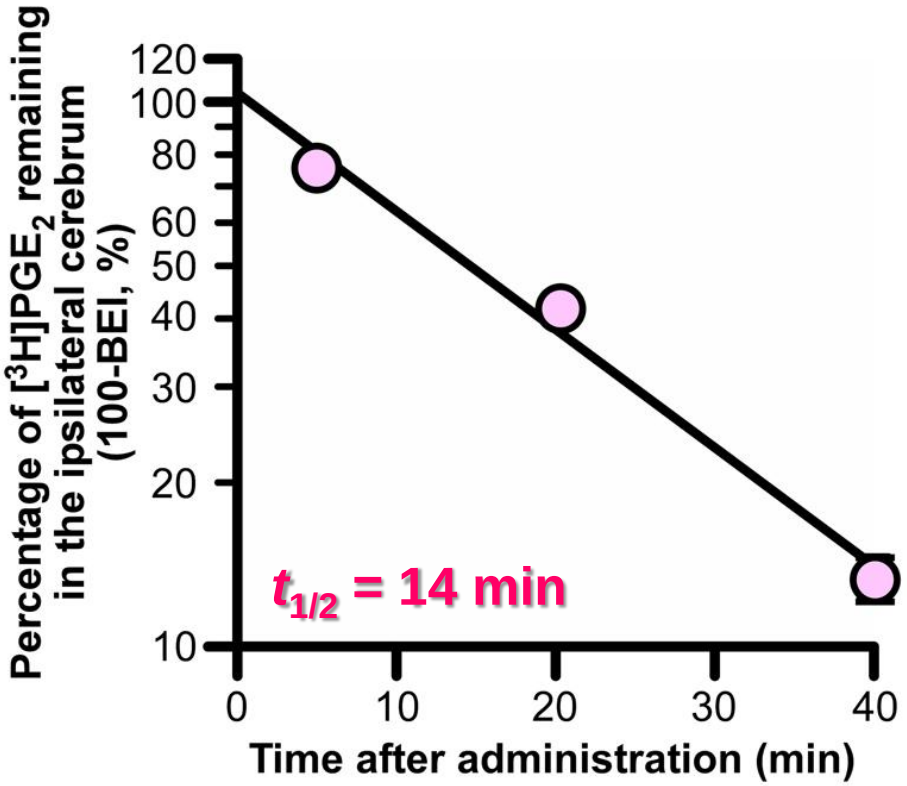
J. Pharmacol. Exp. Ther. **277**, 1550-9 (1996)

PGE_2 elimination across the BBB

Mouse study



Rat study

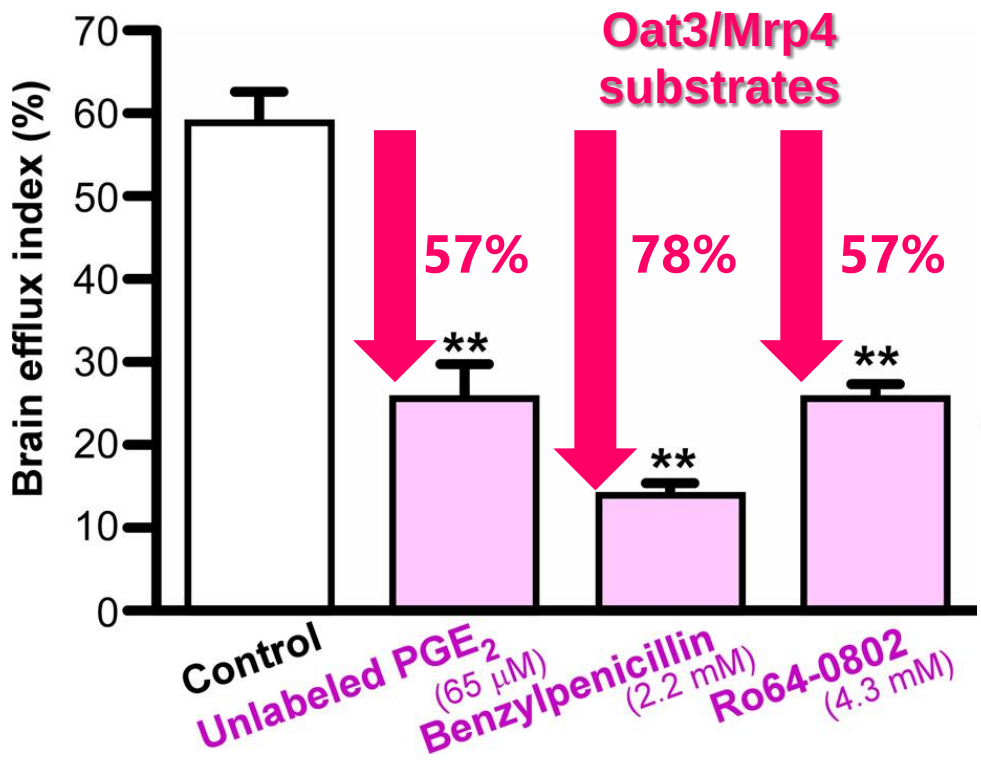


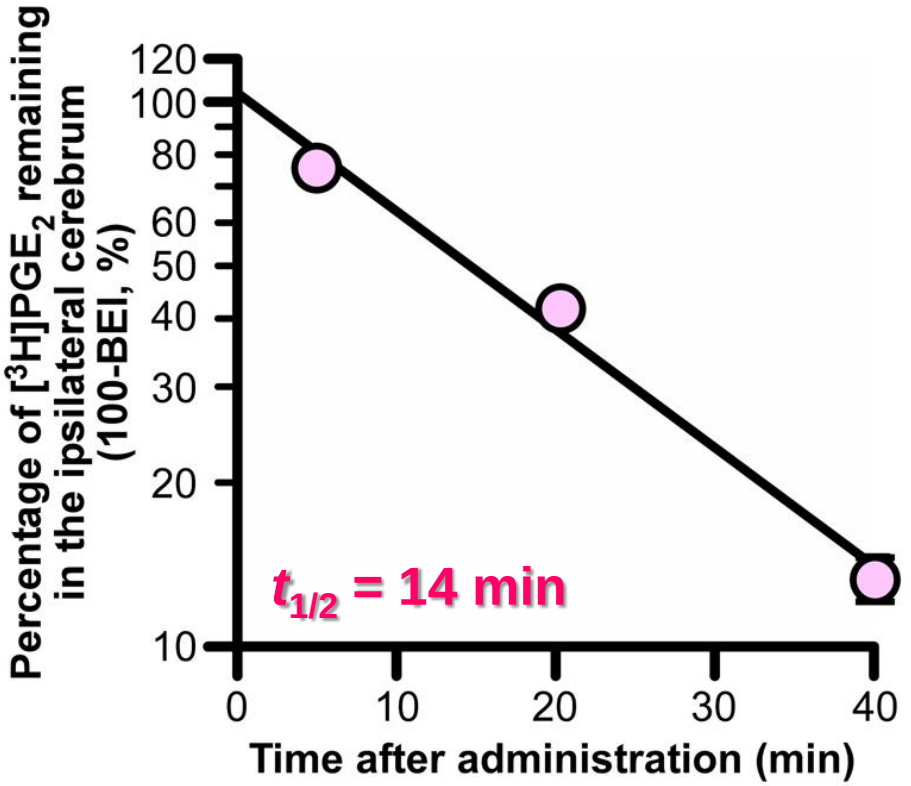
Each point represents the mean ± S.E.M. (n = 3-5).

PGE_2 elimination across the BBB

Rat study

Co-administration (at 20 min)





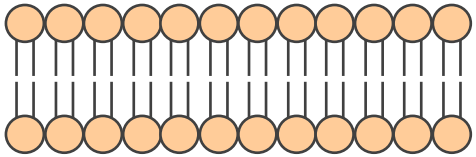
Oat3, organic anion transporter 3
Mrp4, multidrug resistance-associated protein 4

Compounds were co-administered with a mixture of [^{3}H]PGE_2 and [^{14}C]D-mannitol. The brain efflux index value was determined 20 min after intracerebral microinjection of [^{3}H]PGE_2. Each column represents the mean ± SEM (n=4-7). ** p<0.01, significantly different from the control

Each point represents the mean ± S.E.M. (n = 3-5).

Relationship of L-Glu and PGE_2 in the brain

Production of PGE_2



Lipid bilayer

Phospholipase A_2

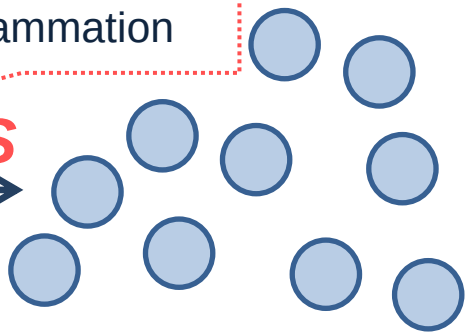
Arachidonate

Cyclooxygenase

PGH_2

PGES

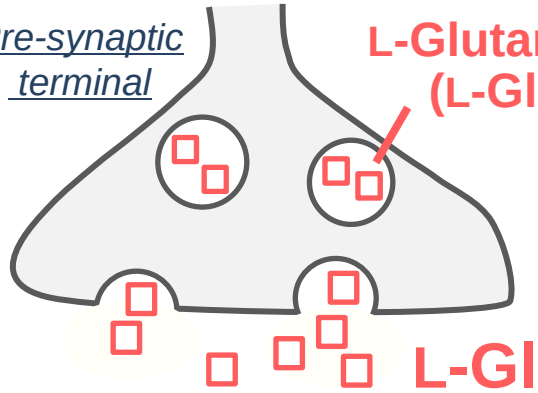
mPGES-1 is up-regulated by inflammation



ProstaglandinE_2 (PGE_2)

2-fold↑

Curr Med Chem. (2008) **15:**1863-9.



Pre-synaptic terminal

L-Glutamate (L-Glu)

L-Glu↑

Metabolite

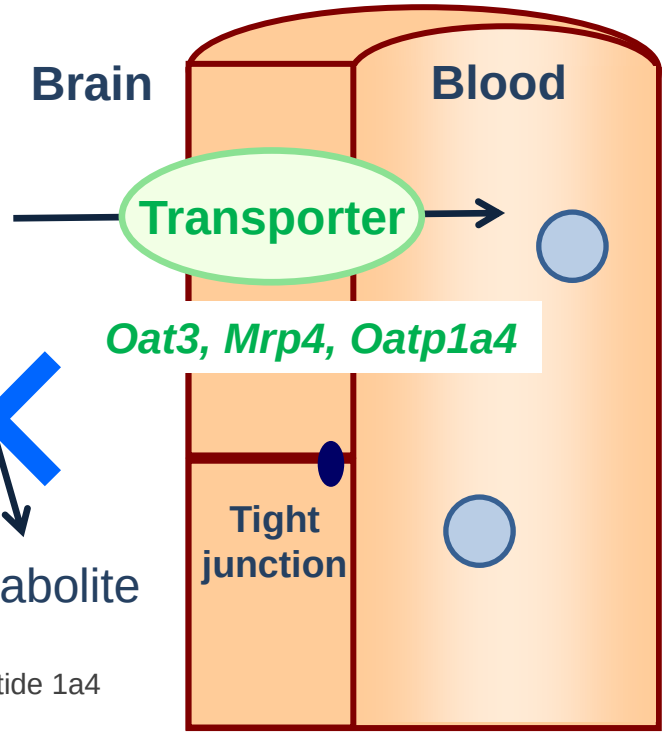
Pathological effects

- **Inflammation**
- **Neurotoxicity**

Ischemia, convulsion

Elimination of PGE_2

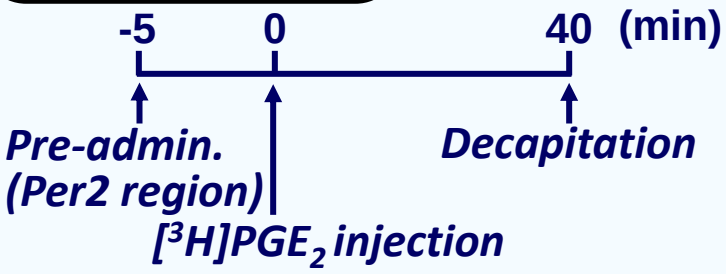
Blood-brain barrier (BBB)



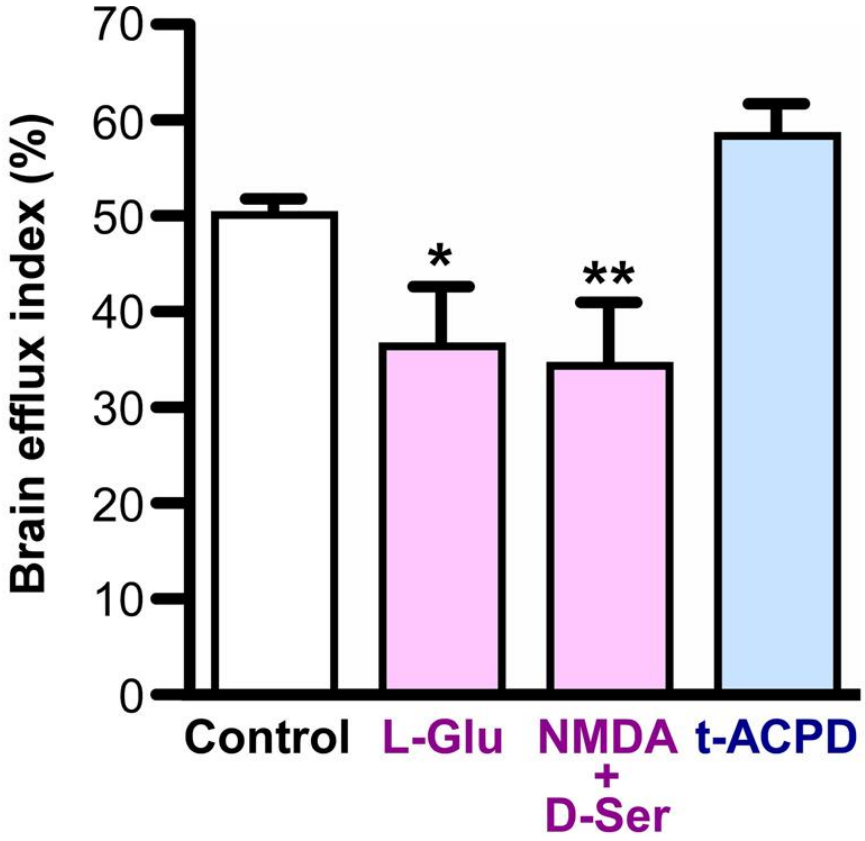
EP; E-type prostanoid receptor, PGES; PGE synthase, Oat3; organic anion transporter 3, Mrp4; multidrug resistance-associated protein 4, Oatp1a4; organic anion transporting polypeptide 1a4

Decrease of the PGE_2 elimination by intracerebral L-Glu

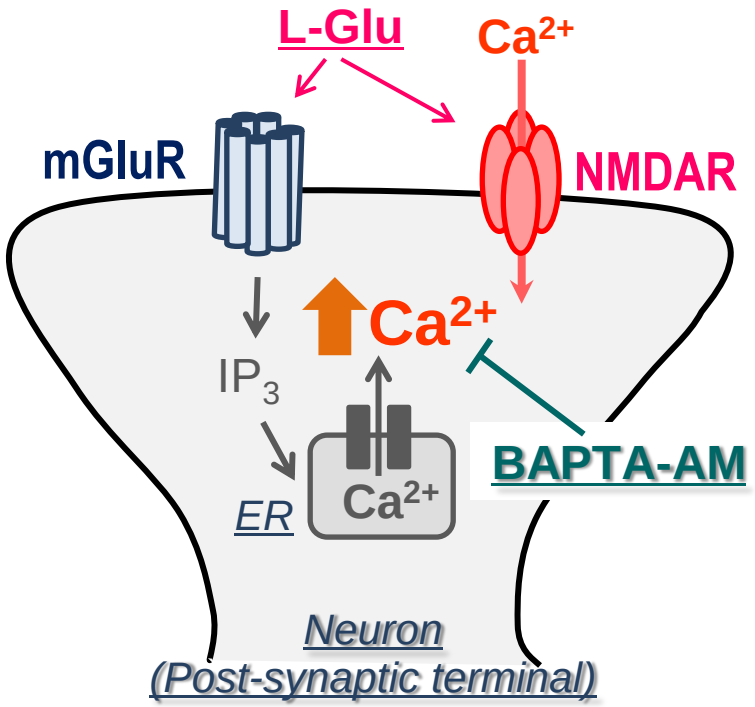
Rat study



Intracerebral agonist admin.



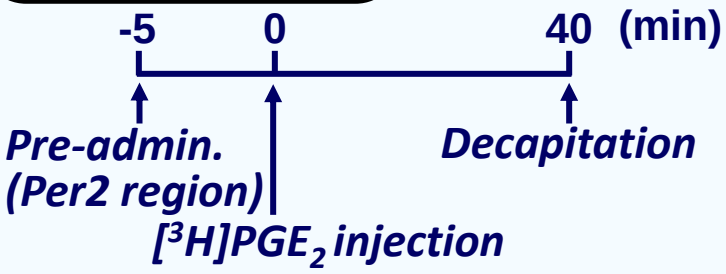
Signal cascade related to L-Glu



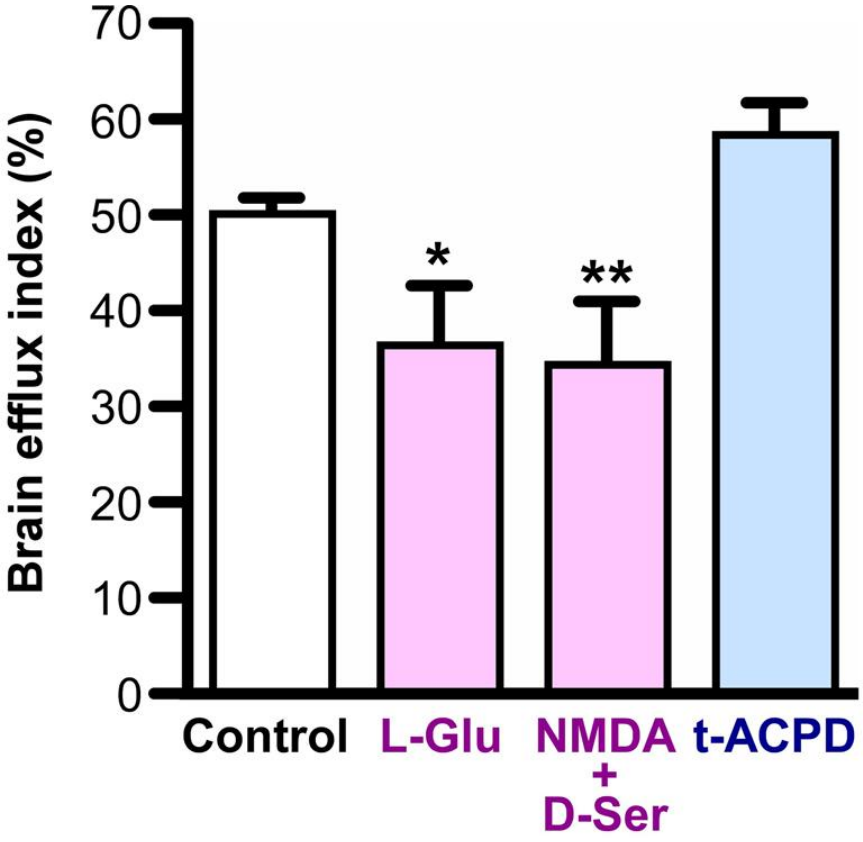
L-Glutamate, a mixture of NMDA and D-Ser, tACPD, or ECF buffer (control) was pre-administered 5 min before administration of a mixture of [^{3}H]PGE_2 and [^{14}C]D-mannitol. ach column represents the mean±SEM (n=3-12). *p<0.05 and **p<0.01, significantly different from the control.

Decrease of the PGE_2 elimination by intracerebral L-Glu

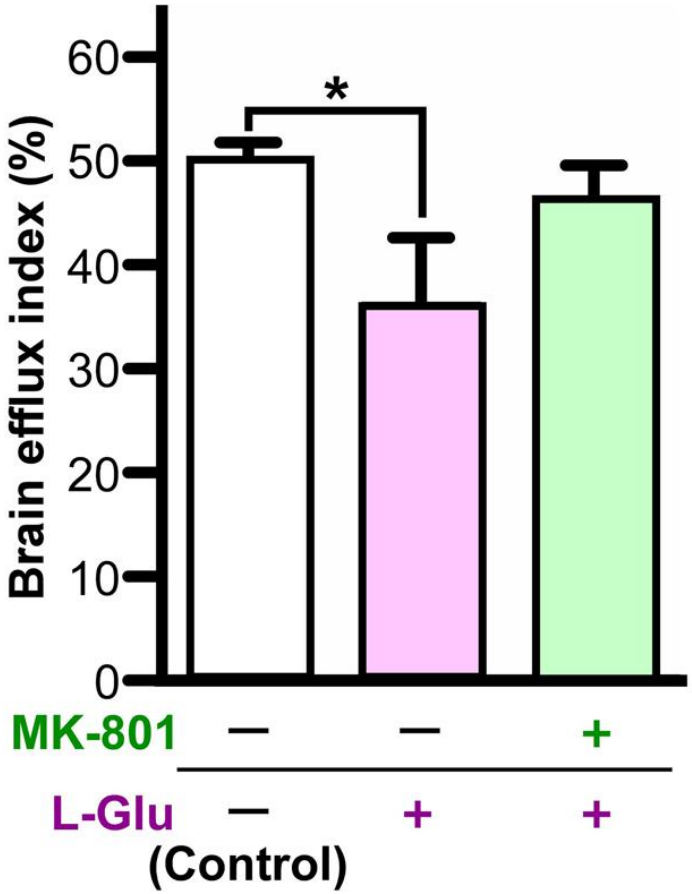
Rat study



Intracerebral agonist admin.

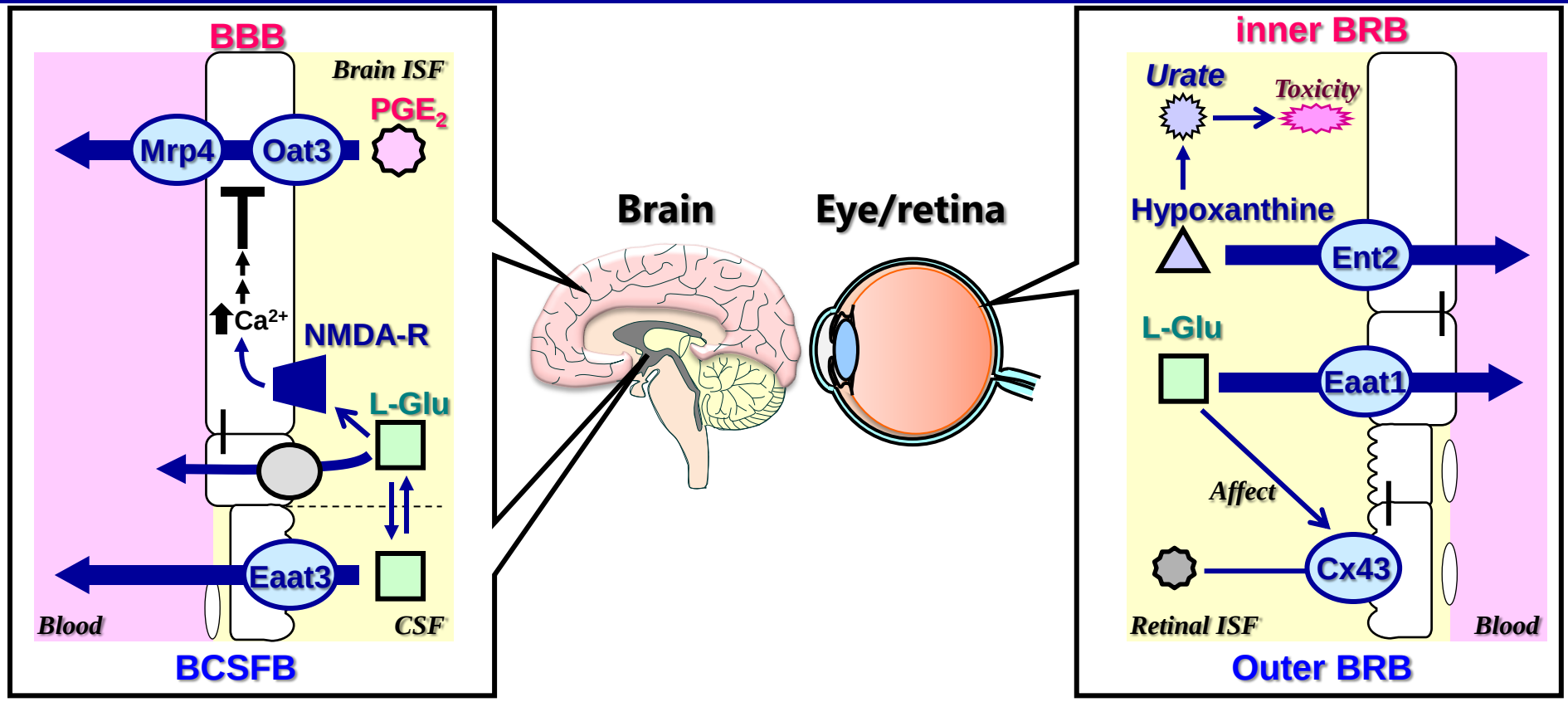


Effect of NMDA-R antagonist



At 5 min before the administration of a mixture of [^{3}H]PGE_2 and [^{14}C]D-mannitol, L-Glu was pre-administered in the absence or presence of antagonists for NMDAR (MK-801). Each column represents the mean ± SEM (n=3-12). *p<0.05, significant difference.

Conclusion and next step of my/our research



J. Pharmacol. Exp. Ther., 333, 912-9 (2010)
Fluids Barriers CNS. 8:24 (2011)
Drug Metab. Pharmacokinet. 29, 387-93 (2014)
Fluids Barriers CNS. 12:11 (2015)
Exp. Eye Res., 168, 128-37 (2018)

Invest. Ophthalmol. Vis. Sci., 54, 1469-77 (2013)
Biol. Pharm Bull. 1087-91 (2015)
Exp. Eye Res., 168, 128-37 (2018)

Administration of drugs interacting with these molecular systems

The neural circumstance gets worse for the treatment of the CNS diseases