Review

Dual effects of the non-esterified fatty acid receptor 'GPR40' for human health

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ABSTRACT

G protein-coupled receptor 40 (GPR40), a receptor for diverse non-esterified fatty acids, is expressed predominantly in the wide variety of neurons of the central nervous system and β-cells in the pancreatic islets. Since deorphanization of GPR40 in 2003, the past decade has seen major advances in our understanding of its role in the insulin secretion. However, there is still a great deal to be elucidated about the role of GPR40 in the brain, because the latter shows the most abundant GPR40 mRNA expression among the human tissues. Since a substantial expression of GPR40 is also seen in the hypothalamus, ‘brain-lipid sensing’ might be involved in the control of insulin secretion and energy balance. The preceding experiments using monkeys after transient global brain ischemia, have highlighted implication of GPR40 for amplifying adult hippocampal neurogenesis. Although GPR40-mediated intracellular signaling was recently found to result in phosphorylation of cAMP response element-binding protein (CREB) necessary for the neuronal differentiation and synaptic plasticity, the signaling cascade is still incompletely understood. Furthermore, in response to conjugated linoleic acids or trans isomers of arachidonic acid, GPR40 was recently demonstrated in rodents to mediate lipotoxicity to β-cells, neurons, or microvessels, which result in diabetes, retinopathy, stroke, etc. However, it still remains undetermined in humans whether and how oxidized, conjugated, or excessive fatty acids evoke lipotoxicity. Although literature about GPR40 is limited especially about the brain or the brain–pancreas interaction, this review aims at summarizing beneficial as well as detrimental effects of this receptor in the brain and pancreas in response to diverse fatty acids.

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Abbreviations:
AA, arachidonic acids; ABCD, ABC transporters; BDNF, brain-derived neurotrophic factor; BKCa channels, Ca2+-activated K+ channels; cAMP, cyclic AMP; CO, carbon monoxide; CLA, conjugated linoleic acids; CNS, central nervous system; CoA, coenzyme A; CREB, cAMP response element-binding protein; DAG, diacylglycerol; DCX, doublecortin; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ER, endoplasmic reticulum; ERR, extracellular signal-regulated kinase; GPCR, G protein-coupled receptor; GPR40, G protein-coupled receptor 40; Gαq, G protein-α-subunit of the Gq family; Gq, G protein of the Gq family; H2O2, hydrogen peroxide; HO, heme oxygenases; Ip3, inositol 1,4,5-trisphosphate; MAPK, mitogen-activated protein kinase; NEFA, non-esterified fatty acids; NGF, nerve growth factor; NFKB, nuclear factor κB; βCREB, phosphorylated cAMP response element-binding protein; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PKD1, protein kinase D1; PLC, phospholipase C; PPAR-γ, peroxisome proliferator-activated receptor-γ; PSA-NCAM, polysialylated-neural cell adhesion molecule; PUFA, polyunsaturated fatty acids; SGZ, subgranular zone; TAA, trans isomers of arachidonic acid; TSP-1, thrombospondin-1; VGCC, voltage-gated Ca2+ channel.

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1. Introduction

All mammalian cells require energy and nutrient supplies for their survival and proper functioning. Aside from glucose, lipids are the major source of energy necessary for the body, and during long-term energy deprivation they are used almost exclusively. Non-esterified fatty acids (NEFA) are the major component of triacylglycerols in the fat, which consist of three fatty acids linked to a glycerol backbone. Hydrolysis of stored triacylglycerols in the adipose tissue by hormone-sensitive lipase liberates NEFA into the blood. NEFA, by binding to albumin, circulate in the plasma to be incorporated into the cell membrane lipid bilayer. The brain shows the highest organ lipid content after the adipose tissue. Because the brain has a lipid content of about 50% of dry weight [20,67,68], longer-chain NEFA are necessary for the neuronal development and function. For example, polyunsaturated fatty acids (PUFA) such as α-3 docosahexaenoic acid (DHA) and α-6 arachidonic acid (AA) are major constituents of neural cell membrane phospholipids [57,36,93].

Since half of the brain content is lipid, fatty acids are among the most crucial molecules that determine the brain's integrity and ability to perform. Essential fatty acids must be obtained from dietary sources for the maintenance of optimal brain health, because they cannot be synthesized by the body. As brain composition and function are sensitive to dietary influences, PUFA deficiency is known to be associated with many psychiatric diseases such as schizophrenia, depression, or Alzheimer's disease [71,89,33]. Furthermore, in the pancreatic β-cells, circulating NEFA are essential for facilitating the glucose-stimulated insulin secretion. The extensive innervation of the Langerhans islets by both parasympathetic and sympathetic nerves indicates an intimate relation between the hypothalamus and the pancreas. Parasympathetic stimulation associated with release of acetylcholine from parasympathetic nerve terminals, activates the M2 muscarinic receptor on the β-cell surface, that stimulates insulin release in a diacylglycerol (DAG)- and protein kinase C (PKC)-dependent manner. The sympathetic nerve is also important, because the sympathetic innervation serves both β-adrenergic agonists and α2-adrenergic agonists; increased β-adrenergic activity enhances insulin release, whereas increased α2-adrenergic activity decreases it. Both pathways act through adenylyl cyclase, resulting in a decrease or increase in cAMP levels, respectively [2,35]. The common characteristic of the hypothalamic neurons and the pancreatic β-cells is that both can respond to longer-chain NEFA, and are related to the regulation of energy balance.

Until recently, it was considered that NEFA must enter the β-cell in order to elicit the majority of their effects. Nowadays, it is clear that NEFA play crucial roles as extracellular signaling molecules, although both the pharmacological mechanism and biological significance still remain mostly unknown. NEFA and their derivatives modulate cell surface or intracellular signaling pathways to activate transcription factors [54]. When non-adipose cells are exposed to chronic elevation of NEFA (50–100 μM for DHA, eicosapentaenoic acid (EPA), AA or palmitic acid), however, cell dysfunction, degeneration, and even cell death occur. For example, NEFA are critical for the normal insulin release, but chronic NEFA exposure to β-cell islets in vitro and in vivo is associated with marked impairments in glucose-stimulated insulin secretion and insulin biosynthesis [70,97]. Prolonged NEFA elevation by a lipid infusion in vivo facilitates insulin resistance and prevents the expected compensatory β-cell response in humans [15]. Prolonged elevation of circulating fatty acids ultimately leads to loss of β-cell viability. Such lipotoxicity may contribute to various pathological conditions including type 2 diabetes and brain disorders [83,85,82,84,1,4]. However, the molecular mechanisms of lipotoxicity especially in β-cells and neurons are not sufficiently understood until now. It is conceivable that generation of reactive oxygen species, abnormal Ca2+ mobilization, as well as lysosomal membrane permeabilization may be key mediators of pathological conditions associated with lipotoxicity. Almaguel et al. [5] demonstrated in nerve growth factor (NGF)-differentiated PC12 (rat adrenal pheochromocytoma) cells that palmitic acid-induced lipotoxicity occurs by lysosomal membrane permeabilization, and cell death is attenuated by lysosomal enzyme, cathepsin inhibitors. Furthermore, they also showed that DHA rescued PC12 cells from palmitic acid-induced lipotoxicity by decreasing lysosomal membrane permeabilization. These data are interesting because during the past decade lysosomes have emerged as a second hub for orchestrating cellular survival and death decisions [92,94,95]. Of particular interest is that lysosomal destabilization was evident not only in NEFA-induced PC12 cell apoptosis [4,5] but also in NEFA-induced hepatic cell apoptosis [23,91]. Although the mechanisms by which NEFA contribute to cell protection and induce cell death are not fully elucidated yet, it would be tempting and reasonable to consider implications of a NEFA receptor for determining cell fate.

Although it is widely accepted that neurons do not utilize NEFA but merely glucose as an energy source, the role of NEFA in the brain, other than as a constituent of cell membranes, had remained unknown for decades. However, recently the concept has emerged that the brain is a NEFA-sensing organ [72], because abundant expression of G protein-coupled receptor 40 (GPR40, also called FFAR1) for NEFA was found in 2003 in the human brain (Fig. 1A), including the hypothalamus [14]. GPR40 belongs to the G protein-coupled receptor family, and was discovered in 1997 by Sawzdargo et al. [73]. It is a seven-transmembrane domain receptor binding with a broad range of medium- to long-chain NEFA. GPR40 shows higher affinity for longer-chain fatty acids with a half-maximal effective concentration (EC50) in the 1–2 μM/L range [31,26]. Deorphanization and characterization of GPR40 in 2003 [14,31,41] unraveled a novel mechanism of NEFA action as extracellular signaling molecules. At the surface of β-cells in the pancreas, for example, GPR40 senses NEFA in the blood and facilitates glucose-stimulated insulin secretion. Furthermore, GPR40 was found to be closely related to adult neurogenesis in the primate hippocampus [93,95]. However, the pharmacological effects of NEFA binding with GPR40 are complex, because NEFA are not infrequently elevated excessively and/or oxidized in the subjects suffering from metabolic syndrome, type 2 diabetes, Alzheimer's disease, or Parkinson's disease, etc. As our understanding of the pharmacology of GPR40 is still incomplete, not only its physiological role but also its pathological role remains obscure. Accordingly, here I review the current state of knowledge and emerging concepts regarding dual roles for GPR40 which is expressed predominantly in the brain and pancreas (Fig. 1A).

2. Predominant expression in the brain and pancreas; Why?

Nutrient-sensitive neurons in the brain sense and integrate information from a range of nutrient signals that are generated after...
that is, the responsible neurons signal that the brain stem are excited or inhibited when exposed to increment in energy status. Glucose-responsive neurons in the hypothalamus monitoring glucose levels provides a signal to the brain regarding acute impairment of the insulin signalling leads to increased food intake and insulin resistance (decreased insulin sensitivity) by impairing peripheral glucose utilization and promoting hepatic glucose overproduction. More importantly, continuously elevated levels of plasma NEFA play a key role in the pathogenesis of β-cell dysfunction [35]. Acute exposure to NEFA stimulates insulin secretion, whereas chronic exposure impairs insulin secretion [78]. The role of excessive fatty acids in the pathogenesis of insulin resistance and type 2 diabetes has been widely accepted [11]. When β-cells are healthy, the adaptive response to insulin resistance, i.e. increased insulin release occurs and normal glucose tolerance is maintained. On the contrary, when insulin resistance is accompanied by dysfunction of β-cells, impaired insulin secretion results in decreased insulin levels and signalling in the hypothalamus, that lead to decreased inhibition of hepatic glucose production and reduced efficiency of glucose uptake in muscle. Accordingly, β-cell dysfunction is critical in defining the risk of impaired glucose tolerance and development of type 2 diabetes. Increased glucose levels together with elevated NEFA levels can synergize to further adversely affect β-cell health, often referred to as ‘glucolipotoxicity’ [35]. However, the exact mechanism of β-cell dysfunction due to its degeneration and/or death is still incompletely elucidated. This knowledge would contribute not only to exploring the molecular and genetic basis of type 2 diabetes but also to developing new approaches to its treatment and prevention.

The amount of insulin release varies according to the nature, quantity and route of administration of the stimulus to β-cells, and the prevailing glucose concentration. When considering an intimate involvement of the hypothalamus in the regulation of energy and glucose metabolism, it is reasonable to assume that the brain has a crucial role in the functional adaptation to changes in insulin sensitivity. Given the recently recognized role of fatty acid metabolism in the brain’s control of energy homeostasis, it would not be surprising if fatty acid receptor GPR40, which is the target of the ingestion of food (Fig. 1B). For example, an increase in the circulating glucose levels provides a signal to the brain regarding acute energy status. Glucose-responsive neurons in the hypothalamus and brain stem are excited or inhibited when exposed to increment in the blood glucose [37]; that is, the responsible neurons signal that food has been ingested. The resultant neuronal output may modulate feeding behaviour, hepatic glucose production, and insulin secretion. Forty years ago, Oomura et al. [60] first demonstrated that NEFA activate lateral hypothalamic neurons in rats, which intriguingly suggested a role for NEFA as neuronal signaling molecules. Nevertheless, less attention has been paid thereafter to the potential role of NEFA in the hypothalamic regulation of energy balance. Twenty-seven years later, however, Obici et al. [58] showed that a 6 h intra-cerebroventricular infusion of the oleic acid reduced food intake as well as hepatic glucose production. Subsequently, Wang et al. [88] confirmed that oleic acid regulates three distinct populations of neurons in the hypothalamic arcuate nucleus in a glucose-dependent fashion. It became apparent that pharmacological manipulation of hypothalamic fatty acid signaling alters the regulation of glucose and energy homeostasis. Daily variations in the plasma NEFA concentrations are currently known to be monitored in the NEFA-sensitive hypothalamic neurons as a cellular messenger informing energy status of the body. Subpopulations of neurons in the ventromedial and arcuate hypothalamic nuclei are selectively inhibited or activated by NEFA in order to control the insulin level [51]. Such ‘brain-lipid sensing’ is involved in the control of feeding behavior, hepatic glucose production, and insulin secretion [58,59,16]. For achieving the ‘brain-lipid sensing’, it would be biologically reasonable to share the same cell surface receptor ‘GPR40’ between the brain and pancreas (Fig. 1).

Since insulin acts in the hypothalamus to regulate body weight, impairment of the insulin signalling leads to increased food intake and body weight gain [76,35]. It is generally accepted that obesity is associated with an increased risk of type 2 diabetes, because adipose tissue releases increased amounts of NEFA which induce insulin resistance (decreased insulin sensitivity) by impairing peripheral glucose utilization and promoting hepatic glucose overproduction. More importantly, continuously elevated levels of plasma NEFA play a key role in the pathogenesis of β-cell dysfunction [35]. Acute exposure to NEFA stimulates insulin secretion, whereas chronic exposure impairs insulin secretion [78]. The role of excessive fatty acids in the pathogenesis of insulin resistance and type 2 diabetes has been widely accepted [11]. When β-cells are healthy, the adaptive response to insulin resistance, i.e. increased insulin release occurs and normal glucose tolerance is maintained. On the contrary, when insulin resistance is accompanied by dysfunction of β-cells, impaired insulin secretion results in decreased insulin levels and signalling in the hypothalamus, that lead to decreased inhibition of hepatic glucose production and reduced efficiency of glucose uptake in muscle. Accordingly, β-cell dysfunction is critical in defining the risk of impaired glucose tolerance and development of type 2 diabetes. Increased glucose levels together with elevated NEFA levels can synergize to further adversely affect β-cell health, often referred to as ‘glucolipotoxicity’ [35]. However, the exact mechanism of β-cell dysfunction due to its degeneration and/or death is still incompletely elucidated. This knowledge would contribute not only to exploring the molecular and genetic basis of type 2 diabetes but also to developing new approaches to its treatment and prevention.

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Fig. 1. GPR40 mRNA expression in the human tissues (A), and brain regulation of glucose and lipid homeostasis (B). (A) The mRNA measurements by RT-PCR show that GPR40 is predominantly, but not exclusively, expressed in the brain and pancreas (cited from [14]). (B) The brain responds to changes in the levels of free (non-esterified) fatty acids (FFA or NEFA) and leptin signals that were released from the adipose tissue (brain-lipid sensing). Leptin is a hormone that is thought to serve as a signal of adiposity to the hypothalamus. The brain responds also to an increase in the levels of circulating glucose, during which hepatic glucose production and storage, pancreatic insulin secretion as well as skeletal muscle glucose uptake are regulated (cited from [72]). As GPR40 is expressed in both K and L cells of the gut, it may play a role in sensing ingested fat within the intestinal lumen and thereby contribute to the incretin-effect by promoting the release of glucagon-like-peptide-1 and glucose-dependent insulinotropic polypeptide into the circulation. Since these peptides can then impinge on β-cells to enhance insulin secretion, FFA (NEFA) may exert both indirect (via incretin release) and direct (via receptors on β-cells) stimulatory effects on the insulin release (cited from [53,66]).
expressed considerably in the hypothalamus [14,46], regulates insulin sensitivity. Although GPR40 was most abundant in medulla oblongata, substantia nigra and spinal cord within the brain [14], it was concentrated at the supraoptic nucleus and paraventricular nucleus in the monkey hypothalamus [46]. NEFA metabolism and/or signaling within discrete hypothalamic regions can function as a sensor for the nutrient availability. In this sense, physiological role for GPR40 in the hypothalamus is presumably to provide acute fine adjustments of insulin secretion to facilitate efficient storage of NEFA [50]. Between the hypothalamus and the pancreas, there is a GPR40-mediated interface that is capable of regulating energy balance and glucose levels. The GPR40-positive neurons in the hypothalamus can directly respond to circulating nutrients and hormones such as NEFA, glucose and leptin to generate efferent nerve activity that directly regulates hepatic glucose production, pancreatic secretion of insulin and glucagon, as well as glucose uptake of the skeletal muscle (Fig. 1B). Accordingly, dysfunction of the NEFA-sensitive neurons may impair neural control of energy and glucose homeostasis, and lead to the development of obesity and type 2 diabetes in the predisposed subjects [21]. It is now widely accepted that NEFA act centrally to modulate not only food intake and body weight but also glucose homeostasis, and lead to the development of obesity and type 2 diabetes in the predisposed subjects [21].

3. **GPR40 expression in the pancreas: beneficial and detrimental**

Glucose homeostasis requires the highly-coordinated regulation of insulin secretion by the pancreatic β-cells, which is primarily mediated by glucose itself and secondarily potentiated by NEFA via GPR40 (Fig. 2A). Glucose-stimulated insulin secretion occurs biphasically within hours, with a rapid but short 1st phase followed by a slower but prolonged 2nd phase [49]. The extended period of lower secretion during the 2nd phase mediated by NEFA actually accounts for the majority of insulin secretion. The glucose-stimulated, rapid but short insulin secretion (1st phase) [49] occurs by the secretion of a readily-releasable pool of secretory granules that were pre-docked at the plasma membrane. Upon stimulation of GPR40, gating of voltage-gated K⁺ channels is impaired, which serves to prolong glucose-induced insulin secretion (Fig. 2A) by opening voltage-gated Ca²⁺ channels (VGCC) and maintaining cytosolic Ca²⁺ at an elevated level [24]. In contrast, the NEFA-augmented, slower but prolonged insulin secretion (2nd phase) [49] occurs by the mobilization of granules from an intracellular pool to the plasma membrane via a process that requires cytoskeletal reorganization (Fig. 3B). GPR40-mediated, Gαq/11/Phospholipase C (PLC)-dependent signaling mechanism is responsible for the NEFA-mediated augmentation of the 2nd insulin secretion phase. Although somewhat weaker than palmitic acid, oleic acid also shows the insulinotropic effect. To explain the mechanism of oleate-mediated insulinotropic effect, Ferdaoussi et al. [25] showed that oleate promotes phosphorylation of the DAG-sensitive, serine/threonine protein kinase D1 (PKD1) for the cortical actin depolymerization which is central to the 2nd insulin secretion phase. DAG promotes phosphorylation of PKD1 which, in turn, activates currently undefined targets implicated in the filamentous (F)-actin remodeling to potentiate the 2nd insulin secretion phase (Fig. 3B). Since a single intracarotid injection of oleate can increase the frequency of neuronal firing rate in the arcuate nucleus of rats [51], it is tempting to
speculate that the 2nd insulin secretion phase might be controlled by the hypothalamic neuronal output.

High intakes of hypercaloric diets have increased alarmingly recently in Western countries. Chronic consumption of high-fat-and-fructose diets is associated with the development of obesity and insulin resistance. Generally, hypercaloric diet, especially rich in trans/saturated fat and cholesterol, and fructose-sweetened beverages seem to increase visceral adiposity and type 2 diabetes. The toxic effects of NEFA upon insulin-producing cells are dependent on chain length and degree of saturation [18]. Unsaturated NEFA, for example palmitic acid exhibit a strong cytotoxic effect upon insulin-secreting cells in response to excess NEFA. Consequently, it is probable that overactivation of GPR40 in response to CLA may induce excessive Ca\(^{2+}\) mobilization and cause cell toxicity. For example, long-term intake of cis-9, trans-11-CLA and trans-10, cis-12-CLA is associated with serious adverse effects such as impaired insulin sensitivity, and ultimately, type 2 diabetes. In this sense, CLA is a highly-efficacious GPR40 agonist, compared to oleate.

Fig. 3. GPR40-mediated signaling in response to oleate and conjugated linoleic acid (CLA). (A) Structures of linoleic acid and CLA. (B) The 2nd insulin secretion phase occurs by the release of insulin granules that were newly recruited to the plasma membrane in response to the normal NEFA (for example, oleate) binding with GPR40. Insulin granules from the intracellular pool are mobilized via a process that requires reorganization of F-actins. This NEFA-stimulated and GPR40-mediated insulin secretion occurs as followings: (1) GDP-for-GTP exchange at G\(_\alpha_{11}\), and the subsequent dissociation from the \(\beta y\) subunit, (2) the resultant activation of PLC, (3) hydrolysis of PIP\(_2\) to produce two second messengers; IP\(_3\), and DAG, (4) IP\(_3\) triggers Ca\(^{2+}\) efflux from ER, simultaneously (5) DAG activates PKD1 which induces F-actin remodeling to recruit insulin granules (cited and adapted from [49]). The main signaling cascade involves activation of PKD1, remodeling of the cortical actin and potentiation of the 2nd phase of glucose-induced insulin secretion. Generally, it is probable that overactivation of GPR40 in response to CLA may induce excessive Ca\(^{2+}\) mobilization and cause cell toxicity. For example, long-term intake of cis-9, trans-11-CLA and trans-10, cis-12-CLA is associated with serious adverse effects such as impaired insulin sensitivity, and ultimately, type 2 diabetes. In this sense, CLA is a highly-efficacious GPR40 agonist, compared to oleate.

The detrimental effect occurs together, it is conceivable that GPR40 does not mediate lipotoxicity to insulin-secreting cells in response to excess NEFA.

\[
\text{H}_2\text{O}_2 + \text{H}_2\text{O} \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

This reaction is catalyzed by the enzyme catalase, which is thought to play a role in protecting cells from oxidative stress. The catalytic activity of catalase is dependent on its molecular structure, and the presence of heme groups in the active site. The heme group reacts with oxygen to produce superoxide radicals, which can then react with other molecules to produce reactive oxygen species (ROS).

The superoxide radical (O\(_2^-\)) yields the highly reactive hydroxyl radical (OH\(^-\)). This causes \(\beta\)-cell dysfunction and ultimately cell death, because of its low antioxidative defense status [27].

Although \(\beta\)-cell lipotoxicity has been subject to extensive research [97,65], intriguingly the molecular cascade has not been elucidated in detail. In particular, implication of GPR40 for the development of \(\beta\)-cell lipotoxicity in response to excessive NEFA has been controversial. Concerning the role of GPR40 in lipotoxicity, some proposed that, by mediating hypersecretion of insulin in response to high-fat diets, GPR40 is indirectly responsible for hyperinsulinemia-induced insulin resistance [78]. On the contrary, others concluded that NEFA-induced hyperinsulinemia represents a mechanism by which the \(\beta\)-cell attempts to compensate for insulin resistance and that this ability is compromised by hyperinsulinemia. Although the mechanism by which the \(\beta\)-cell attempts to compensate for insulin resistance and that this ability is compromised by hyperinsulinemia.

In the first step of \(\beta\)-oxidation, the electrons are transferred to FAD in the mitochondria, while O\(_2\) is the electron acceptor in the peroxisomes, and this leads to the formation of hydrogen peroxide (H\(_2\)O\(_2\)). Accordingly, peroxisomes are thought to be the major site of H\(_2\)O\(_2\) formation in insulin-producing cells, whereas mitochondria are a site of minor contribution. The detrimental effect occurs via an enhancement of peroxisomal metabolism of acyl-coenzyme A (CoA), yielding high levels of H\(_2\)O\(_2\) as a by-product of the \(\beta\)-oxidation (Fig. 2B). Since the pancreatic \(\beta\)-cells almost completely lack the H\(_2\)O\(_2\)-detoxifying enzyme, oxidoreductase catalase [43,81], they are exceptionally vulnerable to H\(_2\)O\(_2\) that was metabolically generated in peroxisomes. If H\(_2\)O\(_2\) is not quickly converted into water and oxygen, it can react in an iron-catalysed reaction with the superoxide radical (O\(_2^-\)) yielding the highly reactive hydroxyl radical (OH\(^-\)). This causes \(\beta\)-cell dysfunction and ultimately cell death, because of its low antioxidative defense status [27].
Both α-3 and α-6 PUFA are essentially not toxic to β cells. In particular, PUFA with a chain length of ≥ C16 show a protective effect against palmitic acid-induced toxicity, and the physiologically-important oleic acid attenuates the toxic effect of palmitic acid [27]. However, linoleic acids after conjugation of their double bonds can become extremely toxic, and GPR40 mediates lipotoxicity in response to the conjugated linoleic acids (CLA). CLA refer to a group of conjugated octadecadienioic acid isomers which are derived from linoleic acids (Fig. 3A). Microbes in the gastrointestinal tract of ruminant animals convert linoleic acids into different isomers of CLA through biohydrogenation. Commercial preparations of CLA are made from the linoleic acids of safflower or sunflower oils under alkaline conditions. Humans most often acquire CLA not only through partially-hydrogenated vegetable oils such as margarine and shortening but also through ruminant meat (e.g., beef), milk and dairy foods. Furthermore, for the purpose of reducing fat stores and increasing muscle mass, CLA have attracted considerable attention as dietary weight loss supplements in Western countries. Such CLA supplements have become a subject of intense debate due to their potential influence on glucose homeostasis and insulin sensitivity by interfering with peroxisome proliferator-activated receptor-γ (PPAR-γ) and activating nuclear factor κB (NFκB) and caspases [62,39]. However, the molecular mechanisms underlying the effects of CLA on the glucose homeostasis are not completely understood. Some workers suggest that CLA may attenuate development of the impaired glucose tolerance and hyperinsulinemia [69,55,52], whereas others suggest that CLA intake is associated with serious adverse effects such as impaired insulin sensitivity, insulin resistance and ultimately, development of type 2 diabetes [64,3,39].

Increased insulin-releasing capacity of pancreatic islets from CLA-fed mice is well-known [63,90]. Intriguingly, it was recently demonstrated in both in vitro and in vivo experiments that the two representative CLA isomers ‘cis-9, trans-11-CLA’ and ‘trans-10, cis-12-CLA’ (Fig. 3A), being contained in foods and commercial supplements, markedly increase glucose-stimulated insulin secretion by targeting and activating GPR40. Each CLA isomer markedly increased glucose-stimulated insulin secretion both in insulin-producing, immortalized rat INS-1E cells that endogenously express GPR40 and in primary pancreatic β-cells of wild type but not GPR40-null mice [75]. In addition, Hsu and his colleagues demonstrated that ‘trans-10, cis-12-CLA’, but not ‘cis-9, trans-11-CLA’, induced apoptosis of TM4t mouse mammary tumor cells by lipid peroxidation and GPCR-dependent activation of the AMP-activated protein kinase pathway [61,29,30]. At present, the molecular mechanisms underlying the effects of CLA on glucose homeostasis are not completely understood and the clinical side-effects of the CLA-mediated GPR40 activation are not accepted worldwide. However, long-term ingestion of CLA potentially causes β-cell dysfunction in humans. One should keep in mind the possible risk that GPR40 is responsible not only for the acute, physiological insulinotropic effects in response to various NEFA (Fig. 2A) but also for the development of insulin resistance and type 2 diabetes after long-term ingestion of highly-efficacious GPR40 agonist; CLA (Fig. 3B).

4. GPR40 expression in the brain: beneficial and detrimental

In the wild-type (GPR40-negative) PC12 cells which can differenti ate into neuron-like cells with nerve growth factor (NGF), 10 μM AA failed to induce intracellular Ca^{2+} mobilization, although a positive control KCl induced it. In contrast, GPR40 gene-transfected PC12 cells showed a transient (~1.5 s) but remarkable Ca^{2+} mobilization in response to the same concentration of AA, which was not inhibited by a Ca^{2+} chelator EDTA [93] (Fig. 4A). This indicates that the Ca^{2+} mobilization occurred not by the influx of the extracellular Ca^{2+}, but by the release from the internal stores as shown in Fig. 3B. Furthermore, in cultured rat neural stem cells transfected with GPR40 gene, DHA induced Ca^{2+} mobilization via the PLC/IP_{3} pathway [48]. In addition, Vettor et al. [86] identified a loss-of-function mutation of the GPR40 gene in human subjects and HeLa cells, that weakens Ca^{2+} mobilization in response to NEFA and prevents the β-cell’s ability to adequately sense lipids as an insulin secretory stimulus. These data suggest that GPR40 can bind with NEFA to induce Ca^{2+} mobilization in neurons.

Expression of GPR40 has been detected in various areas of the human [14] and monkey [46,47,12,13] central nervous system (CNS). In humans, GPR40 mRNA was expressed in the wide variety of brain areas, being most abundant in the medulla oblongata and substantia nigra. Hippocampus and hypothalamus also showed a substantial GPR40 expression [14]. In the monkey brain, GPR40 immunoreactivity was confirmed in the wide variety of neurons including the cerebral cortex, hippocampus, amygdala, hypothalamus, cerebellum, spinal cord, etc. [46]. In addition, GPR40 expression was significantly upregulated in the subgranular zone (SGZ) of ischemia-enhanced hippocampal neurogenesis in adult monkeys. GPR40 immunoreactivity was localized at the neural progenitors, immature neurons, astrocytes and endothelial cells of the SGZ of dentate gyrus [47]. A specific newborn marker, polysialylated neural cell adhesion molecule (PSA-NCAM) and GPR40 double-positive neurons showed a significant increase in the 2nd week after transient whole brain ischemia (Fig. 4B). Furthermore, GPR40/phosphorylated cAMP response element-binding protein (pCREB) double-positive progenitor cells significantly increased in the SGZ on day 15 after ischemia [12,13]. Expression patterns of GPR40 and pCREB were completely identical, and they were coexpressed in both the mature and newborn neurons as well as in the astrocytes residing in the SGZ. It is suggested from these data that PUFA, GPR40, and pCREB may be engaged in the same GPR40 signaling pathway (Fig. 5A) to promote adult neurogenesis in the primate hippocampus [95]. As the expression of GPR40 gene in the rodent brain was initially reported to be negligible compared to humans [31,14,32], analyses focusing the rodent brain have been unfortunately hampered for the past decade. However, recent reports demonstrated ubiquitous expression of GPR40 in the mouse brain [56,96]. Zamarbide et al. [96] confirmed GPR40 mRNA expression by PCR and in situ hybridization, while Nakamoto et al. [56] confirmed GPR40 protein by Western blotting and immunohistochemistry. These reports have paved the way to consider the mouse as a suitable model to study expression and function of GPR40 in the brain.

Despite expression of GPR40 in the mature neurons and adult-born neurons of ischemic monkeys [46,47], it remains to be elucidated whether expression of GPR40 in the hippocampal neurogenic niche is of any importance for learning and memory. Notably, it still remains unknown whether PUFA-GPR40 signaling is crucial for the synaptogenesis, long-term potentiation and synaptic plasticity. All NEFA receptors such as GPR40, GPR41 (predominantly expressed in immune cells), and GPR43 (predominantly expressed in adipocytes) are known to regulate various physiological homeostasis and are linked to activation of extracellular signal-regulated kinases (ERK1/2). Activation of the mitogen-activated protein kinase (MAPK)/ERK pathway is known to be required for the memory acquisition, consolidation and reconsolidation from newborns to adults [7,10,77,74,80,38,19,17,42]. Intriguingly, in the primary cultured mice neurons the selective GPR40 agonist GW9508 increased phosphorylation of CREB, Akt and ERK1/2, and this was blocked by GPR40 antagonist GW1110. Furthermore, a direct GPR40-CREB link was demonstrated by using human GPR40-positive neuroblastoma cells that have been extensively used to investigate CREB activation [96]. Taken together, it is
probable that this membranous receptor can conduct extracellular ω-3 PUFA signals to the nucleus for phosphorylating CREB [13,95] and the GPR40-mediated MAPK/ERK cascade conceivably plays a crucial role in triggering gene transcription for brain-derived neurotrophic factor (BDNF) synthesis that underlies synaptogenesis and synaptic plasticity (Fig. 5B). Accordingly, it is likely that GPR40 is closely related to the adult hippocampal neurogenesis.

Lipids are essential components of a living organism, but in excess or after oxidation, they may show toxicity and cause neurological deficits. For example, high-saturated fat diets impair adult hippocampal neurogenesis in male rats [44]. High-fat diets impair also hippocampal synaptic plasticity and spatial learning ability in middle-aged rats, although involvement of GPR40 has not been indicated [79]. Hypertriglyceridemia observed in the obese mice was shown to mediate cognitive impairment, possibly by disturbing maintenance of the N-methyl-D-aspartate component of hippocampal long-term synaptic potential [22]. These experimental data are consistent with the previous clinical and epidemiological data that excessive energy intake adversely affects the brain, presumably through the increased oxidative damage. Furthermore, adipokines (adipocytokines) such as leptin (Fig. 1B) and adiponectin, being released from the adipose tissue due to obesity by the excess calorie intake, affect brain nuclei important for cognition and energy metabolism. Luchsinger et al. [45] reported that individuals with higher intakes of calories and fats may be associated with a higher risk of Alzheimer’s disease when they carry the apolipoprotein Eε4 allele.

Long-term activation of GPR40 by continuous intake of oxidized ω-6 fatty acids may overstimulate physiological mechanisms, causing excito-toxicity. A characteristic structural feature of AA is a 20-carbon chain containing four cis-double bonds that form a molecule of 5Z, 8Z, 11Z, 14Z-eicosatetraenoic acid. Enzymatic processes convert AA to biologically active lipids such as prostaglandins and leukotrienes, known collectively as eicosanoids. In addition, reactive oxygen radicals oxidize AA to generate a complex mixture of oxidized lipids termed isoeicosanoids that share structural similarity to eicosanoids. Reaction of AA with the nitrogen dioxide radical (NO₂) generates four trans isomers of AA (TAA) via reversible addition of the NO₂ radical to the cis double bonds (Fig. 7A) [9]. TAA is a mixture of AA isomers having one trans-double bond and three cis-double bonds, comprising of 5E-AA, 8E-AA, 11E-AA and 14E-AA [34]. Two of them (5E-AA and 8E-AA) are not found in diets, being endogenously produced [8]. As experimental feeding and clinical studies have supported the concerns that dietary TAA are cardiovascular risk factors, clinical consequences of the endogenous formation of TAA are nowadays thought to be more serious, because many chemical and/or physical stresses would cause cellular AA to isomerize. For example, humans are exposed to various endogenous and exogenous sources of nitrogen...
dioxide radical (\(\cdot{\text{NO}}_2\)). Polluted urban air contains significant amounts of \(\cdot{\text{NO}}_2\), and it is formed in exposure to cigarette smoke, hyperoxia, hypercapnia, air pollution, etc. (Fig. 6). Furthermore, immune responses to invading microorganisms in inflammation stimulate inducible nitric oxide synthase (iNOS) in macrophages that form nitric oxide \(\cdot{\text{NO}}\), which is oxidized to \(\cdot{\text{NO}}_2\). TAA, generated by \(\cdot{\text{NO}}_2\)-mediated isomerization of the AA double bonds, may have profound influence on cellular properties by causing changes of the membrane asymmetry and fluidity [34,9]. Biological effects of TAA especially upon microvessels are diverse (Fig. 6) [9]. Both opening of the \(\text{Ca}^{2+}\)-activated \(\text{K}^+\) (BKCa) channels and activation of heme oxygenases (HO-1/2) by TAA leads
to microvascular relaxation via formation of carbon monoxide (CO) and increase of cGMP levels. On the contrary, TAA induce concentration- and time-dependent apoptotic cell death of microvascular endothelial cells. TAA stimulate formation of the anti-angiogenic factor thrombospondin-1 (TSP-1) and its binding to the CD36 receptor, which leads to apoptotic cell death of microvascular endothelial cells via transient activation of MEKK-ERK1/2 pathway and caspases. One must note that ERK1/2 can have diametrically different effects on cell survival (Fig. 5) and death (Fig. 6), depending on the kinetics and amplitude of its activation and the cellular environment [40]. More importantly, TAA bind also to GPR40 receptor (Fig. 6) and play a significant role as a cause of neonatal retinal microvascular degeneration and ischemia-induced microvascular endothelial cell death and cerebral infarct of rodents through GPR40 activation (Fig. 7B and C) [28]. Since these occurred in the wild-type mice but not in the GPR40 knocked-out mice, role of GPR40 in endothelial cell death is clear at least in the non-primate animals. Although the implications of these observations in humans have yet to be confirmed, it is probable that TAA, originating not only from dietary sources but also from the diet-independent pathobiochemistry of a disease process, can function as a modulator of the PUFA-mediated activation of GPR40 in various pathologies such as retinopathy, infarct, dementia, etc. [9].

5. Summary

The available data from research into GPR40 during the past decade support the following five notions.

(1) GPR40 is responsible for both potentiating glucose-stimulated insulin secretion and mediating the stimulatory effects of long-chain saturated or mono- and poly-unsaturated fatty acids on insulin secretion in pancreatic β-cells.

(2) Abundant expression of GPR40 in the pancreas and hypothalamus may act to facilitate ‘brain-lipid sensing’. The hypothalamus presumably plays an important part in orchestrating appropriate energy metabolism via neuronal GPR40. A better understanding of ‘brain-lipid sensing’ could provide clues for developing new therapeutic strategy for type 2 diabetes and obesity.

(3) By activating ERK1/2 and CREB, GPR40 in the hippocampus may be related to adult neurogenesis and the concomitant synaptic plasticity.

(4) Long-standing, abnormal activation of GPR40 receptors by CLA or TAA possibly causes lipotoxicity of β-cells, brain endothelial cells, or neurons.

Fig. 7. Formation of TAA (A) and their effects upon endothelial cells of the retina (B) and the brain (C). (A) NO2 can bind to a cis double bond of AA and induce cis–trans isomerization by a reversible addition reaction. (B and C) TAA being generated during the nitrative stress induce microvascular degeneration in the rat retina (B) (cited from [40]) and the mice brain (C) (cited from [28]). The role of GPR40 in TAA-induced stroke is obvious, because the lesion is remarkably decreased in the GPR40 knocked-out (KO) mice (C).

Fig. 8. Beneficial as well as detrimental roles of GPR40 signaling in response to the normal fatty acids or CLA/TAA. Non-esterified fatty acids (NEFA), at the physiological concentration, contribute to appropriate brain and pancreas functions, whereas CLA and TAA cause cell degeneration and related diseases.
Accordingly, it may be advantageous to focus on GPR40 as a cause and/or therapeutic target of type 2 diabetes, retinopathy, stroke, etc. in humans.

Whether or how GPR40 expression in some areas of the CNS could have functional consequences remains incompletely understood. Further research using diverse experimental paradigms will be needed to elucidate pharmacological, biological, and therapeu-
tic roles of GPR40. However, the dual (beneficial as well as detri-
mental) roles of GPR40 must be taken into account for considering divergent results in response to un-oxidized fatty acids or those after oxidation or conjugation (Fig. 8).

Conflict of interest

I certify that there is no conflict of interest in relation to this review article.

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