The G-protein-coupled receptor 40 family (GPR40–GPR43) and its role in nutrient sensing

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Abstract
Recent deorphanization efforts have paired the G-protein-coupled receptors GPR40, GPR41 and GPR43 with fatty acids as endogenous ligands. While carboxylic acids have been historically known to serve as fuel sources and biomarkers of disease, these studies demonstrate that fatty acids can act as signalling molecules at the cell-surface level. This receptor subfamily shares approx. 30% identity among members, with some limited cross-over between ligand activities. Generalized expression patterns within the pancreatic β-cell, adipose depots and the gastrointestinal tract infer involvement in energy source recognition, absorption, storage and/or metabolism. GPR40, activated by medium and long-chain fatty acids, has been shown to potentiate insulin secretion at the β-cell, and has been hypothesized to participate in the detrimental effects of chronic fatty acid exposure on β-cell function. GPR41 and GPR43 have been reported to stimulate leptin release and adipogenesis respectively via activation by short-chain fatty acids. These common themes implicate GPR40, GPR41 and GPR43 in playing significant roles in metabolic diseases, such as diabetes, obesity and the metabolic syndrome.

Introduction
Fatty acids serve vital functions as sources of energy, building materials for cellular structures, modulators of physiological responses, and in some cases, biomarkers for disease. SCFAs (short-chain fatty acids) are generated during fermentation of resistant starches and indigestible fibre by anaerobic gut flora, while longer-chain fatty acids are products of dietary intake, adipose recycling and hepatic turnover of neutral fats, cholesteryl esters and phospholipids [1,2]. Recent reports have identified fatty acids as cognate ligands for a small family of G-protein-coupled receptors originally cloned by Sawzdargo et al. [3], thus providing evidence that these receptors function as nutrient sensors [3–9]. Within these reports, fatty acid carbon chain length has been shown to confer activity and specificity, with SCFAs (defined by six or fewer carbon molecules) activating GPR41 and GPR43, and both saturated and unsaturated medium to LCFAs (long-chain fatty acids) activating GPR40 [4–9]. It is important to note that concentrations of SCFAs and LCFAs found to confer activity and specificity, with SCFAs (defined by six or fewer carbon molecules) activating GPR41 and GPR43, and both saturated and unsaturated medium to LCFAs (long-chain fatty acids) activating GPR40 [4–9]. It is important to note that concentrations of SCFAs and LCFAs found to activate these receptors in vitro are in line with those that are physiologically present in the normal animal. Activation of a fourth fatty acid receptor, GPR120, which bears little homology to the GPR40 family, stimulates in vivo and in vitro secretion of the insulinotropic incretin GLP-1 (glucagon-like peptide 1) [10]. This receptor will not be reviewed in depth here. Interestingly, GPR120 shares common agonists with GPR40 in both unsaturated fatty acids and proprietary small molecules [11].

Within the last decade, fatty acids have gained significant attention in the arenas of energy intake and glucose disposal, with obvious implications for the treatment of insulin-resistant diabetes mellitus and obesity. For example, dietary administration of the SCFA propionate (C3) to sheep and chickens has been reported to decrease food intake [12,13]. C3 dosed orally in obese hyperinsulinaemic fa/fa rats on a high cholesterol diet increased satiated fat diet lowered urinary glucose excretion and fasting blood glucose levels, and both oral and rectally infused C3 reduced total hepatic cholesterol [14]. In a recent publication reporting that C3 decreased glucose-stimulated insulin secretion in incubated and perfused rat pancreatic islets, Ximenes et al. [15] hypothesized that this effect in vivo may underlie the reduction of adverse consequences of hyperglycaemia in diabetic patients observed following intake of dietary fibres. However, in this particular study, the authors linked the effect of propionate to a reduction of pyruvate dehydrogenase activity rather than investigating a connection with GPR41 or GPR43 [15].

LCFAs have been shown to play an important role in β-cell function through their regulation of basal insulin secretion and ‘priming’ effects leading to potentiation of insulin secretion after a fast [16–19]. There may be multiple mechanisms underlying the acute effects of fatty acids on the β-cell, and while it has been suggested that intracellular metabolism of the LCFAs is central to the effect, there are also reports suggesting that non-esterified (‘free’) fatty acids play a role

Key words: diabetes, fatty acid, G-protein-coupled receptor, GPR40 family, nutrient sensing, obesity.

Abbreviations used: S-HT, 5-hydroxytryptamine; LCEA, long-chain fatty acid; PYY, peptide YY; RT, reverse transcriptase; SEFA, short-chain fatty acid; siRNA, short interfering RNA.

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[20–24]. Although recent reports suggest that LCFAIs generated as a result of increased lipolysis in the β-cell may function in part as the β-cell compensation for insulin resistance, continual exposure to non-esterified fatty acids is detrimental to β-cell function and survival [25,26]. The demonstrated actions and tissue distributions of the GPR40 receptor family relate to a host of metabolic processes, including insulin action and signalling, adipogenesis and lipolysis, as well as gastrointestinal absorption and motility. Matching these findings with the relatively high ‘drugability’ of G-protein-coupled receptors as compared with many other molecular target classes may provide insight into the treatment of diabetes, obesity and the metabolic syndrome.

GPR40
The relatively specific expression of GPR40 in pancreatic islets across species and its coupling with Gq/11, resulting in elevation of intracellular calcium and stimulation of protein kinase C activity, suggested that the receptor may play a role in the ability of LCFAIs to stimulate insulin secretion [4,6,7]. The initial confirmation of the hypothesis came from studies by Itoh et al. [6], where GPR40 siRNA (short interfering RNA) resulted in reduction of fatty acid-stimulated insulin secretion by Itoh et al. [6], where GPR40 siRNA (short interfering RNA) resulted in reduction of fatty acid-stimulated insulin secretion by Itoh et al. [6]. The recent deorphanization and other publications around GPR41 have elevated its level of interest over the last few years, mainly because of its potential as a therapeutic target. SCFAs have been demonstrated to activate GPR41 in a variety of recombinant cellular systems, including yeast-based reporter gene assays, melanosphere pigment translocation assays, and also in transiently transfected mammalian HEK-293 (human embryonic kidney) and CHO-K1 (Chinese-hamster ovary K1) cell assays [5,8,31]. GPR41 mainly initiates its signalling through coupling with the Gi/Go family of G-proteins, evidenced by the abolition of signal response when transfected cells are treated with pertussis toxin [5,8]. Propionic acid (C3) is the most potent and efficacious ligand for GPR41. Rank order potencies and dose-dependent responses of SCFAs have remained generally consistent between different assay formats and investigators, with propionate, butyrate (C4) and pentanoate (C5) being the most potent agonists and acetate and hexanoate exhibiting measurably lower potencies [5,8,9,31].

In the early published Taqman RT (reverse transcriptase)–PCR human tissue distribution studies, adipose tissue demonstrated the greatest level of mRNA expression across a panel of human tissues, including bone, visceral organs, brain and peripheral blood mononuclear cells [5]. Since then, other laboratories have also reported GPR41 expression in human fat samples, utilizing mRNA detection and immunohistochemistry techniques [5,8,31].

Other studies have revealed the presence of GPR41 mRNA in differentiated adipogenic mouse cell lines (3T3-L1, 3T3-F442A and Ob-Luc) in addition to ex vivo white adipose tissues, while showing little to no expression in brown fat or undifferentiated cells [5,31].

Even though the expression of GPR41 in adipose tissues has been known for some time, its physiological relevance is yet to be understood. The results of Xiong et al. [31] suggested that activation of GPR41 in adipocytes caused leptin production in adipocytes. In this study, using the mouse adipogenic Ob-Luc cell line (one allele of the leptin gene knocked in with a luciferase reporter cassette), SCFAs evoked increases in luciferase activity that correlated well with reported rank order potencies of C2–C6. Further, Xiong et al. [31] were able to significantly knock down propionic acid–induced stimulatory responses in cells overexpressing GPR41 via infection with GPR41 siRNA. Additional experiments indicated that secreted leptin levels increased in primary mouse adipose tissue cultures when treated with C3, C4 and C5, and mice orally gavaged with propionate exhibited increased circulating leptin levels at 8 h post dosing [31]. In contrast with these findings, Hong et al. [32] reported that GPR41 mRNA was undetectable in four separate depots of isolated murine adipocytes or differentiated 3T3-L1 cells, using the same
probes as Xiong et al. [31]. This publication further indicated that GPR41 expression was not detected in human adipose tissues, cultured pre-adipocytes or adipocytes. Although the reasons for these discrepancies are not yet understood, most of the work around GPR41 implies that this receptor does play a role in monitoring energy storage and circulation, perhaps linking GPR41 to obesity. In a recent patent application, Arena Pharmaceuticals published the expression of GPR41 in pancreatic islet cells and its up-regulation in db/db diabetic mice [33]. Therefore GPR41 modulators may have potential in treating diseases of glucose regulation, in particular, Type 2 (non-insulin-dependent) diabetes mellitus, and states of glucose intolerance. GPR41 has also been shown to be present in plasma cells, dendritic cells, lymphocytes and peripheral blood mononuclear cells, as well as artery and arteriolar endothelial cells [5,8].

A fourth family member, GPR42, appears likely to be a non-functional recent gene duplication of GPR41, which exhibits congruency with GPR41 in all but six amino acid positions [3,5,34]. Native GPR42 does not respond to SCFAs; however, mutagenesis to change particular residues to match GPR41 was able to confer SCFA-induced activity. Taqman RT–PCR detected no signal for GPR42 in normal human tissues [5].

**GPR43**

Functional activation of GPR43 by SCFAs has been characterized using a range of the assays described above for GPR41 [5,8]. However, this receptor has shown interesting differences from GPR41 in intracellular signalling, ligand selectivity and tissue localization. Studies by Brown et al. [5] and Le Poul et al. [8] indicate that GPR43 is mainly coupled with G8-proteins, with some evidence for its possible coupling with G12/13 pathways as well. Pharmacologically, C2 and C3 are the most potent activators of GPR43, with lower activity demonstrated by pentanoate (C5) [5,8]. This distinction allows for functional discrimination between GPR41 and GPR43, as C2 is not as potent as C3, C4 or C5 against GPR41.

GPR43 has been shown to be present in a variety of tissues, particularly evident in fat stores, inflammatory cells and the gastrointestinal tract. Interestingly, Hong et al. [32] concluded that C2 and C3 stimulate adipogenesis through GPR43. In this work, GPR43 mRNA expression levels in murine adipose tissues were at higher levels in four separate adipose depots (perirenal, subcutaneous, mesenteric and parametrial) than in any other tissues tested. In vitro, GPR43 mRNA was not found in undifferentiated 3T3-L1 adipocytes, but levels rose over time post-differentiation. Treatment with C2 or C3 increased both GPR43 mRNA expression and Oil Red O staining, indicating increases in lipid accumulation in these cells; staining was reduced when cells were transfected with GPR43 siRNA. Further, treatment with C2 and C3 reduced isoprenaline-induced lipolysis in a dose-dependent fashion. In vivo, GPR43 mRNA was up-regulated in adipose tissues of mice fed a high-fat chow compared with those on normal chow. In a second patent application, Arena Pharmaceuticals indicated expression of GPR43 within 3T3-L1 differentiated adipocytes and mouse adipose, pancreas and large intestine [35]. This patent application also reported that GPR43 was up-regulated in db/db diabetic and ob/ob obese mouse pancreatic islets. In conclusion, modulators of the GPR43 receptor might have therapeutic use in the treatment of numerous metabolic disorders, including diabetes mellitus and obesity.

As SCFAs are at highest concentrations in the distal gastrointestinal tract, it is reasonable that receptors might reside in this location, for recognition of passing ingesta content and indirect monitoring of bacterial flora populations by their metabolic by-products [36]. Using RT–PCR and immunohistochemistry, Karaki et al. [36] detected GPR43 in rat ileal and colonic tissue sublayers, and reported that the receptor co-localizes with PYY (peptide YY) in mucosal epithelium and mast cells, as well as with 5-HT (5-hydroxytryptamine)-containing mast cells in the distal colon. This group argued that SCFA and GPR43 may control gastrointestinal motility and secretion via a 5-HT and/or PYY-mediated effects. GPR43 has also exhibited robust expression patterns in other types of immune cells including, but not limited to polymorphonuclear cells, monocytes, eosinophils and B-lymphocytes [5,8,9]. Since SCFAs have been shown to have effects on the immune response, it is very likely that this receptor (and/or GPR41) may play a role in agonizing or blunting various functions of the leukon such as recognition, recruitment and migration [8].

**Conclusions**

Although the importance of fatty acids in the regulation of metabolism has been appreciated for some time, much research remains to elucidate their functions as they relate to stimulation of cell-surface receptors. The GPR40 family, consisting of GPR40, GPR41 and GPR43, has been identified as a group of G-protein-coupled receptors, with fatty acids now identified as their endogenous ligands. While research activity around LCFA-activated GPR40 is more mature than SCFA-activated GPR41 and GPR43 at this time, relatively little is still known of the exact physiological mechanisms by which any of these receptors work, and in which tissues they exert their respective actions. That being said, GPR40’s presence in pancreatic β-cells and its role in the priming effects of fatty acids on insulin secretion are relatively well characterized. One important issue to overcome is the separation of effects in vivo between GPR41 and GPR43, as they share considerable overlap in their expression pattern and common ligands. The roles of GPR41 and GPR43 on lipid accumulation and disposal have not yet been differentiated, and it is possible that interrelationships exist between these two receptors (for example GPR41 and GPR43 heterodimerization). Further, the presence of GPR41 and GPR43, especially GPR43, in a number of immune cells begs examination, either for therapeutic indications or as potential liabilities for target modulation. It is known that SCFAs activate
a G-protein-coupled receptor on immune cells and change primary immune cell physiology. Taking this into account along with the relatively greater concentrations of GPR43 in immune cells compared with adipose tissue, higher hurdles may exist for the therapeutic use of GPR43 modulators. For the family as a whole, the development of null mouse models and potent target-specific small molecules in-target. For the family as a whole, the development of null mouse models and potent target-specific small molecules incapable of being used as energy sources will ultimately define the participation of these receptors in metabolic and immune system function.

References


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