A putative link of PUFA, GPR40 and adult-born hippocampal neurons for memory

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Abstract

Long chain polyunsaturated fatty acids (PUFA) such as docosahexaenoic and arachidonic acids, which are enriched in the brain, are important for multiple aspects of neuronal development and function including neurite outgrowth, signal transduction and membrane fluidity. Recent studies show that PUFA are capable of improving hippocampal long-term potentiation, learning ability of aged rats, and cognitive function of humans with memory deficits, although the underlying mechanisms are unknown. There have been several reports studying physiological roles of G-protein coupled receptor 40 (GPR40) in the pancreas, but no studies have focused on the function of GPR40 in the brain. As GPR40 was recently identified in neurons throughout the brain, it is probable that certain PUFA may act, as endogenous ligands, on GPR40 at their cell surface. However, the effects of PUFA upon neuronal functions are still not clearly understood. Here, although circumferential, a combination of *in-vitro* and *in-vivo* data is introduced to consider the effects of docosahexaenoic and arachidonic acids on brain functions. GPR40 was found in the newborn neurons of the normal and postischemic hippocampi of adult macaque monkeys, while the positive effects of PUFA upon Ca^{2+} mobilization and cognitive functions were demonstrated in both GPR40 gene-transfected PC12 cells and human subjects with memory deficits. The purpose of this review is to propose a putative link among PUFA, GPR40, and hippocampal newborn neurons by discussing whether PUFA can improve memory functions through GPR40 activation of adult-born neurons. At present, little is known about PUFA requirements that make possible neurogenesis in the adult hippocampus. However, the idea that ‘PUFA-GPR40 interaction might be crucial for adult neurogenesis and/or memory’ should be examined in detail using various experimental paradigms.

Keywords: Adult Neurogenesis; Fatty acid; GPR40; Hippocampus; Memory; Primate; RBANS

Abbreviations: CA1, cornu *Ammonis*-1; DG, dentate gyrus; FABP, fatty acid-binding proteins; GPR, G-protein coupled receptors; IP_3 receptor, inositol 1, 4, 5-triphosphate receptor; PSA-NCAM, polysialylated neural cell adhesion molecule; PUFA, polyunsaturated fatty acids; RBANS, repeatable battery for the assessment of the neuropsychological status; SGZ, subgranular zone

Article Outline

1. Introduction

Involvement of the hippocampus in learning and memory is well known from rodents to primates. Synaptic plasticity within the hippocampal formation and various neurotransmitters such as glutamate and γ-aminobutyric acid are required for the acquisition and retention of memory (Lamprecht and LeDoux, 2004). However, the exact mechanism of memory actually remains unknown, and recently the interests in adult neurogenesis have grown exponentially for considering it. Since the pioneer work of Nottebohm and colleagues about song learning of birds (Goldman and Nottebohm, 1983, Nottebohm, 1985), the adult-born neurons in the subgranular zone (SGZ) of the hippocampus have been assumed to participate in learning and memory, but evidence in favor and against does exist simultaneously (Leuner et al., 2006). Nevertheless, the link between the hippocampal newborn neurons and memory function is becoming reinforced by their increase with enriched environment (Kempermann et al., 1997) and physical exercise (van Praag et al., 1999a,b) as well as their necessity for spatial learning and memory (Madsen et al., 2003, Rola et al., 2004, Snyder et al., 2005). Nowadays, it is widely believed that the continued production of new neurons in the hippocampus is vital for the cognitive and behavioral performance of an individual. For example, growth and maturation of adult-born granule cells are considered closely related to the encoding of time in new memories of rodents (Fig. 1).

PUFA, making up 20% of the brain's dry weight, are critical for the normal brain development, maintenance of the membrane structure and the neuronal function. Omega-6 fatty acid such as arachidonic acid [20:4(n-6)] and omega-3 fatty acid such as docosahexaenoic acid [22:6(n-3)] are known to have an important role for the

hippocampal long-term potentiation and cognitive function of mammals (Fukaya et al., 2007). Arachidonic acid preserves membrane fluidity of hippocampal neurons (Fukaya et al., 2007) and shows anti-apoptotic effect on neural apoptosis (Kim et al., 2000). Docosahexaenoic acid enhances neurite outgrowth of hippocampal and cortical neurons and rat clonal pheochromocytoma (PC12) cells in culture (Calderon and Kim, 2004, Cao et al., 2005, Kawakita et al., 2006). Further, it is likely that PUFA are incorporated into the neuronal membranes to influence the quarternary structure of receptors and transporters (Bourre, 1991, Beltz, 2007). However, the exact mechanism of PUFA modulation of the neuronal function still remains obscure.

G-protein coupled receptors (GPR), a member of the large family with seven-transmembrane domains, are known to play physiological roles in response to peptide hormones, neurotransmitters or free fatty acids. GPR40 is a member of a subfamily of homologous GPR that includes GPR41 and GPR43. Along with GPR41-43, GPR40 was identified downstream of CD22 on human chromosomal locus 19q13.1 (Sawzdargo et al., 1997). Fatty acids are important not only as an energy source, but also as an endogenous ligand for GPR (Briscoe et al., 2003, Itoh et al., 2003, Kotarsky et al., 2003). GPR40 has been shown to be localized in β-cells of the pancreas to modulate insulin secretion in response to free fatty acids. Interestingly, GPR40 gene was identified also in the human brain by RT-PCR (Briscoe, 2003). The ubiquitous distribution of this receptor in the primate brain (Briscoe at al., 2003, Ma et al., 2007a) suggests that PUFA might act as extracellular signaling molecules at the membrane receptor to regulate neuronal function. Briscoe et al. (2003) have identified many kinds of PUFA as ligands for GPR40. To date, however, no receptor for PUFA has been

identified in neurons, but recently the author's group found that GPR40 protein is ubiquitously present in neurons throughout the central nervous system of primates (Ma et al., 2007a). Then, it is probable that long chain PUFA may directly act at the cell surface receptor GPR40 in neurons. Nonetheless, there is a paucity of theoretical and experimental insights into the potential role of PUFA and GPR40 for the brain functions.

For the past decade, adult neurogenesis has been a fascinating biological trait, which has captivated minds of many researchers since its debut 45 years ago (Altman, 1962). Most of the current researches focusing on adult neurogenesis are aimed at understanding how generation of new neurons contributes to learning and memory. However, we still don't have a detailed understanding of how neurogenesis affects the function of neural circuitry in distinct brain regions. The function of this evolutionarily conserved phenomenon still remains elusive especially in the mammalian hippocampus. For predicting the function of GPR40 in the neural progenitor cells of adult hippocampus, the author would unravel the mystery of how PUFA are correlated with the function of GPR40-transfected cells or with the cognitive function of human subjects using *in-vitro* and *in-vivo* approaches. Here a link among PUFA, GPR40, and hippocampal newborn neurons is proposed as a possible mechanism of memory functions.

This review aims at highlighting the new concept that PUFA such as docosahexaenoic and arachidonic acids might improve memory functions by interacting with hippocampal newborn neurons through GPR40. For this purpose the author presents experimental and clinical evidence that (1) expression of GPR40 protein is present in

the normal adult hippocampus and upregulated in the second week after ischemia on Western blots, (2) hippocampal newborn neurons of the postischemic monkeys remarkably increase after ischemia, showing intense GPR40 immunoreactivity compared with the control, (3) PC12 cells transfected with *GPR40* gene show Ca^{2+} mobilization in response to arachidonic acid, and (4) memory function of human subjects is significantly improved with PUFA supplementation. To the best of the author's knowledge, no previous publications are available concerning the role of GPR40 in the brain at present. Accordingly, original data of the author's group are introduced here, and the possibility that PUFA might act on GPR40 to regulate adult neurogenesis for memory functions is discussed with citing related papers.

2.1. GPR40 expression in the monkey hippocampus

In the hippocampal SGZ of the control and day 4, 7, 9 & 15 postischemic monkeys, the anti-GPR40 antibody (Ma et al., 2007a,b) recognized a single band of GPR40 at a molecular weight of approximately 31 kDa (Fig. 2). This was compatible with the band of the pancreas as a positive control. As the internal control protein β-actin revealed a constant expression regardless of the ischemic insult, the densitometric analysis of GPR40/β-actin ratio revealed an increase of the GPR40 protein in the second week after ischemia, being maximal on day 15 after ischemia (Fig. 2).

2.2. GPR40 localization in the monkey hippocampus

GPR40 immunoreactivity was found in the hippocampus of non-ischemic controls, including granule cells of the dentate gyrus and pyramidal cells of the cornu *Ammonis* (CA) 1-4 sectors (Ma et al., 2007a). Polysialylated neural cell adhesion molecule (PSA-NCAM)-positive(+) newborn neurons in the SGZ showed immunoreactivity for GPR40. On day 15 after ischemia, PSA-NCAM and GPR40 double-positive newborn neurons increased significantly, compared with the control (Fig. 3). High magnification showed GPR40 immunoreactivity within the perikarya of newborn neurons. The GPR40 immunoreactivity of newborn neurons was much more intense (Fig. 3 arrows), compared with mature granule cells. In addition, GPR40 was also positive for CD31+ endothelial cells of the proliferating capillaries and S100β+ young astrocytes in the SGZ (Ma et al., 2007b).

2.3. Ca^{2+} mobilization in *GPR40*-transfected rat PC12 cells

Notably, docosahexaenoic acid has a pEC50 (-log molar concentration producing half-maximal response) of 5.37 for GPR40 while arachidonic acid has a pEC50 of 4.92 (Briscoe, 2003). Here the dynamic change of intercellular Ca^{2+} concentration in response to arachidonic acid is introduced as a representative (Fig. 4), using rat pheochromocytoma PC12 cells with *GPR40* gene transfection and the resultant protein expression.

No significant change of intracellular Ca^{2+} was observed in naïve PC12 cells in response to 10 μM arachidonic acid (1.09±0.63, mean±SD), although KCl induced Ca^{2+} mobilization up to 1.3 fold. On the contrary, in PC12 cells transfected with GPR40 gene,

the intracellular Ca^{2+} was significantly increased (1.94±0.11), compared with the wild type. Even after Ca^{2+} was removed from the solution with ethylenediamine tetraacetic acid (EDTA), arachidonic acid-induced intracellular Ca^{2+} mobilization was observed (1.88±0.15), but this showed no significant differences, compared with the control. In contrast, the inositol 1, 4, 5-triphosphate (IP_3) receptor-specific antagonist, xestospongin C (Sigma, St. Louis, MO) blocked arachidonic acid-induced Ca^{2+} increase under the Ca^{2+}-free condition (1.07±0.05). These suggested that GPR40-mediated Ca^{2+} release from the intracellular stores are partially related to the arachidonic acid-induced Ca^{2+} mobilization.

2.4. Cognitive improvement of human subjects with PUFA

For the neuropsychological test, the repeatable battery for the assessment of the neuropsychological status (RBANS: Randolph et al., 1998) was used. A total of 50 subjects (26 females, 24 males, mean±SD: 62.8 ± 12.0 year old) with a complaint of amnesia and RBANS total estimation score less than 40 (average score of each decade being 50) underwent PUFA supplementation for three months (Fig. 5). Each subject was administered 240 mg/day of docosahexaenoic and arachidonic acids by commercially available ARAVITA capsules (SUNTORY Ltd., Osaka, Japan). The Japanese version of RBANS was done before and after the supplementation (Kotani et al., 2006). After the PUFA supplementation, the immediate memory score showed a remarkable improvement (Fig. 5) from 32.7 ± 1.60 to 38.6 ± 1.70. The delayed memory score also showed a remarkable improvement (Fig. 5) from 27.9 ± 2.00 to 36.7 ± 2.01. Then, the

total score of RBANS showed a dramatic improvement from 26.65 ± 1.41 to 33.0 ± 1.66. In contrast, after the supplementation of placebo (olive oil of 240 mg/day), there was no significant improvement of RBANS scores in the age- and RBANS score-matched control group (Kotani et al., 2006).

3. Adult-born hippocampal neurons and memory

Neurogenesis is a critical process in the development of embryonic brain, and is also implicated in the maintenance of adult brain. Because of the resemblance of regulatory mechanisms between embryonic and adult neurogenesis, it is in one sense true that adult neurogenesis is merely a remnant of embryonic neurogenesis rather than an adaptation to adult life. There are, however, essential differences between embryonic and adult neurogenesis, and consequently regulatory mechanisms may have been partly co-opted for a specifically adapted adult function (Lindsey and Tropepe, 2006). The characterization of adult neural stem cells in mammals has been the focus of intense research with two goals of elucidating the memory mechanism and of developing new stem cell-based regenerative treatments for stroke, spinal cord injury, neurodegenerative diseases, etc. Nonetheless, there is a paucity of not only experimental but also theoretical insights into the potential role of neuronal replacement as a mechanism of neural circuit plasticity.

An important concern that has surfaced in the field of adult neurogenesis is the question of how representative are the rodent data when contrasted with the primate data. Rodents generally displayed a remarkable difference in their rate of adult neurogenesis,

compared with macaque monkeys. After transient global brain ischemia, Tonchev et al. (2003, 2007) could identify only 1~3% of neuronal differentiation among the total proliferating cells in the hippocampal SGZ of monkeys. This showed a remarkable contrast to over 60% of neuronal differentiation in rats (Kee et al., 2001). Furthermore, Ngwenya et al. (2006) reported that maturational progression of newborn granule neurons in the monkey takes five times longer to complete, compared to a similar progression in rats. Therefore, one should approach the results of rodents with caution and not take for granted that the same findings will be paralleled in the primates. In this respect, the primate experimental paradigms such as monkey brain ischemia and human PUFA supplementation, as introduced here, may contribute to clarifying the role of newborn neurons in the adult hippocampus.

Environmental influences, daily behavior, and social interactions constitute a number of combined forces affecting on brain plasticity, and are intimately related to the addition of new neurons throughout adulthood (Lindsey and Tropepe, 2006). Seki and Arai (1995) first demonstrated a significant age-related decline in the newborn and developing granule cells in the dentate gyrus of young to old rats. Kempermann et al. (1998) showed in mice that an enriched environment can in part rescue an age-related decline in the production of adult-born neurons. Senescence is generally thought to contribute to the overall decline of memory, and in the context of adult neurogenesis appears to exert an age-related decline in neurogenic capacity (Lindsey and Tropepe, 2006). Although it is possible that hippocampal adult-born neurons may contribute to other brain functions except for memory, such as emotions and stress regulation (Leuner et al., 2006), the most likely and what remains to be established are a definitive link

between adult hippocampal neurogenesis and memory function. This link was strengthened in a mouse Alzheimer model by Dong et al. (2004) who demonstrated that induced amyloid depositions provoke impairment of adult hippocampal neurogenesis being associated with memory deficits.

Microstructural changes of synaptic morphology such as number and size of dendritic spines and post-synaptic densities occur in response to sensory stimuli, and are central for learning and memory formation in the adult brain (Feldman and Brecht, 2005). Thus, the formation, modification and elimination of synapses, being the direct result of removal, generation and replacement of entire neurons, can play a fundamental role in learning and memory. Hippocampal neurogenesis can be altered by various hormones (Gould et al., 1998, Shingo et al., 2003), exercise (Kempermann et al., 1997b, van Praag et al., 1999a,b) and enriched environments in both vertebrates and invertebrates (van Praag et al., 1999b, Scotto-Lomassese et al., 2000). Then, adult neurogenesis may, in fact, have a predominant role rather for modifying circuitry related to the processing of sensory information (Lindsey and Tropepe, 2006). Kempermann (2002) has claimed that the function of newborn neurons in the mammalian hippocampus should be to modify their circuitry in order to enhance capacity for processing information that will eventually be stored as permanent memories. Regardless of the functional significance of adult hippocampal newborn neurons, neural plasticity including cell loss, addition or replacement, is currently receiving more attention given the interest in the field of adult neurogenesis.

In the normal adults, new granule cells in the dentate gyrus are born locally in the

underlying SGZ and migrate a very short distance to integrate into the dentate gyrus (Fig. 1) (Gage, 2000). Dendrites of them receive inputs from the entorhinal cortex via perforant path while their axons send outputs to the CA3 sector via mossy fibers. The addition of new neurons within the SGZ is modified by many factors as mentioned above. Such dynamic regulation of neurogenesis may be important for mediating behavioral tasks that are based on learning or memory (Doetsch and Hen, 2005). Further, the SGZ of monkey hippocampus upregulated neural progenitor cells to generate new neurons especially in the second week after 20 min global ischemia. Pyramidal neurons of the CA1 sector of the monkey hippocampus degenerate after this insult, and lead to the memory impairment of postischemic monkeys (Yukie et al., 2006). Ischemia enhanced neurogenesis in the SGZ, but no neurogenesis occurred in the CA1 sector after ischemia without external therapeutical interference (Yamashima et al., 2000, 2007a,b). One implication of this finding is that homeostatic neurogenesis for the cellular turnover has negligible functional role in the CA1 physiology. In one sense, cellular homeostasis in the postischemic hippocampus can be defined as the insertion of newly recruited neurons in a local circuit of the SGZ in order to compensate for the loss of CA1 neurons.

Though the cellular populations in the postischemic SGZ are spatially isolated, common themes begin to define this neurogenic niche by focusing PUFA and GPR40 (Fig 6): (1) neural progenitor cells and niche cells derive from vascular adventitia, (2) newborn neurons and astrocytes, both expressing GPR40, are essential components of the niche, (3) PSA-NCAM+ young neurons express GPR40 while newborn astrocytes express S100 β , and (4) clusters of S100 β + astrocytes are in intimate contact with

PSA-NCAM+ young neurons (Yamashima et al., 2004). Presumably, astroglia-derived soluble and membrane-bound factors promote proliferation and neuronal fate for hippocampal progenitor cells (Song et al., 2002). Although the postischemic second week SGZ revealed a cluster of S-100 β + young astrocytes within the neurogenic niche (Yamashima et al., 2006), it is not clear to what extent these astrocytes contribute to neural circuit function or maintenance, or whether they play a purely supportive role in ensheathing the progenitor cells and maintaining the blood-brain barrier. Recent evidence has shown that for the maturation of neuronal phenotypes, specific fibroblast growth factors being secreted by astrocytes within their surrounding niche or target migratory site may be required, and that such signaling could be involved in maintaining neurogenesis at later stages of adulthood (Chadashvili and Peterson, 2006). As capillary endothelial cells, astrocytes and adult-born neurons all expressed GPR40 (Ma et al, 2007b), it is probable as well that adult-born neurons respond to PUFA that was incorporated into astrocytes from the blood stream and released into the neuropil of the SGZ.

4. PUFA and brain

PUFA have an important physiological role in the brain. For example, previous studies demonstrated that docosahexaenoic acid is associated with memory and vision (Carlson and Werkman, 1996, Birch et al., 2000), and is useful for the prevention of ischemic brain damages (Tsukada et al., 2000). Further, spatial memory and hippocampal long-term potentiation can be improved with dietary supplementation of arachidonic acid in aged rats (McGahon et al., 1999, Kotani et al., 2003, Okaichi et al., 2005).

Dietary supplementation of such fatty acids presumably affects the intracellular and intercellular signaling, and also the membrane fluidity of neurons (Horrocks and Farooqui, 2004). However, the molecular mechanism of PUFA effects upon brain functions still remains unknown.

Docosahexaenoic and arachidonic acids make up about 30% and 20% of fatty acids in phospholipids of the brain, respectively (Contreras et al., 2000, Lapillonne et al., 2002). In the rat brain, the rate of turnover due to loss followed by replacement is equal to 2~8% per day for docosahexaenoic acid while 3~5% per day for arachidonic acid, with corresponding half-lives (0.693/turnover) being 7~34 and 12~23 days, respectively (Rapoport , 2003). The proportion of docosahexaenoic acid to arachidonic acid in neural membrane phospholipids varies among the subclasses of phospholipids. Docosahexaenoic acid is abundant in synaptic membranes, while arachidonic acid is distributed rather evenly in the gray and white matter and among the different cell types in the brain. As these two PUFA cannot be synthesized de novo from 2-carbon fragments by the mammalian tissue, they must be obtained from dietary sources: α -linolenic acid and linoleic acid in the diet can serve as precursors of docosahexaenoic acid and arachidonic acid, respectively. It is widely accepted that normal brain function and structure depend on a correct balance between omega–3 and omega–6 PUFA, so cognitive and behavioral changes may result if this balance is disturbed (Lands, 1989). Brain events such as apoptosis, gene transcription, neurite outgrowth, membrane excitability, prostaglandin formation, desaturation-elongation, membrane fluidity and elasticity are all thought to depend on the presence of adequate concentrations of docosahexaenoic and arachidonic acids as well as on balanced interactions between

omega–3 and omega–6 PUFA (Contreras and Rapoport, 2002, Rapoport, 2003).

Although abundant in the brain, docosahexaenoic acid cannot be synthesized by neurons and has to be supplied by the cerebrovascular endothelium and astrocytes (Moore, 2001). Then, one of the supporting roles of astrocytes to neurons is to supply docosahexaenoic acid for its enrichment (Kim et al., 2000). Final steps of its biosynthesis in the brain occur in astrocytes (Moore et al., 1991, Garcia and Kim, 1997). They release docosahexaenoic acid that may reach a local concentration sufficient to act as an extracellular signaling molecule (Moore, 1993, Kim et al., 1999), or may support neuronal survival by enhancing membrane phosphatidylserine (Kim et al., 2000). Docosahexaenoic acid thus provided appears to accumulate in neuronal membranes, since this fatty acid has been shown to be resistant to the phospholipase A2 action in neurons (Kim, et al., 1999a,b).

Arachidonic acid is also abundant and necessary in the brain and has various physiological functions. Arachidonic acid plays an important role for the infant brain development (Crawford et al., 2003, Bazan, 2005). It is one of the major components of cell membranes and is of special importance also to the adult brain in both physiological and pathological states. Release of arachidonic acid from membrane phospholipids can serve as an intercellular messenger to activate protein kinase C and modulate ion channels, transporters and receptors as well as synaptogenesis (Kawasaki et al., 2002, Hama et al., 2004). There is a considerable amount of evidence describing the beneficial effects of PUFA on the prevention of ischemic stroke and modulation of epileptic seizure susceptibility. However, the effects of arachidonic acid on the viability of

neurons are still somewhat controversial. Recently, Wang et al. (2006) reported that arachidonic acid can effectively protect rat hippocampal neurons against oxidative stress induced by glutamate or H_2O_2 by enhancing antioxidative enzyme activities.

In the brain, arachidonic acid is esterified mainly into the sn-2 position of phosphatidylinositol or phosphatidylcholine (Rapoport, 2003). After arachidonic acid is released from phospholipids by phospholipase A_2, it enters an unesterified brain pool being largely located at the synapse (Rapoport, 2003). This endogenous arachidonic acid is the precursor pool for conversion to eicosanoids including prostaglandins, leukotrienes, thromboxanes, or hydroxyeicosatetraenoic acids, and does not directly exchange with arachidonic acid in plasma. In contrast, the exogenous unesterified arachidonic acid from plasma is not converted to eicosanoids, and diffuses, by binding to fatty acid-binding proteins (FABP), to the pool at the endoplasmic reticulum (Rapoport, 2003), and from there, it can exchange with arachidonic acid in plasma.

FABP belong to the conserved multigene family of the intracellular lipid-binding proteins having molecular masses around 15 kDa, and are ubiquitously expressed in various vertebrate tissues with peculiar expression patterns. Various functions have been proposed for FABP, including promotion of cellular uptake and transport of PUFA, targeting of them to specific metabolic pathways, and regulation of gene expression and cell growth. It is generally accepted that many FABP participate in cell growth rather than differentiation. Brain-type FABP, also called FABP7, is present in the brain and retina, and is characterized by its strong affinity for n-3 PUFA, in particular docosahexaenoic acid (Haunerland and Spener, 2004). FABP7 is important for the

embryonal neurogenesis, and is strongly expressed in ‘radial glia cells’ of the developing brain, which also behave like stem cells in the nervous system (Feng et al., 1994, Kurtz et al., 1994, Gotz et al., 1998, Anthony et al., 2004). Arai et al. (2005) reported that *Fabp7* is a downstream gene of transcription factor Pax6 (Kukekov et al., 1999) and essential for proliferation of neuroepithelial cells in the developing rat cortex. In our experimental paradigm, no signs of neuronal production were observed in the postischemic hippocampus proper and in particular in the CA1 sector, where the newborn cells were consistently of glial phenotype. As proliferating progenitors in the SGZ but not in the subventricular zone adjacent to CA1 expressed the pro-neural transcription factor Pax6 (Tonchev et al., 2006), Pax6 conceivably controls the fate of progenitor cells of the adult primate hippocampus. Intriguingly, the author’s group has recently identified co-expression of Pax6 and FABP7 in the newborn neurons of the postischemic SGZ. Furthermore, epidermal-type FABP, also called FABP5 was found to be co-expressed with GPR40 in the CA1-4 pyramidal neurons, granule cells and SGZ newborn neurons (unpublished data). Accordingly, it is possible that FABP5 may play a crucial role in the hippocampus in association with GPR40. The exact role of FABP in the adult neurogenesis would be clarified in the near future.

5. Diet and PUFA

Conversion from α-linolenic acid [18:3(n-3)] to docosahexaenoic acid is low in humans with less than 1% of dietary α-linolenic acid converting to docosahexaenoic acid (Burdge et al., 2003). Dietary docosahexaenoic acid, however, is well absorbed and

incorporated into plasma and blood cell lipids, and is readily incorporated into lipids of the developing brain (Innis, 2007). Docosahexaenoic acid is absent from all vegetable fats and oils including nuts, grains and seeds, and poultry and eggs also provide a lower amount while the richest dietary sources are fish and sea foods (Innis, 2003). Neurochemically, enrichment of docosahexaenoic acid in the diet competitively inhibits oxygenation of arachidonic acid by cyclooxygenase thus suppressing the production of pro-inflammatory eicosanoids and pro-inflammatory cytokines (Calder, 2005). Although the ancient diets of human beings had a ratio of arachidonic acid to docosahexaenoic acid of 1 : 1, the present western diets have a ratio of about 15:1. Changes in eating habits and agriculture development within the past two centuries, especially after the World War Ⅱ, caused remarkable changes in this ratio. Both the decreased consumption of docosahexaenoic acid-enriched foods such as fish oil and increased consumption of omega-6 enriched vegetable oil are responsible for the 15 : 1 arachidonic acid to docosahexaenoic acid ratio (Weylandt and Kang, 2005). The consumption of docosahexaenoic acid has numerous beneficial effects on the health of the human brain (Horrocks and Yeo, 1999, Horrocks and Farooqui, 2004). In contrast, not only the decrease of docosahexaenoic acid intake but also the increase of arachidonic acid intake might have adverse effects on the physicochemical properties of neural membranes, because arachidonic acid generates high levels of prostaglandins, leukotrienes, and thromboxanes resulting in neuroinflammation.

Kan et al. (2007) recently demonstrated that docosahexaenoic and arachidonic acids are fundamental supplements for the induction of neuronal differentiation from bone marrow-derived mesenchymal stem cells. The molecular mechanisms underlying the

contribution of docosahexaenoic and arachidonic acids to neurite growth of mesenchymal stem cells are not completely understood. Nevertheless, two major possibilities have been raised, including (1) stimulation of the phospholipid synthesis required for the neurite elongation and membrane expansion, and (2) modulation of the signal transduction pathways involved in the neurite outgrowth (Kan et al., 2007). In adults, decreased intake of omega–3 compared with omega–6 PUFA has been implicated as contributing to the clinical signs of Alzheimer's disease and age-related cognitive disturbances (Simopoulos et al., 1999, Conquer et al., 2000**).** It is probable that such adverse effects of dietary PUFA unbalance might become prominent especially within the newborn neurons of the hippocampus. It is one of world-wide worries of current days that such nutritional unbalance might cause disorders of memory, cognition and/or emotion (e.g. depression) in the aged as well as in the young generations. Diets enriched in omega-3 PUFA increase membrane fluidity, affect signal transduction, and modulate gene expression for brain function (Horrocks and Farooqui, 2004). In addition, the author would like to propose a third possibility that (3) certain PUFA are capable of sending signals to newborn neurons through GPR40.

6. GPR40 and memory

In the last decade, an increasing number of unliganded orphan receptors with unknown function have been identified. GPR respond to a large variety of molecules from inorganic ions to peptides. GPR40 was cloned along with GPR41-43 downstream of CD22 on human chromosomal locus 19q13.1 (Sawzdargo et al., 1997). GPR40-43

belong to a subset of orphan receptors, with GPR40 being 30% identical to GPR41 and GPR43. As GPR responding to fatty acid derivatives such as prostaglandins and leukotrienes have been identified (Coleman et al., 1994, Sarau et al., 1999), it is reasonable to consider that the fatty acid itself may act at a cell-surface receptor. Using a ligand fishing strategy, Briscoe et al. (2003) first demonstrated that medium and long chain saturated and unsaturated fatty acids can activate GPR40 in a dose-dependent manner. The expression pattern clearly differentiates GPR40 from GPR41 and GPR43 (Brown et al., 2003), suggesting that the function of GPR40 has clearly diverged from that of GPR41 and GPR43. It is probable that PUFA may act, as extracellular signaling molecules, at a membrane GPR40 receptor to regulate functions not only of the pancreas but also of the brain. Signaling at the pancreatic islet should be related to the insulin secretion at β-cells, but the role of GPR40 in the brain still remains unknown. As shown in Fig. 4, PC12 cells transfected with GPR40 gene showed arachidonic acid-induced intracellular Ca^{2+} mobilization. Recent *in-vitro* studies have shown that PUFA stimulate cell proliferation through GPR40 in a cultured human breast cancer cell line (Hardy et al., 2000, 2005). As Ma et al. (2007b) recently found expression of GPR40 in the neurogenesis niche of the monkey hippocampus, it is suggested that GPR40 signalling is related to the progenitor cell proliferation. Further, because adult-born neurons in the hippocampal SGZ showed expression of GPR40, it is reasonable to speculate that docosahexaenoic and arachidonic acids not only influenced integration and synaptic formation of newborn neurons by supplying constitutive phospholipids but also directly stimulated their function through Ca^{2+} mobilization by interacting with GPR40.

7. Conclusion

Like in birds and rodents, a close relationship appears to exist between vasculogenesis and neurogenesis in the primate brain as well. In the SGZ, adventitial cells around the proliferating capillaries are spatially and temporally related to hippocampal neurogenesis (Yamashima et al., 2004, 2006). It is likely that GPR40 in endothelial cells and newborn astrocytes of SGZ (Ma et al., 2007b) may be helpful to increase the local concentration of PUFA (Moore, 1993), in order to be sufficient to act as an extracellular signaling molecule. Meanwhile, GPR40 in the neural progenitors, newborn neurons and young granular cells augments PUFA-induced intercellular signaling. An increased PUFA signaling via GPR40 in SGZ is likely to modulate network formation of the adult hippocampus that is indispensable for learning and memory (Fig. 6).

Fat, an important component of diets, provides energy as well as adequate amounts of essential fatty acids to the mammalian body. As the amount of fat in diets is well known to balance growth and development of the infant brain, the author speculates that it might also affect the adult hippocampal neurogenesis through GPR40. Currently, the role of adult hippocampal neurogenesis in learning and memory is highly probable, although not firmly established (Shors et al., 2001, Kempermann, 2002, Prickaerts, 2004). As all data directly linking memory and adult neurogenesis are derived from non-primate experimental models, at present it is not fixed whether the effects of fatty acids on memory in humans could be also mediated via modulation of hippocampal neurogenesis. In fact, strictly speaking, the exact role of adult neurogenesis still remains unclarified in the primate brain. Nevertheless, in concluding this review the author

would like to hypothesize that *'PUFA can modify memory performance via an interaction with GPR40 in the hippocampal newborn neurons'* (Fig. 7*)*. The putative link of PUFA, GPR40 and adult neurogenesis should be studied further using various experimental paradigms to clarify the mechanism of memory.

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Legends

Fig. 1. Growth and maturation of newborn granule cells encoding time as new memories in the adult rodents. Newborn granule cells in the SGZ have few projections

at 3 days, but by 2 months develop maturity with both extension of axons to CA3 and arborizations of many oriented dendrites to perforant path axons. Violet lines indicate perforant path with entorhinal input starting by 2.5 weeks, while green lines indicate mossy fibers from the dentate gyrus (DG) to CA3. γ -aminobutyric acid (GABA, red) is excitatory in immature neurons but becomes inhibitory around the time the excitatory glutamatergic synapses (between violet and green) are established. Involvement of PUFA and GPR40 in such synaptic formations appears to be most likely.

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Fig. 2. Western blotting of GPR40 using a nonischemic control (C), postischemic day 4, 7, 9 and 15 DG tissues and positive control of pancreas. As the internal control protein β -actin shows a constant expression, densitometoric analysis of GPR40/β -actin ratio of band intensities shows upregulation of GPR40 expression in the second week after ischemia, being maximal on day 15. Pan; Pancreas as a positive control of GPR40

Fig. 3. Immunoreactivity of PSA-NCAM (red) and GPR40 (green) in the nonischemic control (C) and postischemic day 15 (d15) hippocampus. PSA-NCAM+ newborn neurons are seen in the control SGZ, and remarkably increase on day 15 after ischemia. They co-express intense GPR40 immunoreactivity, and can be appreciated as merged color of yellow (arrows). SGZ; subgranular zone, GCL; granule cell layer, Scale bar= 50 μm.

Fig. 4. Arachidonic acid (ARA)-induced Ca^{2+} mobilization depends on GPR40.

Rat PC12 cells were transfected with the GPR40 gene, and subsequently ARA was

applied to the medium. This resulted in an increment of the intracellular Ca^{2+} concentration in PC12 cells, and this effect was inhibited by an IP_3 blocker (Xestospongin C). ARA application after mock-infection of GPR40 did not elicit an increase of the intracellular Ca^{2+} concentration.

Fig. 5. Dietary supplementation with arachidonic acid (ARA) and docosahexaenoic acid (DHA) elicits memory improvements. Schematic summary of the data regarding subjects included in the study, dosage and duration of the PUFA supplementation, and improvement of RBANS scores. Both the immediate and delayed memory scores and the total score showed a significant ($p<0.001$) improvement after the PUFA supplementation. Open bars; before supplementation, Black bars; after supplementation, ***; $p<0.001$

Fig. 6. Schematic drawing of the role of PUFA ligand / GPR40 receptor (Y) in each cell and phase of adult neurogenesis niche of primates. Round cells indicate progenitor cells while star-like cells indicate newborn astrocytes. Astrocytes supply PUFA signaling to neural progenitor cells, newborn neurons and mature neurons in the vascular niche. EC; endothelial cells, PUFA; polyunsaturated fatty acids, VEGF; vascular endothelial growth factor, BDNF; brain-derived neurotrophic factor

Fig. 7. A hypothesizing scheme of memory formation via PUFA and GPR40 binding in the hippocampal newborn neurons.