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A trip of peptides to the brain

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Abstract

Dietary di/tripeptides elicit preventive effects against lifestyle-related diseases such as hypertension, and hypercholesterolemia, etc. Although there have been evidential reports that the intake of protein hydrolysate improved impaired memory in human, limited studies on bioavailability, in particular, beyond the blood-brain barrier (BBB) of candidates in hydrolysate may prevent their extensive physiological studies. Thus, this review discusses the updated studies on BBB transport of peptides showing improved cognitive decline. Furthermore, their accumulation in the brain cerebral parenchyma is also introduced.

Keywords: Peptide, Blood-brain barrier, Transport, Cognitive decline

Introduction

The physiological role of peptides is deemed important from the aspect of preventive lifestyle-related diseases, such as hypertension, diabetes, and atherosclerosis (Mine et al. 2010). Clinical evidences prove their effective health benefits in humans, for example, the anti-hypertensive effect shown by daily intake of small (di/tri) peptides, which modulate promoting blood pressure (Hata et al. 1996; Kawasaki et al. 2000). Anti-hypertensive effect of peptides (e.g., Val-Tyr) is closely associated with the suppression of promoting systemic and local renin-angiotensin (RA) systems by inhibiting angiotensin I-converting enzyme (ACE) activity. In addition, the suppression of local RA systems by peptides in the aorta and kidney (Matsui et al. 2003; Dias et al. 2017) extensively leads us to study the potential of peptides in local organs. Researchers have clarified the action of diverse peptides in improving degraded vessel functions (Matsui et al., 2012). Dias et al. (2017) have also reviewed the physiological potential of a dipeptide, Val-Tyr [angiotensin (3–4)], in the kidney through the inhibition of Na^+ reabsorption via the allosteric regulation of angiotensin-related receptors. Collectively, such reports strongly lead us to speculate that our body can accept peptides intestinally in intact form.

An epoch-making study on peptide absorption has been reported by Fei et al. (1994), who successfully cloned a human peptide transporter 1 (PepT1) from the cDNA library of rabbit intestine. PepT1 can recognize the structure of peptide bonding corresponding to di/tri-peptides. Transporters related to peptide transport as a super family of proton-coupled oligopeptide transporters (POT), also known as a solute carrier 15 (SLC15), have been identified as PepT1/2 and peptide-histidine transporters (PHT)1/2 (Daniel and Kottra, 2004). They are located in diverse organs including the intestines, kidneys, spleen, liver, lungs, and brain, whereas their characteristics on peptide recognition at each organ/transporter remain unclear. In particular, the biggest challenge for peptide transport is to account for the role of transporters expressed in the brain because peptide transporters expressed in the brain endothelial cells surrounded by pericytes, astrocyte, and neurons play a crucial role in the efflux (clearance) of peptidic metabolites from the cerebrospinal fluid (Jiang et al. 2009). In contrast, although Banks (2015) reviewed the blood-brain barrier (BBB) peptide transport systems, the uptake of peptides directed from blood to brain is a mystery. In this review, thus, we discuss the possibility of peptide transport through the BBB. Contribution of the preventive potential of peptides on brain functions is also

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reviewed from the point of view on memory and cognitive impairment.

Transportability of peptides across the BBB

Several assays, such as in vitro primary cell membrane, ex vivo sliced brain tissues or ventricle plexus, and in vivo brain perfusion experiments, have been performed for the evaluation of BBB transportability or uptake of compounds in the direction of blood to brain (influx) (Table 1). In vitro cell membrane experiments using rat primary cerebral microvascular endothelial cells are convenient and commercially available in the market. The in vitro transport (influx) assays of targets comprise a transwell chamber system with cells grown into the insert membrane. Carnosine (β-Ala-His; Xiang et al. 2006), Pro-Pro-Leu (Hayes et al. 2016), and cyclo (Phe-Phe) (Tsuruoka et al. 2012) were reported to be transportable by the cell line experiments. Although the results on cell-crossing transport reveal the possibility of compounds capable of crossing the BBB in intact form, great attention should be paid regarding the evaluation of in vitro transportability from the aspect of BBB integrity. Low transendothelial electrical resistance (TEER) of cells cultured on transwell insert compared to in vivo BBB system (Hayes et al. 2016) suggests that the transportability obtained by cell line experiments may not be indicative beyond in vivo BBB barrier integrity or a meshwork of microvessel endothelial monolayer cells surrounded by pericytes and astrocytes, together with the tight-junction (TJ) composition (Hosoya et al. 2002) (Fig. 1). Therefore, in vivo transport experiments by target perfusion in mouse must provide substantial evidence to solve the mystery whether peptides can cross the BBB and accumulate into the cerebral parenchyma. In mouse treated by ligation of thoracic aorta, a perfusion solution containing targets is infused in the left of ventricle of the heart for a few minutes (Fig. 2). Thus, the observed influx profile of targets in the brain tissues by in situ transcardiac brain perfusion experiments demonstrates the intact transport of targets across the BBB under appropriate barrier integrity. Unfortunately, there is no striking evidence on intact influx of peptides beyond the BBB in literatures owing to severely controlled BBB and/or enzymatic degradation by proteases in blood and/or brain microvascular endothelial cells, except for some

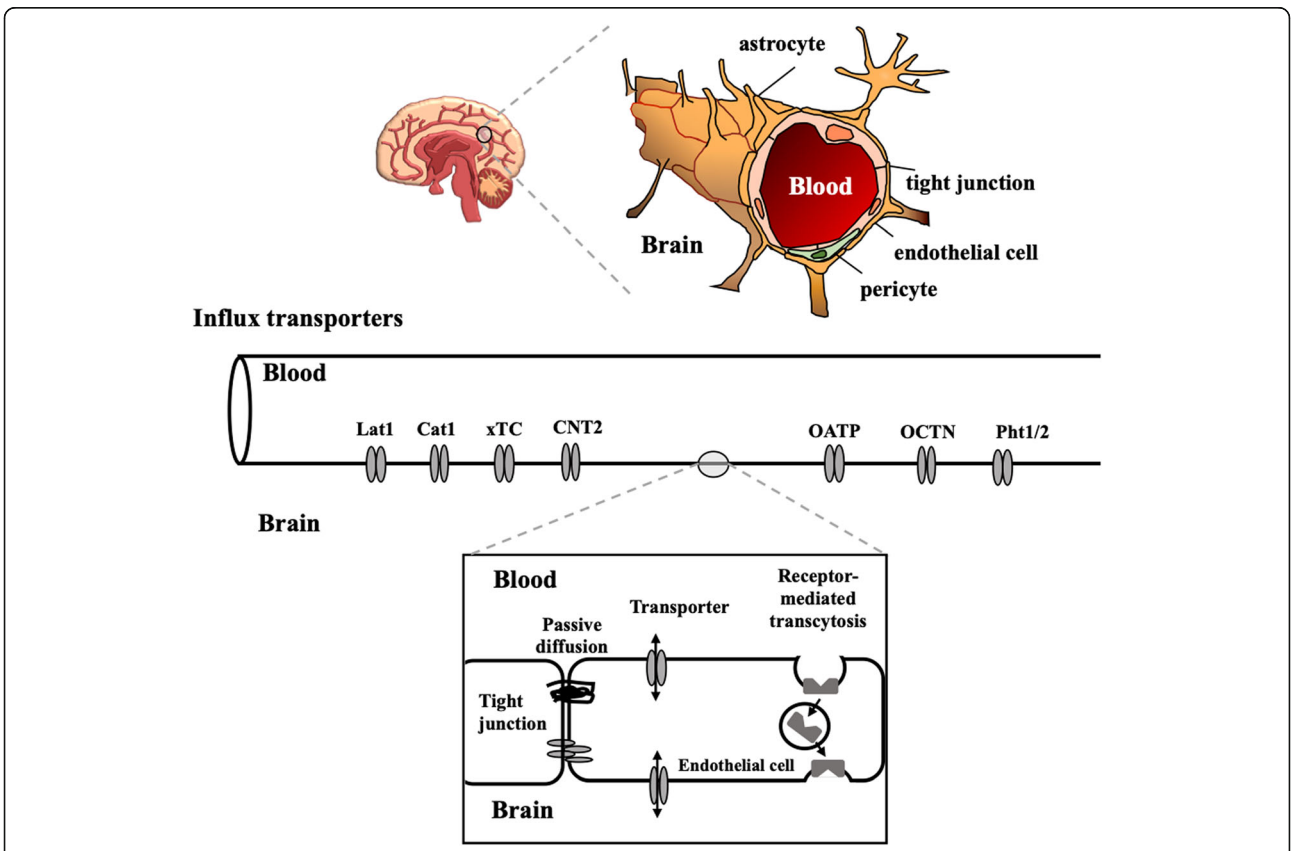

Fig. 1 Blood-brain barrier and transporters for influx routes. Lat1, large neutral amino acid transporter 1; Cat1, high affinity cationic amino acid transporter 1; xTC, cystine/glutamate transporter; CNT2, concentrative nucleoside transporter; OCTN, organic cation/carnitine transporter; OATP, organic anion transporting polypeptide; Pht1/2, peptide/histidine transporter 1/2

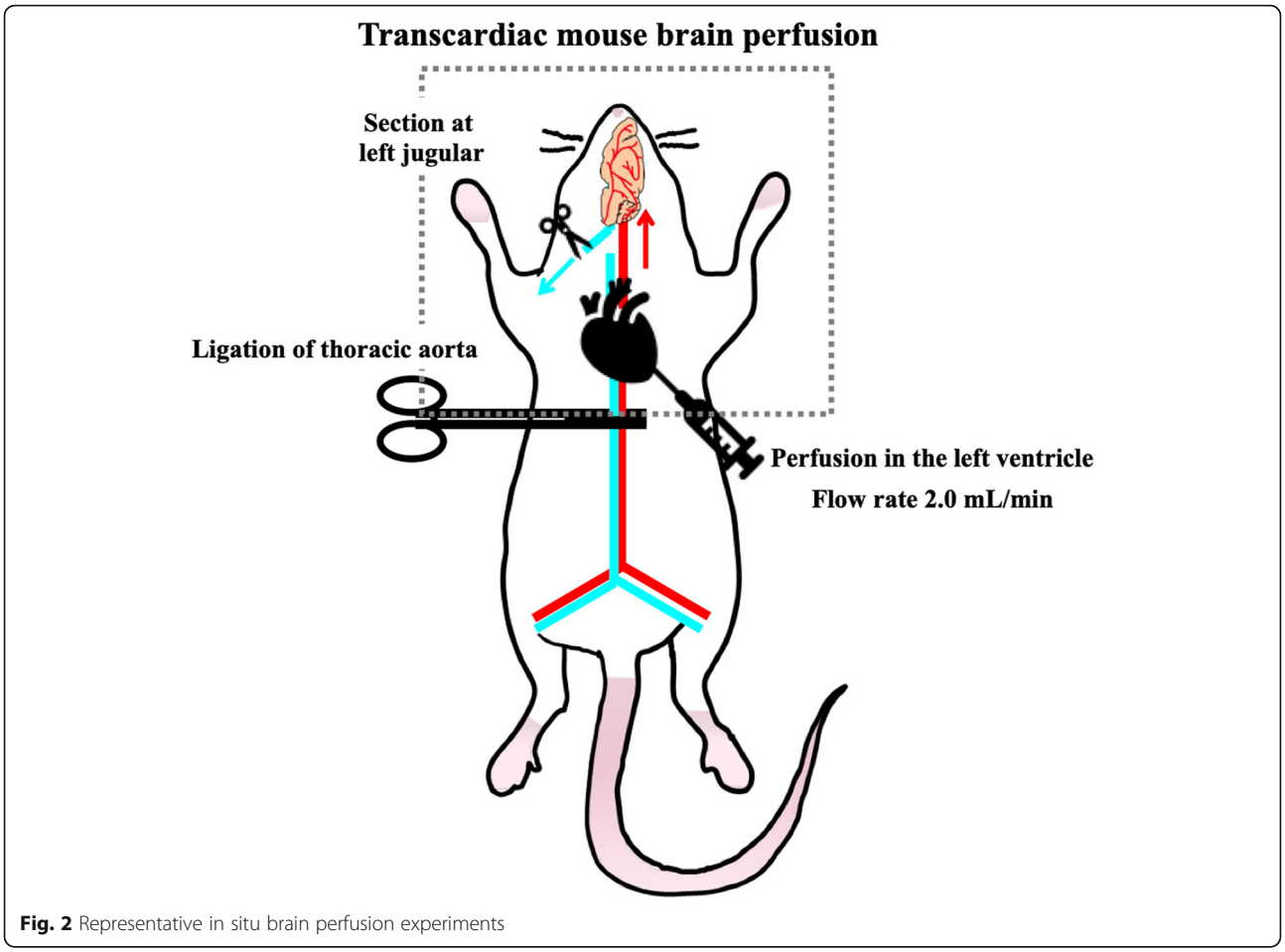

Fig. 2 Representative in situ brain perfusion experiments

BBB transportable dipeptides as introduced in the later section.

Blood-to-brain transporting peptides

To explain whether peptides are of benefit for brain functions, in vivo evidence on peptides capable of influx transporting beyond the BBB must be provided prior to comparative study on transportability by in vitro cell line transport experiments, considering above issues regarding protease degradation and highly integrated barrier of the BBB. Thus, in situ perfusion experiments provide in vivo evidence on possible transportability of peptides in intact form across the BBB. The criteria for the selection of BBB transporting peptides is (1) a dipeptide skeleton [because transporters related to influx transport direction recognize small nutrients such as glucose and amino acids (Ogawa et al. 2020)], (2) PHT-1 substrate or dipeptides recognized by PHT1, which is abundantly expressed in the nerve system (Yamashita et al. 1997), and (3) hydrophobicity that may assist the transcellular diffusive transport (Nau et al. 1994). According to the criteria, the uptake (brain/perfusate ratio, μL/g-brain) of dipeptides listed in Table 2 is evaluated in in situ 2.0-min mouse perfusion experiments (Tanaka et al. 2019). In general, for example, fluorescein isothiocyanate conjugated (FITC)-albumin is used as negative control (or non-BBB transportable compound) to compensate a BBB membrane integrity (brain/perfusate ratio, 6.5 μL/g-brain). Hu et al. (2014) revealed that Gly-*N*-methylated Gly (or Gly-Sar) that can be uptaken into the brain through the PHT1 in rodents (Hu et al. 2014) is preferably transported into mouse brain by 2.0-min perfusion (brain/perfusate ratio, 24.5 μL/g-brain); the significantly high ratio compared to negative control (FITC-albumin) clearly indicates that peptides with at least two amino acid residues are capable of transporting across the BBB in the blood-to-brain direction in vivo. Among the 22 dipeptides, except for Gly-Sar, only two dipeptides (Gly-Pro and Tyr-Pro) show the higher brain/perfusion ratio than that of FITC-albumin; most dipeptides have no ability to cross the BBB (Table 2). In an in vitro incorporation study of histidine in PHT1-transfected oocytes, the uptake was inhibited in the co-presence of some dipeptides [His-Leu, β-Ala-His (carnosine), Met-Met, Gly-Leu, and Gly-Gly], indicating that these dipeptides can be incorporated through the PHT1 (Yamashita et al.

Table 1 The transportability of peptidic compounds across the blood-brain barrier

Penetrant	Cell or animal	Influx or efflux	Time	Data	Doi
cyclo(Phe-Phe)	cell	influx	20, 40, 60 min	P_{app} = ~25 ×10^{-6} cm/s	doi:10.1371/journal.pone.0050824
Pro-HyP[a]	rat	influx	30 min	~0.5 nmol/mL-cerebrospinal fluid (ginger-degraded collagen hydrolysate 600 mg/kg *p.o.*)	doi:10.1096/fj.201902871R
Trp-Tyr	rat	influx	2 h	tissue/plasma ratio : 0.23 (hippocampus)	doi:10.18632/aging.101909
Leu-His	rat	influx	2 h	tissue/plasma ratio : 0.19 (hippocampus)	doi:10.3390/nu11092161
Met-Lys-Pro	rat	influx	15 min	autoradiographic image	doi:10.1371/journal.pone.0171515
Gly-Sar	mouse brain slice	influx	10 min	Uptake = ~110 μL/g (hippocampus)	doi:10.1111/jnc.12687
cyclo(His-Pro)	mousse (*i.v*)	influx	120 min	K_i = 0.179 μL/g-min	doi:10.1152/ajpendo.1993.264.5.E723
kyotorphin (Tyr-Arg)	mouse (*i.c.v.*)	efflux	2-15 min	$t_{1/2}$ = 4.9 min	doi:10.1111/j.1471-4159.2009.06090.x
histidine	mouse brain slice	influx	3 min	Uptake = ~0.09 μL/g (hippocampus)	doi:10.1016/j.bcp.2016.11.012
	mouse(1 nmol/g : *i.v*)	influx	0~30 min	concentration = ~0.5 nmol/g(hippocampus)	doi:10.1016/j.bcp.2016.11.012
oxytocin [CYINQCPLG (Disulfide bridge: 1-6)]	cell	influx	3 h	P_{app} = 3.03 ×10^{-6} cm/s	doi:10.1038/s42003-019-0325-6
neuropeptide Y	mouse	influx	2-30 min	K_i = 0.194 μL/g-min	doi:10.1152/ajpendo.1999.276.3.E479
endomorphin	rat	influx	15-60 min	HPLC Chromatogram	doi:10.1016/j.regpep.2005.12.004
alanine	mouse	influx	5 min	P_{app} = 9.6± 0.8 ×10^{-6} cm/s	doi:10.1016/j.tiv.2004.06.011
L-dopa	mouse	influx	5 min	P_{app} = 3.9± 0.79 ×10^{-6} cm/s	doi:10.1016/j.tiv.2004.06.011
leucine	mouse	influx	5 min	P_{app} = 14.6± 1.9 ×10^{-6} cm/s	doi:10.1016/j.tiv.2004.06.011
apolipoprotein J	mouse	influx	1.0 - 10 min	K_i = 3.75 μL/g-min	doi:10.1016/s0024-3205(96)00685-6
SAM 995 (Tyr-D-Thr-Gly-Phe-Leu-Ser)	rat	influx	5.0 - 20 min	K_i = 1.0 ± 0.2 μL/g-min	http://jpet.aspetjournals.org/content/299/3/967
SAM 1095 (*O*-linked Ser^6-β-D-glucose analog of SAM995)	rat	influx	5.0 - 20 min	K_i = 2.2 ± 0.3 μL/g-min	http://jpet.aspetjournals.org/content/299/3/967
DPDPE (Tyr-D-penicillamine-Gly-Phe-D-penicillamine)	rat	influx	30 min	K_i = 1.46 ± 0.31 μL/g-min	doi:10.1046/j.1471-4159.1996.66031289.x
valproic acid	rat	influx	0.25 - 0.5 min	K_i = ~ 480 μL/g-min	http://jpet.aspetjournals.org/content/276/3/1189
amantadin	mouse	influx	10 - 20 sec	K_i = 102 μL/g-min	doi:10.1002/bdd.2014
clonidine	mouse	influx	0.5 - 3.0 min	K_i = 366 μL/g-min	doi:10.1038/jcbfm.2009.54

[a]*HyP* hydroxyl Pro

1997). However, no uptake of His-Leu in mouse perfusion experiments (Table 2) led us to consider that BBB transportable peptides highly require protease resistance in blood and in brain microvascular endothelial cells in the BBB transporting process. Thus, no uptake (brain/perfusion ratio of less than that of FITC-albumin) of most dipeptides (Table 2) indicates inability to cross the BBB owing to their poor protease resistance in vivo, apart from substrate affinity with BBB transporters and hydrophobicity.

The K_i values of two BBB transportable dipeptides, Gly-Pro and Tyr-Pro, are 3.49 and 3.53 μL/g·min, respectively, showing the lower transportability (7.6 μL/g·min) of the reported BBB transportable model

Table 2 Brain/perfusion ratio of dipeptides in mouse perfusion experiments

Peptide	log *P*	Brain/Perfusate Ratio (μL/g-wet brain)	K_i (μL/g-min)
FITC-albumin		6.46 ± 0.15	- 0.52 ± 0.50
Trp-Leu	1.623	no uptake	-
Leu-Trp	1.019	no uptake	-
Trp-Met	1.004	no uptake	-
Trp-Tyr	0.212	no uptake	-
Tyr-Pro	0.202	10.5 ± 1.3	3.53 ± 0.74
Ile-Tyr	-0.249	no uptake	-
Leu-Tyr	-0.249	no uptake	-
Pro-Tyr	-0.398	no uptake	-
His-Leu	-0.477	no uptake	-
Trp-His	-0.555	no uptake	-
Gly-Pro	-0.602	10.85 ± 0.99	3.49 ± 0.66
Val-Tyr	-0.758	no uptake	-
His-Pro	-0.881	no uptake	-
Met-Tyr	-0.888	no uptake	-
Leu-His[a]	-1.016	no uptake	-
Tyr-His[a]	-1.164	no uptake	-
Tyr-Arg[a]	-1.269	no uptake	-
Ser-Pro	-1.634	no uptake	-
Gly-Sar	-1.651	24.54 ± 1.78	7.60 ± 1.29
Ser-Tyr[a]	-2.371	no uptake	-
Ala-Gln	-2.581	no uptake	-
Ala-Glu[a]	-2.581	no uptake	-
Ser-His[a]	-3.139	no uptake	-

log *P* values are cited from SciFinder (https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf)
no uptake : below the ratio of FITC-albumin of < 6.46 μL/g-wet brain (2 min) or 7.34 μL/g-wet brain (10 min)
[a]10 min-perfusion

dipeptide, Gly-Sar (Hu et al. 2014) (Table 2). The K_i values or transportabilities of Gly-Pro and Tyr-Pro are higher than those of other reported oligopeptides [e.g., 3 μL/g·min for neuropeptide Y (36 amino acids) (Kastin & Akerstrom 1999), 0.84 μL/g·min for growth hormone (191 amino acids) (Pan et al. 2005), and 0.57 μL/g·min for peptide hormone ghrelin (28 amino acids) (Banks 2002)] but much lower than those of chemical drugs (e.g., valproic acid) (Adkison & Shen 1996)(Table 1). The brain/perfusate ratio of 10.5 ± 1.3 μL/g-brain for Tyr-Pro (Table 2) compatible with the BBB-transportable L-DOPA (19.3 ± 5.9 μL/g-brain) (Ko et al. 2015) also suggests acceptable and preferable BBB transportability of Gly-Pro and Tyr-Pro. Although the structure-transportability relationship remains unascertained within the limited transport studies, it seems probably that (1) BBB transportable peptides in the blood-to-brain direction are restrictive to small peptides with high protease resistance (see Fig. 3), (2) peptide sequence, but not hydrophobicity, may determine the BBB transport (as Pro-Tyr, a reversed sequence of BBB transportable Tyr-Pro, shows no uptake; Table 2), and (3) the transportability of peptide is low enough to compare to drugs.

Transport route of BBB transportable peptides

The BBB regulates or limits compounds acceptable for the brain, including amino acids, glucose, organic/inorganic compounds, and vitamins, whereas large compounds fail the BBB transport (Ogawa et al. 2020). Thus, transporters capable of recognizing and transporting nutrients are individually expressed at luminal and abluminal sides of the BBB; typical influx (blood to brain direction) transporters are glucose transporter 1 (GLUT1) for glucose, large neutral amino acid transporter 1 (LAT1), and monocarboxylate transporter 1 (MCT1). For the uptake of peptides across the BBB, PHT1 is probably involved in the BBB transporting route because the incorporation of histidine, a substrate of PHT1, was inhibited by dipeptides in PHT1-expressed oocyte (Yamashita et al. 1997). Ex vivo perfusion experiments using brain tissue slices also demonstrate the BBB transport route of Gly-Sar through the PHT1 (Hu et al. 2014). However, in vivo mouse co-perfusion study of PHT1 substrates with the BBB transportable dipeptide, Tyr-Pro, provides another possible transporting route of peptides; the uptake of Tyr-Pro, but not Gly-Sar, is inhibited by histidine and L-DOPA (Tanaka et al. 2019). Considering the reported substrate specificity of PHT1 and LAT1 for histidine (Alexander et al. 2015), LAT1 for L-DOPA (del Amo et al. 2008), and PHT1 for Gly-Sar (Hu et al. 2014), LAT1 cannot be ruled out for the BBB transport route of dipeptides, together with PHT1. Additionally, a peptide transport system for octapeptide (Peptide T, derived from the human immunodeficiency virus (HIV) envelope glycoprotein) (Barrera et al. 1987) and a transcellular diffusive route by passive or endocytosis penetration pathways (Guidotti et al. 2017) for hydrophobic peptides such as cell penetrating peptides (Rhee and Davis 2006) and delta sleep-inducing peptide (Banks et al. 1986) might be involved in the blood-to-brain uptake of dipeptides (Fig. 1).

Location of peptides in the brain

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) imaging technique is an extensive and advanced visualization tool having wide applications in spatial localization of targets in terms of tissue distribution, such as immunostaining. This is because it possesses great advantage of non-targeting detection for a wide mass range of ionizable compounds

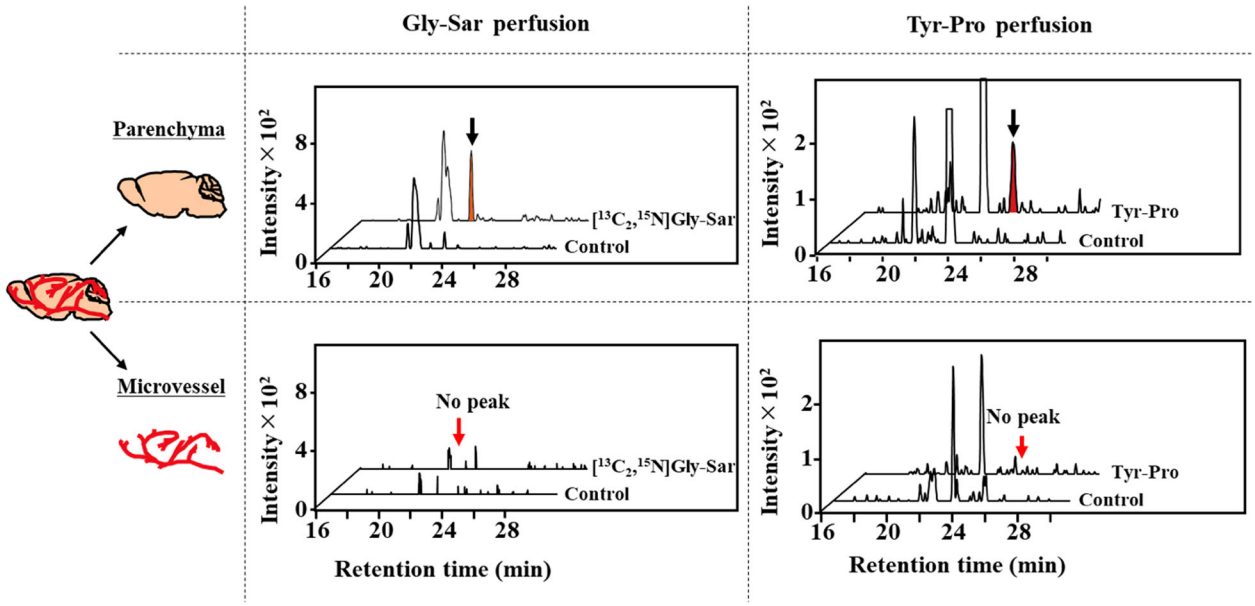

Fig. 3 Transport of dipeptides, Gly-Sar and Tyr-Pro, into the parenchyma of 2.0-min perfused mouse brain. [$^{13}C_2$,^{15}N]Gly-Sar and Tyr-Pro in the parenchyma and microvessel fractions of mouse brain were detected by LC-TOF/MS

such as proteins, lipids, and drug molecules with no use of antigens (Nguyen et al. 2019; Yoshimura & Zaima 2020). The outstanding observation for brain visualization study is the report by Shariatgorji et al. (2014), who simultaneously visualized neurotransmitters, including tyramine, dopamine, and acetylcholine, in mouse brain tissues using MALDI-MS imaging in combination with amine-specific derivatization. Liu et al. (2013) also demonstrate the advantage of MALDI-MS imaging for non-targeting visualization of EGFR inhibitory drug, erlotinib, beyond the BBB in mouse brain, wherein the drug and its metabolites were visually localized at the tumor region of mouse brain. The novelty of the phytic acid-aided MALDI-MS imaging technique (Hong et al. 2013) could be applied for the histological distribution of Tyr-Pro capable of crossing the BBB in intact form (Fig. 3). As depicted in Fig. 4, it seems probable that Tyr-Pro may preferably accumulate in the hippocampus, hypothalamus, striatum, cerebral cortex, and cerebellum of the mouse brain after < 10 min-perfusion. The accumulated regions of Tyr-Pro in the mouse brain are closely associated with memory consolidation and spatial memory (hippocampus) (Squire et al. 2015), food intake control (hypothalamus), and cognition (cerebrum and cerebellum) (Buckner 2013). The accumulation of BBB transporting dipeptides in brain parenchyma is unknown because there have been no comparable reports so far. However, the evidential observation by MALDI-MS imaging could indicate a potential physiological function of dipeptides in the brain.

Perspective

Nowadays, a cognitive dysfunction is a serious social issue in aged population, whereas appropriate therapeutic drugs or preventive food-style are less available. The evidential in vivo reports that a soybean hydrolysate richly containing di/tripeptides improved a delayed memory score in patients with mild cognitive impairment (Maebuchi et al., 2013) and suppressed a cognitive decline in SAMP8 mice (Katayama et al. 2014) strongly lead us to hypothesize that daily intake of some brain-health peptides has physiological potential to prevent the onset of dementia including Alzheimer's disease. Although in this review, peptides crossing the BBB in intact form are discussed, little is known about their brain-benefit roles. Previous reports of di/tripeptides showing in vivo prevention effect of dementia are as follows: Leu-His (reduction of inflammatory cytokines in microglia) (Ano et al. 2019a); Met-Lys-Pro (suppression of inflammatory cytokines and oxidative stress) (Min et al. 2017); Trp-Tyr and Trp-Met (suppression of microglial inflammation) (Ano et al. 2019b); Gly-Arg (increase of brain-derived neurotrophic factor and the number of neurons) (Katayama et al., 2014); Tyr-Trp, Ile-Tyr, and Ser-Tyr (upregulation of catecholamine metabolism) (Ichinose et al. 2015 & Ichinose et al., 2020; Moriyasu et al. 2016); Tyr-Leu, Phe-Leu, Trp-Leu (activation of anxiolytic-related receptors) (Mizushige et al. 2013); and Tyr-Pro (promotion of acetylcholine production) (Tanaka et al. 2020). However, to recognize di/tripeptides as

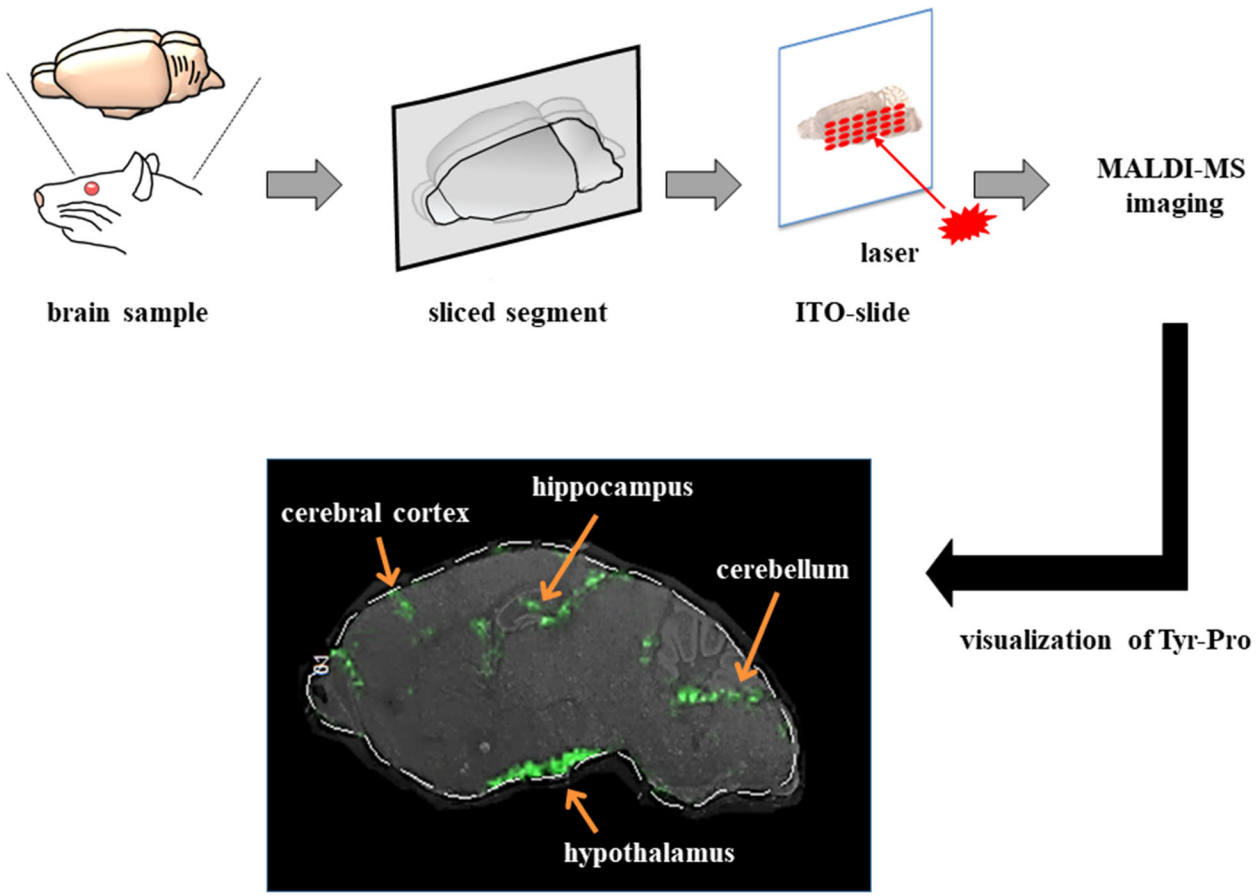

Fig. 4 Visualized distribution of Tyr-Pro in perfused mouse brain by MALDI-MS imaging. MALDI-MS/MS images of Tyr-Pro ($[M + H]^+$, 279.1 > 116.0 *m/z*) display the sagittal tissue and coronal sections of 10-min Tyr-Pro-perfused mouse brain. Scale bar, 2 mm; spatial resolution, 90 μm

brain-beneficial food compounds, further research is required in the following years to clarify the mechanism(s) of BBB transportation and accumulation in brain parenchyma.

Conclusion

It seems likely that some dipeptides may cross the severely controlled barrier, BBB, and may accumulate in the brain cerebral parenchyma in intact form. Animal and human studies also provide their brain-health benefit against cognitive decline. However, we still need studies to clarify the underlying mechanism(s) and to see if the benefit carries over to humans.

Abbreviations

BBB: Blood-brain barrier; RA: Renin-angiotensin; ACE: Angiotensin I-converting enzyme; PepT1: Peptide transporter 1; POT: Proton-coupled oligopeptide transporters; PHT1/2: Peptide-histidine transporters1/2; TEER: Transendothelial electrical resistance; TJ: Tight-junction; FITC-albumin: Fluorescein isothiocyanate conjugated-albumin; GLUT1: Glucose transporter 1; LAT1: Large neutral amino acid transporter 1; MCT1: Monocarboxylate transporter 1; MALDI-MS: Matrix-assisted laser desorption/ionization mass spectrometry

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T. M. designed and conceived the review and all the tables and figures. A. Y. and M.T. contributed to the literature search, the preparation of the tables and figures and the final revision of the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests.

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