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GPR40/FFA1 Free Fatty Acid Receptors and Their Functional Role

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The endogenous ligands of GPR40/FFA1 receptors, which are members of the G protein-coupled receptor class, are saturated and unsaturated free fatty acids (C6–C12) and long-chain (>C12) structures. The highest levels of expression of these receptors are seen in pancreatic β cells and neurons in various parts of the CNS. An enormous amount of experimental data on the functional roles of these receptors in the central and peripheral regulation of the body's metabolic status has been published since the "deorphanization" of these receptors in 2003. This review summarizes current understanding of the mechanisms regulating GPR40/FFA1 receptors by endogenous and synthetic ligands and the intracellular signal transduction systems activated on exposure to free fatty acids. The mechanisms of GPR40/FFA1-mediated effects of free fatty acids on glucose-stimulated insulin secretion by pancreatic β cells are addressed, along with the production of incretins by enteroendocrine cells; the mechanisms of actions on the support of neurogenesis and neurodifferentiation are also considered. Advances and potentials in the use of synthetic ligands of GPR40/FFA1 receptors in the treatment of metabolic disorders are assessed.

Keywords: free fatty acids, GPR40/FFA1 receptors, insulin secretion, incretins, neurons, neurogenesis, allosteric regulation.

The book *Cell Membrane Lipids*, by Academician Evgenii Mikhailovich Kreps, one of the most profound and authoritative researchers of the diversity of natural lipids, was published almost 40 years ago. And today, many years later, this seminal work, unprecedented in the volume of data and diversity of species studied, retains its value in lipidology and comparative neurochemistry. A significant proportion of the research conducted by Kreps and his colleagues addressed esterified fatty acids, which are part of the composition of phospholipids, cerebrosides, and gangliosides. Experimental data obtained in more than 130 fish species living at different temperatures and pressures allowed variations in the set of fatty acids in membrane lipids to be regarded as a major factor in maintaining the stability of the physicochemical state of the membrane and defining the boundaries of the ability of an organism to survive in extreme conditions [1]. By the 1980s, eucosanoids, which are derivatives of polyunsaturated fatty acids, had been discovered and it was understood that the biological function of fatty acids was not confined to their structural role in the organization of biological membranes or their importance as energy substrates. However, nothing was known of the regulatory effects of free fatty acids and receptor science was still at the dawn of its development.

In addition, the opportunities provided by developments in genetics, molecular biology, and bioinformatics at the end of the 20th century led to a colossal jump in studies of the mechanisms regulating physiological functions. Traditionally, studies of signal action algorithms of one regulator or another followed the path from a known ligand to a search for its receptor. The opportunity to analyze genomes allowed the vector of study to be altered to follow the reverse path – structural similarities with already identified receptors led to the discovery of an enormous number of sequences encoding receptors whose ligands were unknown. These receptors were termed orphans. They belonged to two

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main classes – nuclear receptors and receptors functionally coupled to heterotrimeric G proteins (G protein-coupled receptors, GPCR). Among the extensive GPCR superfamily, human genome sequencing identified 720 genes encoding such receptors [2], the ligands for a significant proportion being unknown. Orphan receptors of this type were termed GPCR (G-protein receptors) with the corresponding number.

Large-scale studies to deorphanize GPCR receptors showed that the ligands of four such receptors were free fatty acids – saturated and unsaturated, with different hydrocarbon chain lengths. According to the classification of the International Union of Pharmacology, these deorphanized receptors were termed FFA1-4 [3].* GPR49 (FFA1) and GPR120 (FFA4) receptors are activated by saturated and unsaturated free fatty acids with intermediate (C6–C12) and long (C > 12) fatty acid chains. Agonists of GPR412 (FFA2) and GPR43 (FFA3) receptors are very short fatty acids (C2–C4) formed during fermentation of dietary fibers by the intestinal microbiota. Further studies have demonstrated that a novel type of GPCR activator is involved in regulating a multitude of major physiological processes. The present review is unable to provide even a short consideration of advances in this area, so attention will be focused on the most important and interesting results relating to one of the best studied of the free fatty acid receptors – GPR40.

Deorphanization of the GPR40 Receptor. The nucleotide and amino acid sequences of this protein, allowing it to be assigned to the GPCR family, were first determined in 1997 [4]. PCR was used to amplify a segment of human genomic DNA at chromosomal location 19q13.1 using primers specific for parts of the receptor for the neuroendocrine peptide galanin which are conserved in humans and rats, and this led to identification of four genes encoding unknown GPCR (GPR40, GPR41, GPR42, and GPR43) [4]. GPR40 is a membrane protein containing 300 amino acid resides and has a molecular weight of 31.45 kDa. A comparative study performed some years later addressed the amino acid sequence of this receptor in humans and several mammal species and showed very high levels of conservation [5].

Three independent research groups almost simultaneously deorphanized GPR40 [5–7]. Using CHO cells expressing GPR40 and standard approaches for receptor deorphanization based on assessment of intracellular calcium levels, Iton et al. analyzed more than 1000 potential ligands [5]. At virtually the same time, similar studies were carried out by another group using GPR40 receptors expressed in HEK293 cells [6] and clones of HeLa cells with high-efficiency receptor gene introduction technology [7]. The results were in agreement – free fatty acids with intermediate and long hydrocarbon chains were selective ligands for these receptors and operated in the micromolar concentration range (IC_{50} 1–8 [5] or 4–6 μM [6]). Receptor affinity did not change with increases in chain length or saturatedness. The actions of fatty acids on changes in $[Ca^{2+}]_i$ were dose-dependent and mediated by GTP-binding protein $G\alpha_{q/11}$ and depended critically on the presence of a free carboxyl group at the terminus of the molecule, as fatty acid methyl esters were ineffective [5–7].

The Role of GPR40 Receptors in Glucose-Stimulated Insulin Production. The earliest reports on the deorphanization of GPR40 presented unexpected results from studies of the expression of the mRNA for this protein in different tissues. Rat studies showed that GPR40 mRNA was detected mainly in pancreatic islets of Langerhans, and in situ hybridization and immunohistochemical studies allowed the precise location of GPR40 to be identified as insulin-producing pancreatic β cells [5, 6, 8].

Studies using transformed mouse pancreatic cell line MIN6, which have all the properties of β cells, convincingly demonstrated the ability of these agonists to stimulate insulin production in the presence of high glucose concentrations [5], which allowed this mechanism to be defined as glucose-stimulated insulin secretion. Inhibition of GPR40 expression with siRNA or antisense oligonucleotides in MIN6 cells sharply decreased the effects of fatty acids on insulin production [5, 9, 10]. A significant reduction in the potentiating action of fatty acids on glucose-stimulated insulin secretion was seen in mice with knockout of GPR40 [11], while overexpression of GPR40 in mice on a high-fat diet prevented the development of hyperglycemia and improved insulin secretion [12]. Data from animal models were confirmed by results from analysis of receptor expression in human pancreatic β cells, the GPR40 mRNA expression level showing good correlation with the insulinemic index, a measure of β-cell function [8].

These data led to reconsideration of the action of fatty acids on insulin production. It had long been considered that the stimulating effect of fatty acids on insulin production was based exclusively on their intracellular oxidative catabolism, leading to an increase in the ATP/ADP ratio, opening of ATP-controlled K^+ channels, depolarization of plasma membranes, and opening of L-type calcium channels, leading to an increased intracellular calcium level, rearrangement of the cytoskeleton, translocation of secretory granules to the plasma membrane, and secretion of the insulin within them into the circulation (for review see [13]). Deorphanization of GPR40 showed that free fatty acids have a role in regulating insulin secretion not only as energy substrates, but also as signal molecules having their own receptors on the cell surface. Both mechanisms of action of fatty acids (as ligands and as metabolic substrates) are believed to operate independently and to make essentially equal contributions to potentiation of glucose-stimulated insulin secretion [14]. This follows from the fact that insulin secretion in mice with knockout of GPR40 was reduced by only 50% [15].

Mechanism of the Signal Action of Free Fatty Acids in the Regulation of Glucose-Stimulated Insulin Secretion Mediated by GPR40 Activation. The GPR40-mediated in-

* The current literature makes wide use of various different names for receptors of this type – GPR40, FFA1, or GPR40/FFA1.

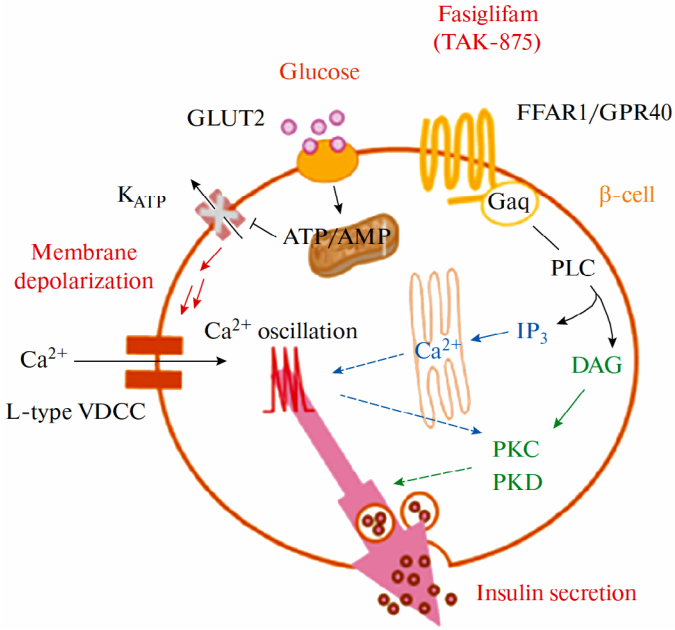

Fig. 1. Involvement of GPR40 receptors in regulating insulin secretion by pancreatic β cells on exposure to free fatty acids and TAG-875 (fasiglifam). GLUT2 – glucose transporter; VDCC – L-type voltage-dependent calcium channel; PKD – phospholipase D; PLC – phospholipase C; PKC – protein kinase C; DAG – diacylglycerol; IP_3 – inositol-1,4,5-triphosphate. From [18] with permission.

tracellular action of free fatty acids on glucose-stimulated insulin secretion by pancreatic β cells is linked with an increase in the cell calcium concentration due to mobilization from the endoplasmic reticulum and enhanced influx into cells through L-type voltage-gated calcium channels [16–18]. Thus, the final target for the effects of fatty acids acting as signal molecules or energy substrates are common and are linked with increased intracellular calcium ion concentrations induced by various mechanisms.

Glucose-stimulated insulin secretion in β cells is a biphasic process – the fast short phase is associated with release of ready granules in the immediate vicinity of the plasma membrane, while the second, longer-lasting, phase is associated with mobilization of insulin granules located far from the plasma membrane and their translocation to the subapical part, which requires reorganization of the cytoskeleton [19, 20]. Free fatty acids are believed to stimulate the second of these phases [14]. The GPR40-mediated action of free fatty acids involves activation of phosphoinositide-specific phospholipase Cβ by the $\alpha_{q/11}$ subunit of $G_{q/11}$ protein, leading to hydrolysis of phosphatidylinositol-4,5-diphosphate to form inositol-1,4,5-triphosphate (IP_3) and diacylglycerol [6, 9]. IP_3 enhances calcium release from the endoplasmic reticulum of β cells [21]. Administration of penetrating analogs of both IP_3 and diacylglycerol (oleoylacylglycerol) to MIN6 cells potentiates glucose-stimulated insulin secretion [18]. Inhibitors of phospholipase Cβ and phorbol-sensitive isoforms of protein kinase C significantly decreased the action of exogenous fatty acids on insulin secretion [16]. It was also shown that exposure of GPR40 to oleic acid led to phosphorylation of protein kinase D via a diacylglycerol-dependent mechanism, which, as demonstrated in [22], is required for activation of F-actin depolymerization, i.e., the cytoskeletal reorganization mediating translocation of insulin granules to the plasma membrane. Inhibition of protein kinase D activity or expression significantly decreased the GPR40-mediated actions of fatty acids on glucose-stimulated insulin secretion [22]. The basic scheme of the intracellular signal pathways for activation of insulin secretion in the GPR40-mediated actions of free fatty acids and the synthetic agonist fasiglifam (TAK-875) is shown in Fig. 1.

The increase in the intracellular calcium concentration on exposure to free fatty acids involves activation of IP_3 receptors on the endoplasmic reticulum membrane. Knockdown of the receptor in MIN6 cells or use of xestospongin C, an inhibitor of IP_3 receptors, in experiments on slices of islets of Langerhans led to a sharp decrease in the effect of fatty acids on glucose-stimulated insulin secretion [23]. However, it was also found that the potentiating actions of fatty acids on insulin secretion also involved store-operated Ca^{2+} entry (SOCE), which is initiated by depletion of the calcium reserve in endoplasmic reticulum structures. The regulator of SOCE is the protein stromal interaction molecule 1 (STIM1), which is located in the endoplasmic reticulum membrane and operates as a sensor for the calcium concentration within the reticulum due to having a specific Ca^{2+}-binding domain. Ca^{2+}-dependent translocation of STIM1 to the plasma membrane activates Orai1 calcium channels within the plasma membrane and supporting the

entry of external calcium [24]. Expression of STIM1 is seen in MIN6 cell and mouse pancreatic β cells and its translocation in these cells is activated by glucose or cAMP [25, 26]. Knockout of STIM1 or Orai1 in MIN6 cells was found to lead to a sharp decrease in the effect of a GPR40 agonist on glucose-stimulated insulin secretion [23]. The critical role of the STIM1-Orai1 mechanism in the GPR40-activated signal pathway has also been demonstrated in knockout mice in which β cells specially lack STIM1 expression [23].

Allosteric Modulators of GPR40 and Their Potential in the Treatment of Type 2 Diabetes Mellitus. The deorphanization of GPR40 by three independent groups and the discovery of the effects of fatty acids on glucose-stimulated insulin secretion became a focus of interest to the pharmaceuticals industry immediately after publication of the experimental data in 2003. The greatest success in seeking and developing pharmacological activators of GPR40 in the treatment of type 2 diabetes mellitus was achieved by the Japanese pharmaceutical company Takeda, whose drug (TAK-875, fasiglifam) has reached phase III clinical trials [27]. In contrast to many other drugs used in type 2 diabetes mellitus, fasiglifam and its analogs stimulate insulin production only when the blood glucose level is elevated, which minimizes the risk of developing hypoglycemia. However, in 2013 the TAK-875 program was stopped because hepatotoxicity was found in a larger patient population [28]; the cause of this is as yet unknown, as GPR40 is not expressed in the liver [5, 6]. Despite this failure, there are now many synthetic GPR40 receptor agonists made by various different pharmaceutical companies (Eli Lilly, Connexios Life Sciences, Japan Tobacco, Piramal, and others), and many are at the preclinical stage of studies in animal models of type 2 diabetes mellitus.

The direction of the search for the optimum structure for such synthetic ligands is largely due to the finding of allosteric modulation of GPR40 activity, which is typical of an enormous number of members of the GPCR family [29]. It was initially believed that fatty acids and synthetic GPR40 ligands interact with a single ligand-binding site in the receptor, consisting of a cluster of hydrophilic amino acid residues (Arg183, Asn244, and Arg258) in hydrophobic transmembrane regions 5, 6, and 7, respectively, with which both the fatty acid carboxyl group and synthetic ligands bind [30]. However, experiments with radioactively labeled ligands demonstrated the existence of different allosteric regulatory sites in GPR40 able to bind synthetic ligands [31]. Some ligands, such as TAK-875, are simultaneously orthosteric and allosteric regulators of GPR40, which apparently determines their high affinity for the receptor, which is 400 times the affinity of the endogenous ligand, oleic acid [27]. The crystal structure of the complex of GPR40 receptor and TAK-875 was determined [32]. As expected, the carboxyl group of the ligand interacted with the arginine residues in transmembrane regions 5 and 7. However, another part of the ligand was located along the lipid bilayer and penetrate into the cell between transmembrane regions 3 and 5. Thus, TAK-875 interacts with a noncanonical binding site through the lipid bilayer rather than in the aqueous phase [32]. The high lipophilicity of TAK-875 is believed to be linked to its hepatotoxicity, and it was for this reason that the phase III clinical trial of this drug was stopped. Thus, one direction in the search for novel insulinotropic GPR40 ligands is linked with the development of compounds with significantly greater polarity than TAK-875 without loss of its high receptor affinity [33].

Most synthetic GPR40 ligands are allosteric modulators whose use is based on the multitude of ligand-binding sites and their functional selectivity. Allosteric modulators show high levels of GPR40 signal plasticity and have advantages over orthosteric agonists in that they can specifically alter or stabilize conformational changes in the receptor, including those induced by orthosteric agonists, to support coupling with various G proteins or even activation of G protein-independent signaling by coupling with regulatory β-arrestin proteins, which may be of critical importance for switching the direction of signaling or its intensity [14]. This phenomenon was termed "ligand-directed signaling." Thus, the insulinotropic effect of TAK-875 is mediated both via $G_{q/11}$ proteins and by recruitment of types 1 and 2 β-arrestins. Knockout of type 2 β-arrestin with siRNA in rat INS832 insulinoma cells weakened the insulinotropic effect of the agonist [34]. GPR40 agonists were synthesized (AM-1638 and AM-5262) which, in the same cells, activate G_s and $G_{q/11}$ proteins, inducing increases in cAMP and IP_3, respectively, and having cooperative insulinotropic effects, though fatty acids, i.e., endogenous GPR40 ligands, act only via $G_{q/11}$ proteins [35]. The construction of allosteric agonists provides for specific actions on those signal pathways required for the therapeutic effect avoiding activation of those which might lead to undesirable side effects. Particularly successful in in vivo experiments in diabetic rat models is the use of a combination of allosteric agonists with different receptor binding sites but cooperative insulinotropic effects [31]. Another promising approach is the construction of "bitopical" ligands [36]. These compounds have two pharmacophores connected together – one binds the orthosteric ligand-binding pocket of GPR40 and the other simultaneously interacts with allosteric sites in the receptor molecule. It is interesting to note that cooperativity is not seen only on use of a combination of synthetic ligands. The insulinotropic effect of TAK-875 in a diabetic rat model decreased in the presence of a lipolysis inhibitor, which led to a decrease in the plasma fatty acid level [37]. This is evidence for the view that synthetic agonists, at least TAK-875, do not displace fatty acids from the receptor but act cooperatively with the endogenous ligands.

The Role of GPR40 in Incretin Secretion. The direct action of fatty acids on pancreatic cells was found not to involve a single mechanism of their role in glucose-stimulated insulin secretion. GPR40 receptors are expressed in intestinal L, K, and I cells which secrete glucagon-like

peptide 1, glucose-dependent insulinotropic peptide, and cholecystokinin, which are involved in controlling insulin secretion [38, 39]. As entry of food into the gastrointestinal tract is a powerful stimulus for insulin secretion, enteroendocrine cells express a whole spectrum of receptors, which are activated by triglyceride lipolysis products. Apart from GPR40, enteroendocrine cells also express free fatty acid receptors GPR120, GPR41, GPR43, and GPR119, whose endogenous ligand is monoacylglycerol [40]. The role of GPR40 in incretin secretion is significantly less well understood than its role in pancreatic β cells, as different types of receptors operate cooperatively [40]. However, GPR40-knockout mice have been shown to have low incretin levels [38, 39, 41]. Not all synthetic GPR40 agonists increasing insulin secretion in β cells, such as TAK-875 and AMG837, are able to influence incretin secretion [42]. It would seem that only full agonists have this effect. It can be suggested that this may be linked on the one hand with significantly lower levels of receptor expression in enteroendocrine cells and on the other with activation of different signal cascades stimulated by full agonists [43]. Thus, agonists which are effective in relation to incretin secretion (AM1638 and AM5262) activate both $G\alpha_{q/11}$- and $G\alpha_s$-mediated signal pathways, while ineffective agonists activate only both $G\alpha_{q/11}$-mediated signal pathways.

GPR40 Expression and Its Functional Role in Brain Structures. Brain lipids occupy a special place in science. Brain tissue in all vertebrates differs from other tissues in having very high lipid contents, lower only than fatty tissues, and in having long-chain polyene fatty acids – docosahexaenoic and arachidonic acids, the total of which in the human brain accounts for about half of all fatty acids. As the brain has a very limited ability to synthesize polyunsaturated fatty acids, high levels of these compounds in this tissue are attained by constant delivery from the plasma. Thus, there is no surprise in the fact that the brain is characterized by an extremely high level of expression of GPR40 receptors. The earliest studies on the deorphanization of this receptor established that of all organs studied, two stood out in terms of the GPR40 expression level – the pancreas and the brain [6]. Subsequent studies showed that GPR40 was located in neurons in a variety of brain structures – the hypothalamus, hippocampus, hypophysis, cortex, caudate nucleus, substantia nigra, and medulla oblongata [6, 44, 45]. Application of the two main experimental approaches in in vivo models – use of selective agonists/antagonists and GPR40 knockout mice – convincingly demonstrated that these receptors mediate many important effects of fatty acids in CNS structures. In contrast to pancreatic β cells, the actions of free fatty acids on GPR40 receptors in neurons is linked with changes in the expression of genes mediating activation of Akt kinase and ERK1/2 kinase and the phosphorylation of cAMP-activated transcription factor CREB [46–48].

Neurons control the body's energy balance, monitoring plasma fatty acid concentrations. One of the most important mechanisms processing information on metabolic status is associated with intracellular fatty acid metabolism in neuronal cells, along with β oxidation, ATP production, and activation of ATP-regulated potassium channels. Changes in fatty acid metabolism in the arcuate nucleus of the hypothalamus (inhibition of fatty acid transporters, carnitine-palmitoyl-transferase I, acyl-CoA synthase) induced by pharmacological agents lead to changes in feeding behavior, body weight, and insulin secretion [49, 50]. Intracerebroventricular administration of oleic acid but not short-chain fatty acids decreased food consumption and hepatic glucose production [51]. A major role in monitoring free fatty acid levels in plasma is played by neurons in the arcuate nucleus of the hypothalamus, which produces neuropeptide Y and proopiomelanocortin (POMC) [52]. These neurons show the highest levels of GPR40 receptor expression and it has been convincingly demonstrated that the effects of fatty acids on neuron activity and the control of energy status are mediated not only by their intracellular metabolic products, but also by surface receptors [53, 54]. The hypothalamus also expresses GPR120 receptors, which are similar to GPR40 in terms of the chemical structure of ligands, though GPR120 are located mainly on microglial cells [54]. The specific localization of these two types of fatty acid receptors in the hypothalamus has also been seen in cell cultures – GPR40 but not GPR120 is expressed in CLU189 hypothalamus neuronal cells, while cultures of BV2 microglial cells express only GPR120 [54].

Differences in the locations of GPR40 and GPR120 in hypothalamic cells are probably associated with their specific functional roles. High-fat diets, promoting obesity and type 2 diabetes mellitus, are known to activate inflammatory processes in many body tissues, including the hypothalamus [55] and hippocampus [56]. Inflammatory reactions develop particularly sharply in response to excess consumption of saturated fatty acids [57], such as palmitic 16:0, stearic 18:0, and arachidonic 20:0. In the hypothalamus, activation of proinflammatory genes is seen within a few hours of consumption of high-fat foods [58], while longer-lasting high-fat diets lead to endoplasmic reticulum stress [59], mitochondrial damage, and apoptosis of neuronal cells [60]. The inflammatory effects of saturated fatty acids have been shown to be linked with activation of TLR4-mediated signal pathways which involve the "classical" proinflammatory kinases and transcription factors [61, 62]. In contrast to saturated fatty acids, polyunsaturated fatty acids have powerful neuroprotective actions which have been demonstrated in many in vivo and in vitro studies. Supplementation of the diet of obese mice with polyunsaturated fatty acids or direct administration of these compounds into the hypothalamus decreased the course of inflammatory processes and improved metabolic indicators [63]. Both types of free fatty acid receptor (GPR40 and GPR120) are believed to have roles in anti-inflammatory effects in the hypothalamus, though only GPR40 agonists increased proopiomelanocortin expression

and decreased body weight and calorie intake [54]. This is evidence that monitoring of the plasma free fatty acid level and regulation of energy status by NPY- and POMC-ergic neurons is mediated at least partly by GPR40 receptors.

Metabolic impairments have close etiological links with cognitive disorders and mental diseases. Many statistics have provided evidence that obesity and metabolic syndrome, due, among other factors, to the spread of the "western diet," with its high content of saturated fatty acids and low content of ω3 fatty acids, contribute to degradation of cognitive functions and the development of dementia [64, 65]. Although the mechanisms of this relationship remain unclear, GPR40-mediated signaling attracts ever more attention among neuropharmacologists and GPR40 receptors are regarded as a target for the treatment of cognitive disorders.

Thus, studies using mouse models of type 2 diabetes mellitus (high-fat diet and *db*/*db* mice) have demonstrated that these animals, as compared with healthy animals, have reduced GPR40 expression in the hippocampus and decreased cognitive capacities [48]. Brain-derived neurotrophic factor (BDNF) is an important linking element in these pathological processes, and is also one of the most important growth factors in the CNS; it takes part in the CREB-dependent mechanism of memory formation and has a role in controlling energy balance [66, 67]. BDNF expression is significantly decreased in diabetic mice [48]. The negative actions of a high-calorie diet on spatial learning in mice have been shown to be linked with decreases in BDNF expression in the hippocampus [68]. Inhibition of BDNF expression in the hypothalamus induces hyperphagy and obesity in mice, providing evidence that BDNF also acts as an anorexigenic signal molecule [69]. Various approaches using primary cultures of cortical neurons have shown that docosahexaenoic acid increases BDNF expression via GPR40 receptors, activating ERK1/2 and MAPK-mediated signal cascades. Addition of docosahexaenoic acid or GW9508, a synthetic GPR40 agonist, to the diet of diabetic mice led to normalization of the level of BDNF expression and improvement in cognitive functions, while intracerebroventricular administration of the GPR40 antagonist GW1100 to diabetic mice weakened the useful effects of the fatty acid on improvements in cognitive functions and BDNF expression in the hippocampus [48]. Intracerebral administration of the GPR40 agonist GW9508 in mouse models of Alzheimer's disease improved memory and the animals' learning ability. Receptor activation led to phosphorylation of transcription factor CREB, with significant increases in the production of various neurotrophic factors in hippocampal neurons, and stimulated neurogenesis [47].

Polyunsaturated fatty acids, particularly ω3 fatty acids, play a major role in supporting neurogenesis and the functioning of nerve cells at all stages of ontogeny, in the processes of memory formation, learning, and mental behavior. A deficit of polyunsaturated fatty acids in the body leads to serious impairments to CNS operation, causing neurodegeneration and mental disorders [70–72]. Although the mechanism of this remains incompletely understood, there are data on the involvement of GPR40 in controlling a wide circle of mental processes. Thus, female mice reared on a balanced fat diet but with GPR40 knockout showed impairments to emotional status, social behavior, locomotor activity, and care for offspring [73].

The GPR40-dependent signal pathway activated by docosahexaenoic acid has been shown to play an important role in neurogenesis and neurodifferentiation in neurogenic niches, maintaining the regenerative potential of the brain [44]. In cultures of rat neural stem cells, docosahexaenoic acid, acting via GPR40 receptors, stimulated neuron differentiation and axon growth. The effect of the fatty acid in this cell type is mediated by the classical mechanism of this type of receptor – activation of phospholipase Cβ and mobilization of intracellular calcium [74].

Data have been reported showing that GPR40 receptors play an important role in the modulation of nociception. Docosahexaenoic acid and the synthetic GPR40 agonist GW9508 have antinociceptive effects: they inhibit the transmission of pain stimuli, increasing β-endorphin production in the arcuate nucleus of the hypothalamus and activating the opioid system [45]. Studies in mice with GPR40 knockout showed that dysfunction of this receptor system led to the development of chronic pain [75].

The Role of GPR40 in Lipotoxicity. Many results have clearly indicated that increases in free fatty acid levels induced by excessive fatty tissue are, if not the main, then at least one of the more important causes of insulin resistance. Most patients with obesity, metabolic syndrome, and type 2 diabetes mellitus have elevated free fatty acid levels, leading to insulin resistance in many tissues. Prolonged incubation of pancreatic β cells with free fatty acids leads to loss of β-cell viability and decreases in glucose-stimulated insulin production [76, 77]. This same effect was seen in vivo on prolonged maintenance of a high plasma fatty acid level [78, 79]. The decrease in glucose-stimulated insulin production by pancreatic cells seen in this situation is linked with the intracellular effects of fatty acids, for example, an imbalance in the Randle cycle [76], production of reactive oxygen species [80], and endoplasmic reticulum stress [81].

Although the toxic effects of chronic exposure to high fatty acid concentrations have been convincingly demonstrated, the question of the involvement of GPR40 in metabolic impairments induced by high plasma fatty acid contents is far from a definitive answer and the experimental data are contradictory. Some data indicate that GPR40 receptors mediate the lipotoxic effects of fatty acids. Thus, β cells harvested from mice with GPR40 knockout were not subject to the adverse actions of fatty acids on long-term exposure, while increases in receptor expression in these cells led to damage and reductions in insulin release [82]. Prolonged incubation of MIN6β cells with palmitic acid induced endoplasmic reticulum stress and apoptosis, though

pharmacological inactivation of GPR40 receptors protected cells from the adverse effects of the fatty acid [81].

However, other data indicate that the toxic effects of free fatty acids are not mediated by GPR40 receptors. A high-fat diet induced damage to pancreatic β cells and inhibited glucose-stimulated insulin production to identical extents in wild-type mice and mice with GPR40 knockout [83]. In addition, the fact of large differences in the toxic effects of fatty acids with different structures is contradicted by their similar receptor affinities [80].

It is important that the toxic action is typical only of saturated fatty acids with chain lengths of C > 14. Unsaturated fatty acids, regardless of chain length and the number of double bonds, are not only nontoxic for cells, but also protect cells from the harmful actions of saturated fatty acids [80, 84]. Nonetheless, some of their derivatives may have toxic effects. Fatty acids in the trans configuration are high-affinity GPR40 ligands and have damaging actions on cells, as does conjugated linoleic acid [77, 85, 86]. Some proportion of trans isomers can enter the body as part of the diet, though they can also be formed endogenously, for example, on exposure to the reactive nitrogen radicals forming in many pathologies. Studies using mouse models of ischemia have shown that trans-arachidonic acid induces degeneration of cerebral microvessels, this effect being mediated by GPR40 and being absent in in mice with knockout of this receptor [86].

Conclusions. A great diversity of lipid mediators acting via auto/paracrine mechanisms though activation of GPCR has now been identified. These include numerous derivatives of arachidonic and various other polyunsaturated fatty acids – prostaglandins, thromboxanes, and leukotrienes – discovered long before many others. Products of phospholipid metabolism – endocannabinoids, various types of lysophospholipids, plasmalogens, monoacylglycerol, and sphingosine-1-phosphate – are GPCR agonists. The formation of each mediator depends on a sequence of network of fine regulatory metabolic reactions whose occurrence and, thus, the quantity of mediator, depends on the activity or level of expression of the components of the corresponding enzyme systems.

Free fatty acids as signal molecules have fundamental properties distinguishing them from other lipid mediators. These include stable high concentrations of nonesterified fatty acids in the plasma and interstitial fluid. Their delivery into the blood, where they immediately bind with albumins, is mediated by relatively simple reactions – lipolysis of triglycerides obtained from dietary fats or intrinsic fat reserves. However, this external simplicity hides questions remaining far from being answered and showing that the problem is more complex than it seems at first sight. The standard view is that the process of receptor activation by an agonist is preceded by the appearance of the agonist or an increase in its condition. The EC_{50} value for agonists of free fatty acid receptors are in the micromolar concentration range. How might receptor activity and signal transmission be regulated in conditions of stable high agonist concentrations, which can be several μM in normal conditions? The authors of one of the first studies on the deorphanization of GPR40 showed that the receptor is activated by fatty acids not bound with serum albumins, as in the presence of BSA at conditions of 0.01% of more, fatty acids completely lost the ability to activate the receptor and stimulate insulin production [5]. However, the plasma non-albumin-bound fatty acid concentration is in the nanomolar range [87] and is too low to activate the receptor; furthermore, in vitro experiments have shown that the receptor is activated by complexes of fatty acids with albumin. Is it not possible to suggest that in this "ligand-receptor" pair, the state of the receptor itself and its accessibility/agonist sensitivity might be subject to regulation?

However, despite these unanswered questions, convincing data provide evidence that free fatty acids represent a separate class of lipid mediators acting on GPCR activating different signal transduction systems and having a wide range of regulatory effects. GPR40 and other free fatty acid receptors are involved in the pathogenesis of the commonest impairments to human health in the modern world – metabolic syndrome, obesity, type 2 diabetes mellitus, neurodegenerative pathologies, and cognitive and mental disorders. This fact produces continuing interest in studies of fatty acid receptors both from the point of view of basic science and on the part of the pharmaceutical industry.

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