Biological roles and therapeutic potential of G protein-coupled receptors for free fatty acids and metabolic intermediates

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Abstract Several G protein-coupled receptors that transmit signals in response to dietary free fatty acids (FFAs) as well as endogenous metabolites, such as lactate and 3-hydroxybutyrate, have been discovered. These receptors are shown to sense levels of energy substrates or metabolic states of the body, and regulate metabolism and endocrine functions of various organs to maintain energy homeostasis. The receptors for FFAs and hydroxy carboxylic acids (HCAs) appear to be involved in several metabolic disorders including obesity, diabetes and atherosclerosis. In this review, we summarize the functions of FFA and HCA receptors in physiology and pathophysiology, and discuss their implications for the treatment of metabolic diseases.

Keywords: G protein-coupled receptor, free fatty acid, ketone body, obesity, diabetes

Introduction

G protein-coupled receptors (GPCRs) constitute one of the largest gene families so far identified and are important regulators of several cellular functions and physiological actions1). They have initially been described as a family of receptors activated by hormones and neurotransmitters, and the structural mutation or unsuitable expression of the receptors are related to numerous diseases. GPCRs are reported to account for about 20% of the clinical drug targets thus far2) and significant efforts have been made to develop novel therapeutics.

Obesity is a growing health problem throughout the world. Compared with people of normal weight, those who are obese may have increased risk for many diseases, including diabetes, high blood pressure, cardiovascular diseases, stroke, and several types of cancers3-5). Since obesity results from a chronic imbalance between energy intake and energy expenditure, it is commonly understood that excess adipose tissue predisposes toward the development of metabolic disorders. On the other hand, lipodystrophy, a medical condition characterized by the loss of adipose tissue, causes similar metabolic consequences as obesity, i.e. insulin resistance, hyperglycemia, and dyslipidemia. Thus, adipose tissue, as a storage place for lipids and adipocyte-derived secretory substances, are important components in the maintenance of a healthy energy balance. Fatty acids (FAs) are nutritional components and metabolic intermediates that contribute to a wide range of cellular functions. FAs are also known to produce a variety of both beneficial and detrimental effects on metabolic and inflammatory processes. Previously, FAs were believed to produce their physiological actions through intracellular targets such as peroxisome proliferator-activated receptors (PPARs) or fatty acid-binding proteins (FABPs)6,7). Recently, several GPCRs activated by long-chain FAs have been identified8-10). In addition, there are receptors activated by short-chain FAs, such as acetate, propionate, and butyrate, that are produced by microbial anaerobic fermentation of dietary fibers in the gut11,12), as well as intermediates of lipid metabolism13-18). This review focused on the functions of these receptors in physiology and diseases, and discusses their potential uses as targets for prevention and therapeutic treatments.

Free fatty acid (FFA) receptors

The genes encoding FFA1, also known as GPR40, and family members FFA3 (GPR41) and FFA2 (GPR43) were isolated in 1997 as a group of tandemly clustered genes downstream of the CD22 gene residing on human chromosome 19q13.119). FFA1 was shown to be activated by a wide range of saturated, mono- and polyunsaturated medium- and long-chain FAs8,9,20) (Fig. 1). The EC50 values for activation of the receptor by different fatty acids are estimated to be in the μM range, which is comparable with plasma FFA concentrations under various physiological states. FFA2 and FFA3 are the primary mediators of SCFA signaling. Although these two receptors are closely related, with 43% amino acid identity21), differences in ligand potency and selectivity are observed between human and mouse FFA2 and FFA3 orthologs (Fig. 1). The EC50 values for activation of the receptor by different fatty acids are estimated to be in the μM range, which is comparable with plasma FFA concentrations under various physiological states. FFA2 and FFA3 are the primary mediators of SCFA signaling. Although these two receptors are closely related, with 43% amino acid identity21), differences in ligand potency and selectivity are observed between human and mouse FFA2 and FFA3 orthologs (Fig. 1). Human FFA2 can bind to acetate (C2), propionate (C3), and butyrate (C4) with EC50 values approximately 10^7 M, whereas human FFA3 preferentially binds to propionate and butyrate with relatively higher affinities than
acetate\textsuperscript{11,12,22}. In mice, however, acetate can activate both FFA2 and FFA3, and propionate shows a clear selectivity for FFA3 compared to FFA2\textsuperscript{23}. These substantial differences among species in the relative affinity of FFA2 and FFA3 for SCFAs may have resulted from specific adaptations to differences in diet as well as metabolism. FFA4, also referred to as GPR120, was first identified as a new orphan GPCR belonging to the family of rhodopsin GPCRs by searching the human genome databases\textsuperscript{24}. It was eventually shown to be notably expressed in the human gastrointestinal tract, as well as in adipocytes and macrophages, where it recognizes long-chained FFAs including omega-3 FAs\textsuperscript{10,25} (Fig. 1). The gene encoding FFA4 is located on human chromosome 10q23, and consists of four coding exons\textsuperscript{26}. FFA4 is recognized to mediate beneficial metabolic effects, in particular glucose homeostasis, lipid metabolism and inflammatory responses.

1. **FFA1 and insulin secretion**

FFA1 is primarily expressed in pancreatic β-cells\textsuperscript{8,9)}, and also in enteroendocrine cells\textsuperscript{27-30}, immune cells\textsuperscript{31)}, osteoclasts\textsuperscript{32}, taste buds\textsuperscript{33}, osteocyte\textsuperscript{34,35} and the central nervous system\textsuperscript{8,36-38}. FAs are known to have complex and divergent effects on β-cell function. Under physiological conditions, FFAs potentiate insulin secretion from pancreatic β-cells in the presence of glucose\textsuperscript{39,40}. On the other hand, prolonged exposure to elevated FFA levels results in secretory defects and cell death, which is a phenomenon termed as “lipotoxicity”\textsuperscript{41}. FFA1 has been proposed to be involved in the regulation of FA-potentiation of glucose-stimulated insulin secretion (GSIS) from β-cells\textsuperscript{9}. siRNA or oligonucleotide-mediated reduction of FFA1 causes impaired FFA potentiation of insulin secretion\textsuperscript{42,43}, and disruption of the FFA1 gene in mice reduces fatty acid augmentation of insulin secretion \textit{in vivo}\textsuperscript{44,45}. In contrast, transgenic overexpression of FFA1 and administration of FFA1 agonists augment insulin secretion\textsuperscript{46-48}. These data suggest that FFA1 activation is a valuable therapeutic strategy in type 2 diabetes, however, the potential contribution of FFA1 to the deleterious effects of FAs on β-cell function still remains controversial\textsuperscript{49,50}. FFA1 is expressed in endocrine cells of the gastrointestinal tract.

![Fig. 1 Structures of endogenous ligands for the FFA and HCA receptors.](image-url)
and mediates FFA-stimulated glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) secretion\textsuperscript{27,28}, suggesting that FFA1 modulates FFA-stimulated insulin secretion from β-cells not only directly, but also indirectly via regulation of incretin secretion. This is supported by the finding that a novel FFA1 agonist including AM-1638 and AM-6226 stimulates GLP-1 and GIP secretion from intestinal enteroendocrine cells\textsuperscript{51} (Fig. 2). Several studies have been conducted to understand the signaling mechanisms linking FFA activation of FFA1 to insulin secretion. FFA1 predominantly couples to the G protein subunit Gαq/11\textsuperscript{11,52}. Activation of Gαq/11 promotes hydrolysis of membrane phospholipids by phospholipase C and the production of diacylglycerol (DAG) and inositol triphosphate (IP\textsubscript{3}). DAG and IP\textsubscript{3} subsequently serve as second messengers to activate protein kinase D\textsuperscript{53} and trigger cytosolic influx of extracellular Ca\textsuperscript{2+}\textsuperscript{54-56}, respectively. Coupling of FFA1 to Gαi or Gαs is also demonstrated by using its synthetic agonists such as TUG-424 and AM-5262\textsuperscript{57,58} (Fig. 2). Although the potential therapeutic applicability of FFA1 in type 2 diabetes has been validated via several clinical studies, the discontinuation of TAK-875 in the phase III clinical trial brings about a risk of adverse side effects. More detailed understanding of the FFA1 receptor in biology and pharmacology is required to design new therapeutic strategies.

There is a substantial volume of literature exploring the link between dietary lipids and bone density. Bone is a dynamic organ that is constantly being restructured in a process called remodeling, which requires the coordinated action of different types of bone cells such as osteocytes, osteoclasts, and osteoblasts\textsuperscript{59}. Recently Wauquier et al. demonstrated that FFA1 knockout mice exhibit osteoporotic phenotype\textsuperscript{35}. It is also reported that FFA1 mediates an inhibitory effect on osteoclastogenesis\textsuperscript{52,53,55}, and the knockdown of FFA1 by RNA interference protects from thiazolidinedione (TZD)-induced osteocyte apoptosis\textsuperscript{34}, indicating a beneficial role of FFA1 in preventing osteoporosis. TZDs are well known as synthetic compounds used in the treatment of type 2 diabetes, and the insulin-sensitizing effect is mediated by the nuclear receptor PPAR-γ. Interestingly, osteoporosis and type 2 diabetes responses via neuronal network and peripheral hormones. There is strong evidence for the expression of FFA3, but not FFA2 in sympathetic ganglion as well as enteric neurons\textsuperscript{70}. FFA2, but not FFA3, is expressed in several immune cells including neutrophils, monocytes, and intestinal regulatory T cells\textsuperscript{11,12,22,71}. Several lines of evidence suggest that FFA2 plays an important role in chronic inflammatory diseases; however, whether the receptor exert pro- or anti-inflammatory effects remains unanswered\textsuperscript{71,76}. Maslowski et al. demonstrated that FFA2 deficient mice showed exacerbated inflammation in models of colitis, arthritis and asthma\textsuperscript{71}. In contrast, Sine et al. reported that FFA2 KO mice showed protection against inflammatory tissue destruction in a chronic colitis model\textsuperscript{70}. Further studies are required to validate the therapeutic potential of FFA2 in inflammatory diseases.

The nervous system is a master regulator that detects metabolic transition to coordinate tissue-specific responses via neuronal network and peripheral hormones. Ketone bodies are produced by the liver during fasting and prolonged exercise, and used peripherally as an energy source when glucose is not readily available. In addition, 3-hydroxybutyrate possesses a variety of signaling functions that regulate broad cellular responses\textsuperscript{70}. It antagonizes FFA3 signaling and thereby inhibits sympathetic activity\textsuperscript{77}; whereas, an electrophysiological study later described that 3-hydroxybutyrate acts as an agonist of FFA3\textsuperscript{70}. The physiological consequences of 3-hydroxybutyrate actions via FFA3 to regulate energy homeostasis and metabolic adaptation remain to be demonstrated that SCFAs exert beneficial effects on host energy metabolism as signaling molecules via FFA2 and FFA3\textsuperscript{65,66}.
explored.

Although FFA2 has been found to be expressed in adipocytes of white adipose tissue\(^8\), whether or not FFA3 is expressed in adipocytes is still debatable. Early studies demonstrated that FFA3 is expressed in mouse and human white adipose tissues\(^11,22,81\), but other groups have provided inconsistent results\(^7,77,80,82\). A growing body of evidence suggests that FFA2 promotes adipogenesis\(^80,83\) and reduces lipolysis in adipocytes\(^80,83\). As compared with wild type mice, a decreased amount of lipid droplets in brown adipose tissue and lower macrophage content in epididymal white adipose tissue from high fat diet (HFD)-fed FFA2 KO mice were observed\(^85\), suggesting that antagonists of FFA2 may provide a new strategy for treating type 2 diabetes. More work is needed to understand the molecular mechanisms of the SCFA/FFA2 signaling system for controlling energy expenditure and metabolic inflammation.

Co-expression of two functional SCFA receptors is also observed in pancreatic islets\(^68\). They have been shown to be coupled to Gαi/o, and activation of the receptors leads to inhibition of intracellular cAMP accumulation, which is necessary for GSIS. Therefore, FFA2 and FFA3 are supposed to reduce insulin secretion from pancreatic \(\beta\)-cells, which is consistent with the previous \textit{in vitro} experiment showing that propionate induces a significant decrease in insulin secretion\(^89\). As is the case with FFA4, studies on the metabolic function of FFA2 and FFA3, with the genetic knockout mice, have provided controversial results\(^6,69,85,90-92\). This may be attributed to the overlap of gene expression and/or ligand specificity between FFA2 and FFA3. It is also possible that their functional redundancy compensates for the lack of one receptor. To solve the problem, Tang et al. generated mice with double knockout lacking both FFA2 and FFA3\(^88\). HFD-fed mice with a combined deficiency of the receptors showed increased insulin secretion and improved insulin tolerance. HFD-fed mice with pancreatic \(\beta\) cell-specific double knockout displayed the same phenotype, but intestinal cell-specific deletion of FFA2 and FFA3 had no effect. They proposed that acetate has an autocrine role in the pancreas, and elevated acetate acts on both FFA2 and FFA3 under diabetic conditions. Interestingly, a recent study showed that different FFA2 agonists could either accelerate or inhibit GSIS\(^93\). This is probably because both G protein subunits can be simultaneously activated by FFA2, and G\(\alpha_q/11\)- and G\(\alpha_{i/o}\)-dependent pathways in \(\beta\)-cells seem to exert opposite effects. The use of specific agonists/antagonists may help unravel the physiological functions of the receptors and their involvement in various metabolic disorders\(^94,95\) (Fig. 2).

**Fig. 2** Structures and potencies (pEC50/pIC50) of synthetic agonists/antagonists for the FFA and HCA receptors. AM-1638\(^51\), AM-5262\(^175\), AM-6226\(^171\), TUG-424\(^176\), TAK-875\(^177\), GW9508\(^170\), TUG-891\(^179\), GLPG0974\(^178\), Acifran\(^122\), MK-0354\(^180\), MK-1903\(^145\), Monomethylfumarate\(^136\), SCH900271\(^181\).
3. FFA4, GLP-1 secretion and inflammation

The human FFA4 gene, consisting of 4 coding exons, is subject to alternative splicing, which is different from the rodent gene. The two splice variants with or without 16 amino acid insertion have different signaling properties. The short isoform is coupled to Gαq/11 as well as the β-arrestin pathway, whereas the long isoform loses its ability for G protein coupling99. Activation of the short variant of the FFA4 receptor, but not the longer one, raises the intracellular Ca\textsuperscript{2+} levels. This is probably due to the fact that the insertion is located in the third intracellular loop of the receptor which is a critical cytoplasmic element interacting with specific effector molecules97,98. Several Ser and Thr residues in the third intracellular loop as well as the C-terminal tail of the receptor were identified as being phosphorylated. Although phosphorylation at these sites is involved in specific receptor functions99,100, further studies on the impact of receptor phosphorylation in its physiological responses are needed.

A biological action of FFA4 was first identified in the enteroendocrine L cells, in which its activation by FFAs causes the secretion of GLP-110). GLP-1 is an incretin hormone that enhances insulin secretion and promotes β-cell survival and proliferation. FFA4 expressed by other enteroendocrine cells mediate the FA-induced release of CCK from I cells and GIP from K cells101,102. Involvement of FFA4 in the postprandial ghrelin suppression is also reported103,104. These hormones play pivotal roles in intestinal response to nutrients and energy homeostasis.

Unlike FFA1, whether insulin secretion is directly regulated by FFA4 in pancreatic islets remains unclear. Tanaka et al. reported that the long-term administration of α-linolenic acid promoted pancreatic β-cell proliferation and insulin secretion105. On the other hand, Stone et al. demonstrated that FFA4 was specifically expressed in pancreatic δ-cells106. Another report using FFA4 KO mouse demonstrated that FFA4 is important for FA-potentiated glucagon release from α-cells but not insulin release from β-cells107. The effect of FFA4 agonist on GSIS in isolate islets is likely due to FFA1 activation or indirect effect from inhibition of somatostatin secretion108. Further studies are needed to clarify the overall role of FFA4 in pancreas.

Regulation of glucose and lipid homeostasis is a key function of adipocytes. FFA4 regulates not only adipocyte differentiation but also glucose metabolism in adipocytes. The expression level of FFA4 was increased according to the progress of adipogenesis, and knockdown of the receptor by the RNA interference inhibited differentiation of 3T3-L1 preadipocyte109. The activation of FFA4 led to an increase in glucose transporter 4 (GLUT4) translocation to the plasma membrane and glucose uptake in adipocyte via a Gαq/11-dependent pathway25. This stimulatory effect was blocked by siRNA-mediated knockdown of FFA4109. Hudson et al. showed the involvement of FFA4 in glucose metabolism in adipocytes by using the specific agonist TUG-891110 (Fig. 2).

There is increasing evidence showing that chronic inflammation is highly relevant to insulin resistance and is one of the primary causes of diabetes111. Adipose tissue macrophages play a pivotal role in the regulation of the obesity-induced inflammation112,113. FFA4 is highly expressed in adipose tissue macrophages and its expression is increased by a high-fat diet. Activation of FFA4 by omega-3 FAs blocks proinflammatory alteration induced by TNFα or lipopolysaccharide stimulation25. The anti-inflammatory effects of FFA4 in macrophages are mediated by the β-arrestin-2/TAB1 interaction that disrupts the TAB1/TAK complex and inhibits the downstream NFκB and JNK activation25. Interestingly, the G protein coupling is dispensable for the anti-inflammatory effects, suggesting that the biased downstream signaling of the receptor is linked to distinct physiological responses.

In 2014, Yore et al. reported the discovery of a novel class of lipid mediators, i.e. branched fatty acid esters of hydroxy fatty acids (FAHFAs)114 (Fig. 1). FAHFAs are endogenous FFA4 ligands released by adipocytes, and its levels are reduced with diet-induced obesity and insulin resistance. It is also demonstrated that FFA4 activation induced by FAHFAs leads to enhanced insulin-stimulated GLUT4 translocation and glucose uptake in adipocytes, and suppression of proinflammatory cytokine release from adipose tissue macrophages. Although the precise pathways responsible for the synthesis of FAHFAs remain to be determined, restoring the reduced FAHFAs levels may have therapeutic effects to ameliorate insulin resistance and type 2 diabetes.

The gene knockout technique is a valuable tool to examine the role of a gene in physiological processes. FFA4 receptor is a multifunctional protein that improves many aspects of metabolic homeostasis. However, studies in FFA4-deficient mice have provided inconsistent and incomprehensible results with respect to body weight, plasma glucose and insulin levels, glucose tolerance, and insulin resistance25,109,115,116. It is conceivable that the different effects of FFA4 deficiency on body weight gain and other metabolic phenotypes result from several variable factors such as the experimental protocols, the genetic background of mice or dietary fat composition. The development of selective FFA4 agonists or antagonists will provide valuable insight into the integrated physiological function of FFA4, excluding compensatory mechanisms by FFA1 (Fig. 2).

**Hydroxy carboxylic acid receptors as nutrient sensors**

The hydroxy carboxylic acid receptors (HCA1-3) are a family of GPCRs that are activated by endogenous metabolic intermediates117. In 1993, the HCA3 receptor was cloned from a human monocyte cDNA library screened with degenerate oligonucleotide primers derived from human leukocyte chemoattractant receptors118. The HCA1
1. HCA1 and lactate

Lactate is produced from glucose and glycogen through glycolysis, and serves as an energy source to many tissues in the body. In 2008, lactate was identified as activating the HCA1 receptor, leading to an anti-lipolytic effect in adipocytes\(^{16,17}\). The EC\(_{50}\) value for lactate is approximately 5 mM, which is within the normal physiologic levels of lactate, suggesting that lactate is an endogenous ligand for HCA1. Ahmed et al. demonstrated that insulin-induced inhibition of lipolysis and an insulin-induced decrease in adipocyte cAMP levels were strongly reduced in mice lacking HCA1\(^{125}\). They proposed that lactate, a glycolytic metabolite, is involved in the anti-lipolytic effect of insulin as a novel link between carbohydrate and lipid metabolism in adipose tissue. Other than adipocytes, expression of HCA1 has been reported at lower levels in various organs including the liver, kidney and skeletal muscle\(^{17,122,126}\). Exercise studies with wild type and HCA1-deficient mice showed no differences in FFA or glycerol levels between the two groups, suggesting that HCA1 is unlikely to have a significant impact on the regulation of lipolysis during intensive exercise\(^{125}\). The physiological function of HCA1 in skeletal muscle during exercise remains to be determined. Recently, HCA1 has been demonstrated to be present in the brain\(^{119,127,128}\). The
potential involvement of the receptor regulating neuronal activity has been suggested\(^{20}\).

The Warburg effect is a phenomenon in which cancer cells exhibit enhanced glucose uptake and prefer aerobic glycolysis to oxidative phosphorylation, even under normoxic conditions, which results in increased lactate production\(^{16}\). Lactate contributes to acidosis, signals for angiogenesis, acts as a cancer cell metabolic fuel, and induces immunosuppression\(^{31}\). Roland et al. reported that HCA1 is highly expressed in pancreatic cancer cells and plays a critical role in sensing extracellular lactate\(^{32}\). Xenografted cancer cells in which HCA1 was silenced showed a significant decrease in tumor growth and metastasis in vivo. The expression of HCA1, as well as HCA3, is also increased in human breast cancer patient tissue\(^{33}\). Thus HCA1 has attracted more attention as a potential drug target for cancer treatment.

2. HCA2, nicotinic acid, and ketone body

HCA2 is expressed in adipose tissue as well as various immune cells including macrophages, neutrophils, dendritic cells, and epidermal Langerhans cells\(^{13,120-122,134-136}\). HCA2 is also present in retinal pigment and colonic epithelial cells, and keratinocytes\(^{12,16-138}\). Its natural ligand 3-hydroxybutyrate is produced in the liver, through \(\beta\)-oxidation of FA, and utilized as an alternative energy source for the brain as well as other tissues during prolonged fasting. It reaches millimolar concentrations, which are sufficient to activate HCA2 to inhibit adipocyte lipolysis. Since the rate of hepatic ketogenesis is partially limited by the rate of adipocyte lipolysis, the negative feedback of 3-hydroxybutyrate via HCA2 promotes efficient utilization of stored energy and prevents the development of ketoacidosis. Activation of HCA2 on adipocytes results in \(\mathrm{G}_\alpha\)-mediated inhibition of adenyl cyclase activity and a subsequent decrease in production of \(\mathrm{cAMP}\)^{3,121}. In adipocytes, \(\mathrm{cAMP}/\mathrm{PKA}\) signaling plays a critical role in modulating the activity of lipolytic enzymes including hormone-sensitive lipase. Thus the activation of HCA2 causes a rapid reduction in lipolysis and reduced release of FFAs from adipocytes. This is consistent with the finding that the anti-lipolytic effect of 3-hydroxybutyrate observed in adipocytes is abolished in adipocytes isolated from HCA2-deficient mice\(^{15}\).

Although nicotinic acid has been used for decades to treat dyslipidemia and prevent atherosclerosis\(^{39,140}\), molecular identification of specific receptors for nicotinic acid is quite recent. Nicotinic acid activates both HCA2 and HCA3, with EC50 values of 0.1 \(\mu\)M and 100 \(\mu\)M, respectively\(^{15,121}\). It has been known to have profound effects on blood lipoproteins by decreasing very low-density lipoproteins (VLDL) and low-density lipoproteins (VLDL), and increasing high-density lipoproteins (HDL), resulting in cardiovascular risk reduction\(^{41,142}\). Several synthetic agonists of HCA2 (MK-0354, MK-1903 and SCH900271) failed to produce an altered lipid profile similar to nicotinic acid, suggesting that HCA2-mediated anti-lipolytic effects may not be related to the beneficial anti-dyslipidemic effects of nicotinic acid\(^{43-45}\). Studies in HCA2 deficient mice provide evidence that the anti-atherosclerotic action of nicotinic acid is lipoprotein-independent and involves anti-inflammatory mechanisms in immune cells expressing HCA2\(^{146,147}\).

The therapeutic value of nicotinic acid is limited by a cutaneous vasodilation called flushing, which is mediated by HCA2 expressed in epidermal Langerhans cells and keratinocytes\(^{13,146,149}\), as mice lacking this receptor no longer respond to nicotinic acid with flushing\(^{134}\). The mechanism of the cutaneous flushing by nicotinic acid is involved in the activation of cyclooxygenase and subsequent formation of prostaglandin D2 and E2\(^{138}\). Walters et al. reported the adverse side effect of nicotinic acid, caused by phosphorylation of ERK and subsequent activation of phospholipase A2, which was mediated by \(\beta\)-arrestin 1, whereas its beneficial anti-lipolytic effect was mediated by G-proteins\(^{150}\). Consistent with this finding, a partial agonist of HCA2 having anti-lipolytic, but no vasodilatory activity fails to activate ERK phosphorylation\(^{43,151}\). The separation of downstream effectors mediating therapeutic responses from those inducing side effects may deliver a new generation of useful medicines.

The ketogenic diet, which contains high fat and low carbohydrate, is known to induce the hepatic production of ketone bodies. Ketone bodies can cross through the blood-brain barrier and act as an alternative energy source for the brain. The ketogenic diet has been used for almost a hundred years to treat refractory childhood epilepsy. In recent years, the ketogenic diet has not only treated epilepsy, but also shown neuroprotection for many neurological disorders including Alzheimer’s disease\(^{152-154}\), Parkinson’s disease (PD)\(^{155,156}\), stroke\(^{157,158}\), and amyotrophic lateral sclerosis\(^{159}\). PD is a chronic neurodegenerative disorder characterized by progressive loss of dopaminergic neurons in the substantia nigra\(^{160}\). The most common treatment for PD is dopamine replacement therapy using L-DOPA or dopamine receptor agonists. Fu and colleagues have demonstrated that 3-hydroxybutyrate exerted protective effects on dopaminergic neurons by inhibiting microglial activation, and the motor dysfunction of the PD model rat was significantly improved by 3-hydroxybutyrate treatment\(^{161}\). An \textit{in vitro} mechanistic study revealed that the neuroprotective effect of 3-hydroxybutyrate on dopaminergic neurons against inflammatory challenge is mediated by HCA2. Ischemic stroke represents one of the leading causes of death and disability. The inflammatory responses induced after ischemic stroke often cause ischemic lesion. The cellular events, characterized by activation of resident microglia and infiltration of several types of immune cells including neutrophils and monocyte/macrophages, are attributed to ischemic brain injury. Rahman et al. showed that HCA2 is required for the neuroprotective effect of 3-hydroxybutyry-
ate and a ketogenic diet in a stroke model, and nicotinic acid reduces infarct size via an HCA2-mediated mechanism\(^{158}\). Since many synthetic HCA2 agonists with better pharmacokinetic and pharmacodynamic properties have been developed so far, it is necessary to assess their efficacy in treating neurological disorders.

Butyrate is an SCFA produced by anaerobic bacterial fermentation of dietary fibers and has been suggested to function as a tumor suppressor by inhibiting histone deacetylases\(^{162}\). The expression of HCA2 in intestinal epithelial cells and the presence of millimolar levels of butyrate in the colonic lumen lead to the concept that butyrate exerts anticancer effects via HCA2. Recent studies show that the expression of HCA2 is silenced in human colon cancer, as well as several colon cancer cell lines, by DNA methylation\(^{123,163}\). Functional expression of HCA2 in cancer cells induces apoptosis in the presence of butyrate and nicotinic acid. In addition, Singh et al. reported that HCA2 deficient mice are susceptible to developing colon cancer and nicotinic acid suppresses colon cancer in an HCA2-dependent manner\(^{164}\). These results suggest that HCA2 mediates the tumor-suppressive effect of the bacterial metabolite in colon cancer. Butyrate is also present at high levels in the mammary gland, in which butyrate is produced during lactation. The protective effects of butyrate against breast cancer have been reported\(^{165,166}\). HCA2 is silenced in human breast tumor tissue and murine mammary tumors, and ectopic expression of HCA2 induces apoptosis in breast cancer cells\(^{167}\). Meanwhile deletion of HCA2 in mice increases tumor incidence, consistent with the notion that HCA2 functions as a tumor suppressor. As cancer cells predominantly rely on increased glycolysis (the Warburg effect), glucose is the main source of energy for cancer cells. For this reason, a ketogenic diet plan has been recognized as a logical therapeutic strategy to kill cancer cells by inducing the selective starvation of cancer cells while supplying ketone bodies as alternative energy substrates to normal cells\(^{168-171}\). Synthetic ligands of HCA2 need to be evaluated as a potential therapeutic target for cancer treatment.

### 3. HCA3 and β-oxidation intermediate

HCA3 is closely related to HCA2, but it does not bind nicotinic acid or 3-hydroxybutyrate with practical affinity. Comparison of the two genes shows 15 nucleotide changes and 5-nucleotide insertion/deletion at the 3’ end of the gene, resulting in 15 amino acid conversions and an extra sequence of 23 amino acids at the C-terminus, respectively\(^{121,122}\). Although HCA3 was once reported to be a low-affinity receptor for nicotinic acid\(^{122}\), Ahmed et al. provided evidence that as a natural ligand for HCA3, 3-hydroxy-octanoate has anti-lipolytic activity\(^{18}\). Under certain conditions such as fasting or diabetic ketoacidosis, 3-hydroxy-octanoate reaches the level sufficient to activate HCA3\(^{172,173}\), suggesting that HCA3 plays an important role in regulating the FFA supply from adipocytes to avoid excessive lipolysis resulting in a waste of energy. HCA3 is also expressed in various immune cells and epithelial cells in the colon\(^{18,121,174}\), however, the physiological significance of HCA3 in immune or absorptive functions remains to be determined.

### Conclusion

FFAs and metabolic intermediates such as lactate and 3-hydroxybutyrate modulate several biological effects through the activation of GPCRs of the FFA and HCA families. These receptors act as dietary and metabolic sensors controlling energy metabolism as well as inflammatory response. As chronic inflammation is relevant to a number of diseases including insulin resistance, diabetes and atherosclerosis, to elucidate the physiological functions of the receptors, not only in metabolic tissues but also immune cells, provides new avenues for therapeutic intervention in metabolic disorders.

Omega-3 FAs display anti-inflammatory properties and reduce systemic insulin resistance via FFA4. The antiatherogenic effects of HCA2 agonists are attributed to both anti-lipolytic activity in adipocytes and anti-inflammatory effects in macrophages. In addition, activating the FFA1 receptor, which shares an overlapping spectrum of ligands with FFA4, enhances GSIS from islet β-cells and stimulates incretin secretion from intestinal endocrine cells (Fig. 3). It is well recognized that receptor signaling proceeds through heterotrimeric G proteins as well as G protein-independent mechanisms, resulting in distinct biological responses. Thus, the synthetic biased ligands that can activate the specific signal pathways represent an opportunity for the discovery of new drugs. The SCFA receptors, FFA2 and FFA3, are involved in the regulation of energy homeostasis in pancreatic islets and endocrine cells of the intestinal epithelium (Fig. 3). Novel synthetic agonists/antagonists of the SCFA receptors need to be developed as targets for the treatment of metabolic disorders.

There is still much to learn about the physiological roles and pathological implications of both HCA1 and HCA3. HCA3 agonists might create a unique class of drug candidates in regulating lipid metabolism since the receptor is present only in higher primates. In the process of investigation, we can learn much about uncharacterized biological phenomena that are important in human health and disease.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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