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Review

Role of free fatty acid receptors in the regulation of energy metabolism

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ABSTRACT

Free fatty acids (FFAs) are energy-generating nutrients that act as signaling molecules in various cellular processes. Several orphan G protein-coupled receptors (GPCRs) that act as FFA receptors (FFARs) have been identified and play important physiological roles in various diseases. FFA ligands are obtained from food sources and metabolites produced during digestion and lipase degradation of triglyceride stores. FFARs can be grouped according to ligand profiles, depending on the length of carbon chains of the FFAs. Medium- and long-chain FFAs activate FFA1/GPR40 and FFA4/GPR120. Short-chain FFAs activate FFA2/GPR43 and FFA3/GPR41. However, only medium-chain FFAs, and not long-chain FFAs, activate GPR84 receptor. A number of pharmacological and physiological studies have shown that these receptors are expressed in various tissues and are primarily involved in energy metabolism. Because an impairment of these processes is a part of the pathology of obesity and type 2 diabetes, FFARs are considered as key therapeutic targets. Here, we reviewed recently published studies on the physiological functions of these receptors, primarily focusing on energy homeostasis.

1. Introduction

Free fatty acids (FFAs) are essential nutrients that contribute to various cellular functions. Several epidemiological and physiological studies have examined the beneficial or harmful effects of FFAs [1,2]. FFAs exert biological effects through several signaling pathways; however, the precise mechanisms remain unclear. FFAs are associated with intracellular and nuclear proteins such as FA-binding proteins and peroxisome proliferator-activated receptors (PPARs) [3,4]. Activation of G protein-coupled receptors (GPCRs) by FFAs has been predicted because some physiological functions of FFAs are difficult to describe. During the past decade, several FFA receptors (FFARs) have been identified. Ligand profiles of FFARs depend on the length of the carbon chain of FFAs. Thus, FFA2 and FFA3 receptors are activated by short-chain FFAs, whereas FFA1 and FFA4 are activated by medium- and long-chain FFAs. In contrast, GPR84 is activated by medium-chain FFAs but not by long carbon chains. Expression and functional studies of FFARs have shown that these receptors are strongly associated with energy metabolism (Table 1). Therefore, FFARs have received considerable attention as potential therapeutic targets for energy metabolism disorders such as obesity and type 2 diabetes. However, the ligand profiles of short-chain and medium- to long-chain FFA receptors are similar to each other, and the expression profiles of these receptors in pancreas, intestine, and immune cells partly overlap. Therefore, the development of selective ligands (Fig. 1) and gene knockout studies would be essential to reveal the precise physiological functions of these receptors. Particularly, in addition to the basic properties such as distribution, signaling pathways, and ligands, it is important to focus on how these receptors are orchestrated in the whole body and systemically contribute to the pathogenesis of disease, which in turn might provide novel insights into therapeutic options. This review summarizes the crucial and basic properties previously reported, as well as the recent advances in FFAR functions in relation to energy metabolism.

2. Short-chain FFAs (SCFAs)

SCFAs consisting of chains less than 6 carbons have various physiological functions. Acetate (C2), propionate (C3), and butyrate (C4) are major products in the bacterial fermentation of dietary fiber with gut microbiota and are used as an energy source in epithelial cells of the intestinal tract and in the liver [5,6]. SCFAs are therefore considered as endogenous ligands for SCFA receptors expressed in the intestines. In addition, the increase in the plasma levels of SCFAs from micromolar to millimolar levels activates the expression of SCFA receptors in leukocytes and sympathetic nerves.

3. FFA3 (GPR41)

3.1. Ligands and signal transduction

Several research groups have characterized the pharmacology of FFA3. Ligand screening showed that FFA3 is activated by SCFAs such as propionate (C3), butyrate (C4), and valerate (C5) [7,8]. FFA3 activates

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Table 1
Expression and functional profiles of FFARs.

Expression	FFARs	Physiological functions of each FFAR
Pancreas	FFA1, FFA4	Insulin secretion (FFA1), glucagon secretion (FFA1)
Intestine	FFA1, FFA2, FFA3, FFA4	GLP-1 and GIP secretion (FFA1, FFA2, FFA3)
		Intestinal motility (FFA2)
		GLP-1 secretion (FFA4)
Adipocyte	FFA2, GPR84, FFA4	Adipogenesis, lipolysis (FFA2)
		Adiponectin expression (GPR84)
		Differentiation (FFA4)
Sympathetic nerve system	FFA3	Sympathetic activation
Immune cells	FFA2, GPR84, FFA4	Anti-inflammatory
		Neutrophil, eosinophil (FFA2)
		Cytokine production
		Monocyte, macrophage (GPR84)
		Anti-inflammatory
		Macrophage (FFA4)

the $[Ca^{2+}]_i$ response and the phosphorylation of extracellular signal-regulated kinase (ERK)1/2, but inhibits cAMP production. Furthermore, these responses are inhibited by pertussis toxin (PTX) treatment, suggesting that the regulation system is coupled to Gα(i/o) [9]. On the other hand, several synthetic compounds have been also reported as FFA3 agonists or antagonists. Arena Pharmaceuticals have described the selective agonist (Compound 1) and antagonist (Compound 2) for FFA3 [10]. Schmidt et al. also reported small carboxylic acids, including the bulky structure of cyclopropane in the structure (Compound 3) that showed a 100-fold selectivity for FFA3 against FFA2 [11].

3.2. Physiological functions

3.2.1. Intestine

FFA3 expression has been confirmed in several types of cells in the intestines [12–14]. Intestinal L-cells typically secrete peptides YY (PYY) and GLP-1, which are gut hormones involved in energy homeostasis, whereas the neurotensin-positive cells of the proximal colon express FFA3 mRNA. PYY and GLP-1 secretion is reduced in the primary culture of cells derived from FFA3 knockout (KO) mice [14]. The effects of another synthetic compound designated as AR420626, that has the similar basic structure of compound 1, on GLP-1 secretion from colonic crypt cultures was confirmed by stimulation of FFA3 or FFA2 using specific synthetic ligands. AR420626 also showed the IP3 accumulation in COS-7 cells transiently expressing FFA3 [10,15]. FFA3 KO mice showed significantly lower body and fat pad weight and lower plasma leptin concentrations than wild-type (WT) mice [16]. In contrast, the intestinal transit rate and SCFA content in the feces of KO mice were higher than those in the feces of WT mice. However, these differences between WT and FFA3 KO mice have not been confirmed under germ-free conditions. Furthermore, germ-free WT mice, but not FFA3 KO mice, were colonized by specific microbes and showed an increase in PYY levels. These results indicate that the production of SCFAs by bacterial fermentation is essential for the activation of FFA3 function and that PYY modulation of gut motility is important for the absorption of SCFAs. Bellahcene et al. earlier confirmed the phenotypic differences between male and female FFA3 KO mice [17]. The rate of energy expenditure and lean body mass in male KO mice supplemented with high-fat diet was lower than those in similarly treated female KO mice. In addition, compared to female KO mice, body fat mass and the plasma leptin and glucose levels were elevated in male KO mice. These differences could be explained by the effect of sex hormones on the metabolic regulation in the central or peripheral nervous systems and on the distribution of adipose tissues. Furthermore, chemokine and cytokine levels were increased by stimulation of intestinal epithelial cells with SCFAs, and the recruitment of leukocytes and activation of effector T cells were induced [18]. In addition, the inflammatory responses of KO mice were reduced compared to those of WT mice. The administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) in KO mice, which induces acute inflammatory responses, showed lower levels of cytokines such as IL-6 and IL-12, and lower levels of neutrophil infiltration in the colon. In a *Citrobacter rodentium* infection model that evaluates immune responses to bacterial pathogens, the clearance of pathogens from the intestines was slower in FFA2 and FFA3 KO mice. The levels of IL-6 and neutrophil recruitment, as well as the frequencies of Th1 and Th17 cells were also reduced in the KO mice. These results indicate that SCFA receptors are important for the regulation of the immune system of the intestines such as the recruitment of leukocytes and cytokine production via T-cell activation [18].

3.2.2. Nervous system

We have previously reported that the sympathetic ganglia abundantly express FFA3 mRNA in FFA3 WT [19]. Furthermore, because tyrosine hydroxylase protein, which is the enzyme for catecholamine production, and sympathetic nerve innervation were significantly lower in FFA3 KO mice than WT mice, FFA3 could be a critical molecule for sympathetic nerve growth.

Propionate-induced elevation of heart rate and oxygen consumption was blocked by propranolol (a β-adrenergic receptor blocker) treatment but not by hexamethonium (a nicotinic acetylcholine receptor blocker) treatment. These findings suggested that propionate affects sympathetic nerve system activity through FFA3 at the ganglion level.

In vitro studies of precise signaling mechanisms for sympathetic nerve system activation through FFA3 have demonstrated that FFA3 activates Gβγ–PLC β-3–ERK1/2 pathways, leading to the phosphorylation of synapsin 2-β at serine 426. Synapsins are a family of synaptic vesicle-associated proteins implicated in the secretion of neurotransmitters. Therefore, propionate-activated FFA3 may promote noradrenalin release from sympathetic neurons and regulate energy expenditure.

However, FFA3 contributes to the suppression of energy expenditure under fasting conditions. β-hydroxybutyrate (β-HB), a ketone body produced in the liver during food deprivation, exhibits an inhibitory effect on ERK1/2 phosphorylation and cAMP production through FFA3. This antagonistic effect has been confirmed in cultured sympathetic neurons and in mice in vivo. Hence SCFAs produced by bacterial fermentation under fed conditions activate FFA3, leading to an increase in energy expenditure. In contrast, the ketone body β-HB produced under fasting conditions inhibits FFA3 signaling, suppressing energy expenditure [19,20].

Recently, a transgenic mouse study using monomeric red fluorescent protein as the reporter for FFA3 revealed that FFA3 is expressed in neuronal cells of the submucosal and myenteric ganglia [15]. Pluznick et al. reported that Olfr78 and FFA3 are expressed in smooth muscle cells of small resistance vessels. Interestingly, modulation of blood pressure was observed in both Olfr78 and FFA3 KO mice under antibiotic treatment that causes the reduction of SCFAs derived from the gut microbiota fermentation. Thus, we should consider Olfr78 as the novel type of SCFAR with the therapeutic potential for and on the novel mechanisms for blood pressure modulation via SCFARs including Olfr78 [21]. Q4
To elucidate the precise mechanism of FFA3 in the nerve, conditional KO mice or specific ligands to discriminate FFA3 expressed in the nerve and other tissues are required.

4. FFA2 (GPR43)

4.1. Ligands and signal transduction

In 2003, FFA2 was identified as a receptor for SCFAs. During the ligand screening using bioactive compounds in Ca^{2+} assays, the authors reported that FFA2 was activated by acetate in transfected cells [8,22]. In vitro, FFA2 is also activated by other SCFAs such as formate (C1), propionate (C3), butyrate (C4), and pentanoate (C5). The potencies of FFA2 activation can range in the following order: C2 = C3 > C4 > other SCFAs [7,8]. In G protein signaling, FFA2 displays dual coupling through the

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