



FATTY ACIDS AS REGULATORS OF HIPPOCAMPAL NEUROGENESIS: THE CASE OF GPR40

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*The hippocampus of adult mammals including humans continues to generate neurons throughout life. The neural stem/progenitor cells, the cellular phenotype responsible for this regenerative capacity, are a subject of intensive research focused on the mechanisms of their regulation. Intriguing recent data implicate an unexpected molecular player in this regulation: free fatty acids. Polyunsaturated fatty acids (PUFA) such as docosahexaenoic and arachidonic acids were identified to act on the pancreas via a novel receptor, named G-protein coupled receptor 40 (GPR40). However, the pancreas appears not to be the single target organ of PUFA/GPR40 actions. Very recent findings have discovered GPR40 expression in the hippocampal dentate gyrus, including its progenitor cell niche responsible for neuronal renewal throughout life. These data open a possibility that PUFA may regulate adult hippocampal neurogenesis and thus become a therapeutic tool to treat neurological disorders in humans. **Biomed Rev 2007; 18: 69-73.***

INTRODUCTION

The adult brain preserves albeit limited regenerative capacity to regenerate its neurons via multipotent cells named neural stem/progenitor cells (1). The process of neuronal generation by these cells in the adult brain is designated adult neurogenesis, and at present it is widely recognized to occur in two regions of the mammalian central nervous system (CNS), one of these being the hippocampal dentate gyrus (2). The dentate gyrus harbors progenitor cells located in its subgranular zone (SGZ), a thin band of tissue adjacent to the innermost layer of granule neurons (2). Numerous molecular signals have been identified to modulate SGZ neurogenesis

(3). Here we shall provide evidence suggesting the existence of a new player in the molecular framework regulating progenitor cells in the adult hippocampus: the polyunsaturated fatty acids (PUFA). Fatty acids such as PUFA are able to bind and activate a cell membrane receptor known as G-protein coupled receptor 40 (GPR40). This signaling system is classically known for its effects on insulin secretion from the β -cells of the pancreas (4,5). However, there might be more than that. In addition to the pancreas, the receptor GPR40 is also expressed by various cell types within the CNS of adult monkeys (6), and PUFA supplementation improves memory in humans (7). Given the emerging link between

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memory and hippocampal neurogenesis (8,9), it is tempting to investigate the possible involvement of PUFA/GPR40 signaling in this process. Below we shall summarize and discuss this putative link.

INVOLVEMENT OF PUFA IN NEURAL FUNCTIONS

PUFA make up to 20% of the brain's dry weight and are critical for the normal brain development, maintenance of the membrane structure and the neuronal function (10,11). Omega-6 fatty acid such as arachidonic acid [20:4(n-6)] and omega-3 fatty acids 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 (12). Arachidonic acid preserves membrane fluidity of hippocampal neurons (12) and shows antiapoptotic effect on neural apoptosis (13). Although abundant in the brain, docosahexaenoic acid cannot be synthesized by neurons and has to be supplied by the cerebrovascular endothelium and astrocytes (14). Docosahexaenoic acid enhances neurite outgrowth of hippocampal and cortical neurons and rat clonal pheochromocytoma (PC12) cells in culture (15-17). Further, it is likely that PUFA are incorporated into the neuronal membranes to influence the quaternary structure of receptors and transporters (18,19).

In the brain, arachidonic acid is preferentially esterified on sn-2 position of phosphatidylinositol or phosphatidylcholine (20). After its release from phospholipids by phospholipase A₂, arachidonic acid enters an unesterified brain pool predominantly located at synapses (20). This endogenous pool is a precursor 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 (FABPs), to the pool at the endoplasmic reticulum (20), and from there, it can exchange with arachidonic acid in plasma.

FABPs 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 in a specific manner. The FABPs are involved in cellular uptake and transport of PUFA, targeting of them to specific metabolic pathways. Brain-type FABP, also called FABP7, is present in the brain and retina, and is characterized by its strong affinity for n-3 PUFA, and in particular - docosahexaenoic acid (21). FABP7 is possibly involved in neurogenesis as it is strongly expressed in radial

glia cells, a major type of progenitor cells in the developing CNS (22-24).

Overall, despite the fact that the precise mechanisms of PUFA-mediated modulation of neuronal function still remains obscure, PUFA appear to possess multiple neural effects implicating them as diverse CNS regulators.

THE RECEPTOR GPR40

The last decade has witnessed the identification of an increasing number of orphan receptors with unknown functions. Among them, the G-protein coupled receptors (GPRs) are a member of the large family of seven-transmembrane receptors, and are known to play important physiological roles in response to a large variety of molecules, including free fatty acids such as PUFA. In particular, fatty acids have been identified to bind the GPR members GPR40 and GPR41-43 (4,5,25). Along with GPR41-43, GPR40 was identified downstream of CD22 on human chromosomal locus 19q13.1 (26). Subsequent studies demonstrated GPR40 mRNA expression in human tissues including pancreas and brain, and reported its activation by long chain FFA (4,5). In the pancreas, GPR40 affects insulin secretion (5,25,27,28), but its involvement in the CNS remained unknown at the time. Fatty acid carbon chain length has been shown to confer activity and specificity, with short-chain fatty acids (defined by six or fewer carbon molecules) activating GPR41 and GPR43, and both saturated and unsaturated medium to long-chain fatty acids activating GPR40 (4,5,25,29). Medium and long chain saturated and unsaturated fatty acids can activate GPR40 in a dose-dependent manner (4). This expression pattern clearly differentiates GPR40 from GPR41/GPR43 (29), suggesting that the function of GPR40 is probably divergent from the function of GPR41 and GPR43. Overall, there was a possibility that PUFA may act as extracellular signaling molecules, regulating function via membrane GPR40 receptor in various tissues, including not only of the pancreas but also of the brain.

EXPRESSION OF GPR40 IN THE BRAIN

Quantitative mRNA analysis assays had identified GPR40 expression in RNA samples from human tissues including pancreas and brain (4,5). However, detailed data on the GPR40 expression in the CNS of mammals were unavailable until recently. Using a novel antibody, we were the first to show GPR40 protein expression in diverse locations of the adult monkey CNS (Table 1) (6). GPR40-positive cells were neurons or astrocytes, suggesting a role of the receptor in both neuronal and astroglial biology.

Table 1. Localization of GPR40 protein in adult monkey brain (based on ref. 6)

- Spinal cord (gray matter, white matter, pia)
- Cerebellum (Purkinje cell layer, granule cell layer)
- Neocortex (layers 2-5)
- Hippocampal formation (cornu Ammonis, dentate gyrus)
- Hypothalamus
- Amygdala
- Subventricular zone (lateral ventricle)

EXPRESSION OF GPR40 IN HIPPOCAMPAL PROGENITOR CELL NICHE

Identifying GPR40 protein in adult monkey hippocampus, we focused our next analyses on the hippocampal progenitor cell niche, a location of continuous neuronal production throughout life (1,2). Neurogenesis in the hippocampus is thought to be tightly associated with angiogenesis (30,31). We thus explored whether GPR40 is expressed by blood vessels in the SGZ of the dentate gyrus, the site of active stem/progenitor cell proliferation. Indeed, co-labeling studies revealed GPR40 signal in vascular wall (32). Using cell type-specific markers (33), we explored the cellular phenotypes comprising the SGZ precursor cell niche for their putative expression of GPR40 – neural progenitors, immature neurons, SGZ astrocytes, and granule cell neurons. We detected GPR40 expression in a type-specific manner (32), and thus were able to construct a map of the putative sites of action of PUFA in the dentate gyrus (Fig. 1).

PUFA/GPR40 AS A PUTATIVE SIGNALING SYSTEM IN THE BRAIN

Similarly to the Ca^{++} -mobilizing effect of PUFA upon binding GPR40 in the pancreas, signal transduction of the PUFA/GPR40 axis was detected in neural cells. Using PC12 cell system, it was observed that the PUFA arachidonic and docosahexaenoic acids were able to increase intracellular Ca^{++} only upon transfection of GPR40 in the PC12 cells (34). Another piece of evidence came for studies in aged human volunteers in whom dietary supplementation with arachidonic and docosahexaenoic acids lead to improvements of cognitive functions, including hippocampus-dependent memory (35). Although the latter effects could be due to synaptic plasticity (36) rather than neuronal generation, the event of neurogenesis driven cognitive improvements by PUFA/GPR40 mechanisms might also be considered.

CONCLUSION

Three lines of evidence indicate that an involvement of PUFA/GPR40 system in adult neurogenesis and/or memory may be considered: (i) GPR40 is a membrane receptor for PUFA such as arachidonic and docosahexaenoic acids, (ii) GPR40 is expressed by cells in the hippocampal progenitor cell niche including the precursor cells themselves, and (iii) PUFA may signal in neural cells via GPR40 and PUFA supplementation improves hippocampus-dependent memory in which neurogenesis is thought to be involved. Future studies will provide direct evidence whether PUFA may affect cognitive functions via activating neurogenesis, and furthermore, whether PUFA acting via GPR40 may be useful tools in cellular therapies for neurological disorders.

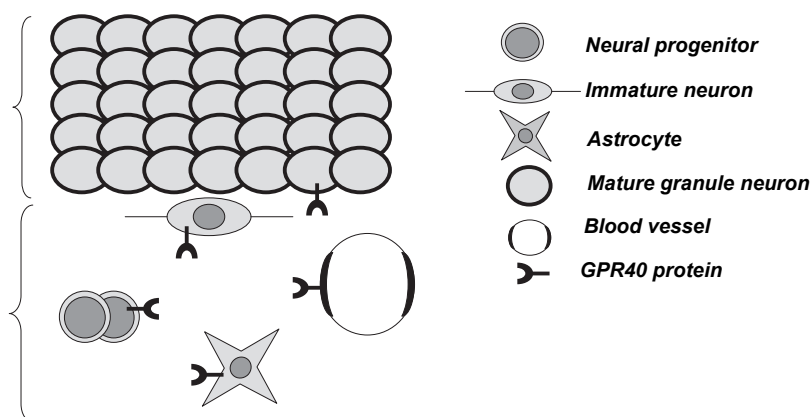


Figure 1. The cellular composition of the adult hippocampal progenitor niche, and the phenotypes expressing GPR40. SGZ, subgranular zone (based on ref. 32).

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