Current Topics

Physiology and Pharmacology of the Gut Nutrient Perception

Free Fatty Acid Receptors and Drug Discovery

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Utilizing the human genome database, the recently developed G-protein-coupled receptor (GPCR) deorphanizing strategy successfully identified multiple receptors of free fatty acids (FFAs) and is proposed to play a critical role in a variety of physiologic homeostasis mechanisms. GPR40 and GPR120 are activated by medium- and long-chain FFAs, whereas GPR41 and GPR43 are activated by short-chain FFAs. GPR40, which is preferentially expressed in pancreatic β -cells, mediates insulin secretion. On the other hand, GPR120, which is abundantly expressed in the intestine, functions as a receptor for unsaturated long-chain FFAs and promotes the secretion of glucagon-like peptide-1 (GLP-1). In this review, we summarize the identification, structure, and pharmacology of the receptors and speculate on the respective physiologic roles that FFA receptor family members may play.

Key words G protein coupled receptor; free fatty acid; insulin; G protein receptor 40; G protein receptor 120

1. INTRODUCTION

Free fatty acids (FFAs) are not only essential nutritional components but contribute to many cellular functions. By coordinating the expression of proteins involved in lipid uptake, synthesis, transport, storage, degradation, and elimination, nuclear receptors, including peroxisome proliferator-activated receptors (PPARs), work as FFA “sensors” that maintain homeostasis under physiological and pathophysiological conditions.³⁾ However, not all biological effects reported can be explained by these mechanisms. Some of the mediator effects appear to be PPAR independent and are instead characteristics of cell surface receptor involvement.^{4,5)} Recently, the G-protein-coupled receptor (GPCR) deorphanizing strategy⁶⁾ has successfully identified multiple receptors for FFAs, which function on the cell surface and play significant roles in nutrition regulation (Table 1). G-protein receptor (GPR)41 and GPR43 are activated by short-chain FFAs, whereas GPR40 and GPR120 can be activated by medium- and long-chain FFAs. This review gives an update on recent advances in our understanding of recently deorphanized FFA receptors and their physiologic functions.

2. GPR41, GPR42, AND GPR43

Genomic Structure During a search for novel galanin receptor subtypes, a cluster of four GPCR genes, GPR40, GPR41, GPR42, and GPR43, were identified as tandemly encoded genes present on human chromosome 19q13.^{3–7)} GPR40, GPR41, and GPR43 genes, but not the GPR42 gene, are present on the murine and bovine chromosome. The members of this subfamily share *ca.* 30–40% identities with each other, except that human GPR42 (hGPR42) differs from human GPR41 (hGPR41) only at six amino acid positions. Four of the six amino acid positions that differ between hGPR41 and hGPR42 are conserved among the rodent and bovine orthologues. The orthologues match hGPR41 at two or three of these positions, but match hGPR42 at only one, suggesting that hGPR42 occurred as the result of a gene duplication of hGPR41 that happened after the human lineage diverged from the rodent and bovine lineages.⁸⁾

Ligands To identify the ligands for GPR41 and GPR43, several ligand-fishing strategies were performed using heterologous expression systems in yeast, mammalian, and *Xenopus* cells. GPR41 and GPR43 are activated by short-chain FFAs, such as formate, acetate, propionate, butyrate, and pentanotate.^{8–10)} The two receptors, however, differ in their specificity for ligands with varying carbon chain

Table 1

Nomenclature	GPR41	GPR43	GPR40	GPR120	GPR42 Pseudo gene
Antagonist	—	—	—	—	—
Agonist (FFA)	Short chain, C3>C4>>C2	Short chain, C3~C4~C2	Medium-long	Medium-long	No activation
(Other)			Thiazolidimiedione		
G protein coupling	Gi/o	Gq/11, Gi/o	Gq/11	Gq/11	—
Gene/chromosomal	GPR41	GPR43	GPR40	GPR120	GPR42
Localization	19q13.1	19q13.1	19q13.1	10q23.33	19q13.1
Protein (human)	NP_005295, 346 a.a	NP_005297, 330 a.a	NP_005294, 300 a.a	NP_859529, 377 a.a	NP_005296, 346 a.a
Expression	Adipose tissue		Pancreatic β -cell	Colon	
Physiological role	Leptin production		Insulin secretion	GLP-1 secretion	

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lengths. GPR41 is equally activated by propionate, butyrate, and pentanotate, whereas GPR43 prefers propionate over other short-chain FFAs.^{8,9} These two receptors are coupled to inositol 1,4,5-trisphosphate formation, intracellular $[Ca^{2+}]$ mobilization, activation of extracellular signal-regulated kinase 1/2, and inhibition of intracellular cAMP accumulation.⁹ However, they exhibit differential coupling to G-proteins; GPR41 couples exclusively through the pertussis toxin-sensitive Gi/o family, whereas GPR43 couples through the Gi/o and pertussis toxin-insensitive Gq families.⁹ GPR42 is not activated by short-chain fatty acids, even though it shares 98% identity with GPR41.⁸ Mutational analysis of GPR41 in yeast identified Arg174 of hGPR41 (Trp in GPR42) in the second extracellular loop as essential for ligand activation, presumably responsible for forming a salt bridge with the carboxylate ligand. Thus GPR42 appears to have acquired loss-of-function mutations after gene duplication. RT-PCR analysis using GPR42-specific primers detected no signal for GPR42 mRNA in RNA samples from normal human tissues.⁸ Because the GPR42 gene is reportedly present in only a subset of individuals as a polymorphic insert,⁷ GPR42 may be a pseudogene in the human genome.

Physiological Function GPR41 is abundantly expressed in adipose tissue⁸ and has been implicated in short-chain fatty acid-stimulated leptin production by adipocytes.¹¹ The mouse adipocyte cell line Ob-Luc produces leptin upon stimulation with short-chain fatty acids. Leptin production by short-chain fatty acids: increased by exogenous overexpression of GPR41 and abolished by knockdown of GPR41 expression with siRNA.¹¹ Acute oral administration of propionate increases circulating leptin levels in mice, suggesting that the effects of propionates are mediated *via* GPR41 *in vivo*. Since leptin is known to be a potent anorexigenic hormone that suppresses food intake *via* receptors in the central nervous system,¹² propionate may likely be associated with an inhibitory effect on food intake by causing increased leptin release. Recently, Hong *et al.*¹³ and Ge *et al.*¹⁴ have reported that GPR43, but not GPR41, is expressed in adipose tissue and functions in adipogenesis. Further studies are needed to resolve the differences in these results.

GPR43 mRNA is predominantly expressed in immune cells, particularly in polymorphonuclear (PMN) cells.^{8–10} Short-chain FFAs have been described as displaying activities on leukocyte populations, particularly PMN cells.^{15–17} Stimulation of PMN cells by propionate or acetate results in a transient increase in intracellular calcium.⁹ Short-chain FFAs also evoke the chemotactic response in PMN cells,⁹ suggesting that GPR43 might be involved in the neutrophil activation induced by short-chain FFAs. GPR43 is also reported to be induced during the differentiation of leukocyte progenitor cells to monocytes or neutrophils and is found mainly in hematopoietic tissues, suggesting that GPR43 could have an important function in the differentiation and/or activation of leukocytes.¹⁸ Karaki *et al.* reported that GPR43 is expressed in peptide YY (PYY)-containing enteroendocrine cells and 5HT-containing mucosal mast cells,¹⁹ which is consistent with physiologic data showing that short-chain FFAs stimulate the release of PYY²⁰ and serotonin²¹ from the ileum and colon. Recently, Ge *et al.*¹⁴ have reported that adipocytes treated with acetate and propionate exhibit a reduction in lipolytic activity. This inhibition of lipolysis is

the result of GPR43 activation as this effect is abolished in adipocytes isolated from GPR43-knockout animals. Activation of GPR43 by acetate results in the reduction in the plasma FFA level.¹⁴ This result is consistent with the previous *in vitro* experiment showing that activation of GPR43 results in the reduction of lipolysis and activation of adipogenesis. These results suggest a potential role for GPR43 in regulating plasma lipid profiles and perhaps aspects of metabolic syndrome.¹³

3. GPR40

Ligands While GPR41 and GPR43 are activated by short-chain FFAs, GPR40 is activated by medium- and long-chain saturated and unsaturated FFAs, as reported by three independent groups almost simultaneously.^{22–24} A variety of fatty acids were found to act as agonists to GPR40 in the micromolar concentration range with eicosatrienoic acid representing the most potent analogue.²² Interestingly, the potency of the saturated fatty acids was dependent on chain length, with pentadecanoic acid (C15) and palmitic acid (C16) being the most potent, whereas carbon chain length or degree of saturation did not appear to correlate with potency among unsaturated fatty acids.²² Briscoe *et al.*²² tested about 40 different saturated and unsaturated fatty acids, which all displayed agonistic activity.

Signal Transduction In CHO cells, exogenously expressed GPR40 is coupled to inositol 1,4,5-trisphosphate formation, intracellular $[Ca^{2+}]$ mobilization, and activation of extracellular signal-regulated kinase 1/2. Feng *et al.* showed that linolenic acid reduces the voltage-gated K^+ current in rat pancreatic β -cells through GPR40-mediated cAMP levels and protein kinase A activity, leading to enhanced β -cell excitability and insulin secretion.²⁵

Expression Expression analysis of GPR40 using RT-PCR, immunohistochemistry, and *in situ* hybridization revealed high expression in insulin-producing pancreatic islets.²³ GPR40 was found to be enriched 2- to 100-fold in pancreatic islets as compared with the whole pancreas.²² We identified GPR40 in splenocytes, THP-1 cells, and human peripheral blood mononuclear cells using anti-GPR40 monoclonal antibody.²⁶ GPR40 knockout mouse analysis showed that insulin secretion in response to intralipid was reduced by approximately 50%, suggesting that GPR40 is probably expressed in the intestinal tract.²⁷

Physiological Function FFAs are known to have pleiotropic effects on pancreatic β -cells. Although acute administration of FFAs stimulates insulin release, chronic exposure to high levels of FFAs results in the impairment of β -cell function and secretory capacity. Steneberg *et al.* showed that GPR40 mediates both acute and chronic effects of FFAs using GPR40 knockout and transgenic mice.²⁸ FFAs are recognized to play an important role in both maintaining basal insulin secretion and potentiating glucose-stimulated insulin secretion in the fasting state in rodent and human islets^{22,29–33}; however, the mechanism of action is not clearly understood. Itoh *et al.* revealed that long-chain fatty acids amplify glucose-stimulated insulin secretion from pancreatic β -cells by activating GPR40.²³ When the expression of GPR40 was inhibited by small interfering (si)RNA, the increase in insulin secretion after fatty acid stimulation was

Fig. 1. Schematic Diagram of the Internalization Based Ligand-Screening System

eliminated, clearly confirming the involvement of GPR40 in this process. GPR40-deficient β -cells secrete less insulin in response to FFAs, and the loss of GPR40 protects mice from obesity-induced hyperinsulinemia, hepatic steatosis, hypertriglyceridemia, increased hepatic glucose output, hyperglycemia, and glucose intolerance. Conversely, overexpression of GPR40 in β -cells of mice leads to impaired β -cell function, hyperinsulinemia, and diabetes. These results suggest that GPR40 plays an important role in the chain of events linking obesity and type 2 diabetes.

Polymorphism For human GPR40, two nucleotide substitutions, an Arg211His polymorphism and a rare Asp175Asn mutation of GPR40, were identified. Both variants showed EC_{50} values similar to the wild type. Maximum efficacy of Asp175Asn was 39% lower compared with the wild type. At present, these variants do not appear to be associated with type 2 diabetes or insulin release alterations.³⁴⁾

Synthetic Ligand Because of its biological activity and tissue distribution, GPR40 is an attractive drug target for type 2 diabetes, and its development has been investigated. A GPR40 agonist, GW9508, that activates both GPR40 and GPR120 and stimulates glucose-stimulated insulin secretion (GSIS) in insulin-secreting MIN6 cells (but not in isolated islets) and a selective GPR40 antagonist, GW1100, that reverses the effects of GW9508, were described.³⁵⁾

Antidiabetic thiazolidinediones, troglitazone and rosiglitazone, and the experimental anti-obesity compound MED-ICA16 also activate GPR40.²⁴⁾

4. GPR120

Ligands GPR120 is an orphan GPCR, which we isolated from mouse and human genomic DNA fragments. Using the receptor internalization assay (Fig. 1),³⁶⁾ we identified endogenous ligands for GPR120 as medium- to long-chain FFAs. Apparent stimulatory activities were detected for saturated FFAs with chain length of C14 to C18, and for unsaturated FFAs with chain length of C16 to C22. Despite similar-

Fig. 2. Phylogenetic Tree Analysis of Free Fatty Acid Receptors and Typical G-Protein Coupled Receptors (Rhodopsin-Type Receptor)

The primary amino acid sequences from various organisms were obtained from the Genbank database and analyzed using the CLUSTAL program. An unrooted dendrogram is shown. Note that phylogenetic analysis suggests a distant evolutionary relationship between the GPR120 and GPR40 families.

ity in the ligand specificity, GPR120 shared only 10% amino acid identity with human GPR40, which was far away on evolutionary tree, as shown in Fig. 2. This might be a result of convergent evolution.

Signal Transduction We could not detect either a stimulatory or an inhibitory effect of long-chain FFAs on cAMP production in HEK 293 cells transiently expressing mouse

Fig. 3. Free Fatty Acid Receptor Actions in Nutritional Regulation

Stimulation of fatty acid receptors by the long-chain fatty acid (LCFA) or short-chain fatty acid (SCFA) controls metabolism through the secretion or production of peptide hormones.

and human GPR120 cDNAs. Recent analysis of the anti apoptotic effects through GPR120 activation showed that GPR120 coupled through pertussis toxin-insensitive Gq families. This observation does not necessarily correspond to the analysis of the signal transmission concerning GLP-1 secretion, and further studies are needed.³⁷⁾

Physiological Function in the Intestine GPR120 is highly expressed in the human and mouse intestinal tract and mouse enteroendocrine STC-1 cells. Previously, Sidhu *et al.* showed that mouse enteroendocrine STC-1 cells secrete GLP-1 and cholecystokinin (CCK) upon challenge with FFA.³⁸⁾ The FFA-mediated GLP-1, CCK secretion, and $[Ca^{2+}]_i$ response can be inhibited by transfection with the RNAi expression vector specific for GPR120 but not for GPR40.³⁹⁾ It was also noted that oral administration of FFA and direct administration to the colon³⁷⁾ increased circulating GLP-1 and insulin levels in mice, and it is tempting to speculate that the FFA effects are mediated *via* GPR120 *in vivo*. As GPR120 and GPR40 are activated by similar properties of FFAs, and GPR40 directly and GPR120 indirectly promote glucose-stimulated insulin secretion, both GPR120 and GPR40 will be important for assessing the mechanism of FFA-mediated insulin secretion and for the treatment of diabetes.

Physiological Function in Other Tissues The expression of GPR120 was confirmed in other tissues, and it is suggested that GPR120 plays a physiological role in these tissues. GPR120 is also expressed in a number of tissues including adipocytes, taste buds, and lungs, however, the functional consequences of GPR120 activation in these tissues is not clear.

Gotoh *et al.* showed that GPR120 is also highly expressed in human and mouse adipose tissues. The expression of GPR120 mRNA was higher in adipocytes compared with stromal-vascular cells. The level of GPR120 mRNA increased during adipocyte differentiation in 3T3-L1 cells. Moreover, the use of siRNA to downregulate GPR120 expression resulted in inhibition of adipocyte differentiation.⁴⁰⁾

These results indicate that GPR120 has an important role in adipogenesis by functioning as a factor that facilitates maturation of adipogenesis *in vitro*. However, the precise molecular function of GPR120 in adipocytes is unclear. Further studies of the relationship between obesity and GPR120 should be of considerable interest.

Matsumura *et al.* detected the mRNA and immunoreactivity of GPR120 in rat taste buds. This result suggests that GPR120 is expressed in the taste cells of the circumvallate papillae to sense dietary fat, like the receptor expressed in the enteroendocrine cells.⁴¹⁾

5. CONCLUSION

Newly deorphanized receptors for FFAs belong to the nutrient-sensing GPCRs⁴²⁾ that directory monitor the level of nutrients in the extracellular environment and feed back through the secretion or production of peptide hormones. Some of the nutrients sensing GPCRs have lower binding affinity (in the micromolar or millimolar range) for their nutrient ligands compared with the classical high-affinity ligands such as hormones or growth factors.⁴³⁾ Because of the lower binding affinity (in the micromolar range) for FFAs and nonspecific binding for other fatty acid-binding proteins, it is difficult to show direct evidence for the binding of FFAs to FFA receptors. Further studies are needed to probe the direct interaction between FFA receptors and their ligands. Further deorphanization studies are needed to determine whether other GPCRs for FFAs might still be included in the receptors considered to be orphan GPCRs.

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