Artykuł przeglądowy *Review*

Free fatty acid receptors and their physiological roles in the colon*)

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Summary

Free fatty acids (FFAs) are not only an important source of energy but they also play key roles in regulating various physiological responses. FFAs including short-chain fatty acids (SCFAs) have recently been demonstrated to act as ligands of several G-protein-coupled receptors (GPCRs) (FFA1, FFA2, FFA3, GPR84 and GPR120). FFA1 and GPR120 are activated by medium- and long chain fatty acids. GPR84 is activated by mediam-chain, but not long chain FFAs. On the other hand, FFA2 and FFA3 are both activated by SCFAs. Tissue distribution studies have indicated that FFA2 and FFA3 function as chemical sensors in the colon. For the involvement of SCFAs in the regulation of colonic motility, propionate and butyrate concentration--dependently induced phasic and tonic contractions in rat colonic circular muscle. The responses were not observed in mucosal free preparation. Thus, FFA2 and FFA3 are important molecular devices to monitor the chemical composition in colonic lumen. For the local function of SCFAs, it should be stressed that individual SCFA has different mode of actions on colonic smooth muscles. These different effects may be due to the relative contribution of FFA2 and FFA3 on the control of intestinal muscle activity. In this article, we have reviewed the expression and functions of these molecules, especially FFA2 and FFA3 on the regulation of colonic motility.

Keywords: G-protein coupled receptor, Short-chain fatty acid, FFA2, FFA3, human colon, rat colon, colonic motility

The gastrointestinal (GI) mucosa comes in direct contact with a vast majority of potentially beneficial or harmful substances in the lumen and acts as a sensory organ by detecting luminal components and sending messages to the nervous system to initiate the appropriate response of digestion and absorption of nutrients or neutralization and expulsion of drugs, toxins and microorganisms. Such physiological response of the GI tract to incoming nutrient is very important to be coordinated to allow correct processing of an ingested meal. Sensing of luminal content is also important for food intake control via gut-to-brain signaling pathways. This complex process of chemosensory perception is regulated by different sensors, including enteroendocrine cells, brush cells and neural pathways (8, 11, 17, 25). Since nerve terminals do not reach the intestinal lumen and do not enter the mucosal lining, enteroendocrine cells or specialized epithelial cells serve as the first level of integration of information from the gut lumen. Despite its physiological importance, the molecular recognition events sensing the chemical composition of the luminal contents of the GI tract have yet to be elucidated.

Recently, many G protein-coupled receptors (GPCRs) have been deorphanized. Among them, free fatty acid (FFA) receptors are identified as membrane receptors and play significant roles in nutritional regulation. Each of the FFA receptors is expressed differentially, and they may play different functional roles. This finding has prompted reevaluation of the mechanism of actions of FFAs in health and disease. FFA1 (originally termed GPR40) and GPR120 are activated by medium- and long-chain FFAs, whereas FFA2 (previously designated GPR43) and FFA3 (previously designated GPR41) are activated by short chain fatty acids (SCFAs) (53). In this review, we will summarize the recent knowledge on the roles of deorphanized FFA receptors, especially, FFA2 and FFA3 and their contributions for the regulation of colonic motility based on our studies.

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Production of FFA

The major components of both dietary and storage fat consists of triacylglycerols. About 90% of the dietary lipid is in the form of water insoluble triacylglycerols containing mostly long-chain fatty acids (16 to 18 carbon atoms). In monogastric animals, the GI tract breaks down ingested triacylglycerols into FFAs. Both pancreatic lipase and bile salts are required for the normal digestion and absorption of triacylglycerols. Exogenous triacylglycerols from the diet are absorbed as chylomicrons. Pancreatic lipase degrades triacylglycerol to one monoglyceride and two FFA molecules. These two digestion products are the form in which fat enters the intestinal epithelium. FFAs are composed of long carbon chains (14 to 24) with a carboxyl terminus, and they can be either saturated with hydrogen atoms or unsaturated. From 30% to 40% of plasma FFA molecules are oxidized. Once monoglycerides and free fatty acids enter the epithelial cells, they are resynthesized to triacylglycerols by intracellular enzyme systems. The newly synthesized triacylglycerols are aggregated into droplets, which become progressively larger during passage through the cells.

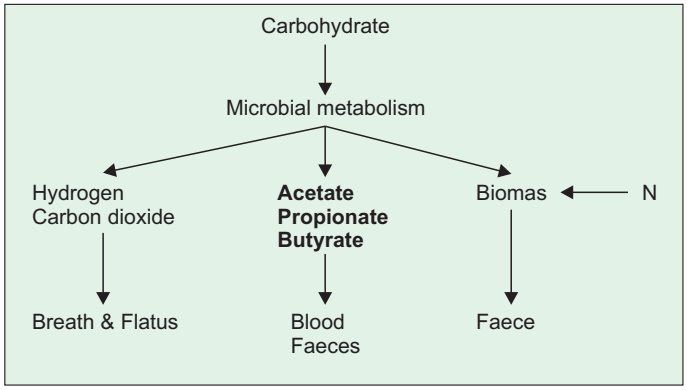
Table 1 presents the average basal concentrations of the most important plasma lipids. Although FFAs circulate at the lowest concentration, the concentration of FFAs in the postabsorptive state is ~0.5 μM and can be substantially increased after ingestion of a fatty meal (19). The circulating FFAs are bound with serum albumin such that the concentration of unbound FFAs is in the micromolar range (50).

Tab. 1. Average lipid concentrations in postabsorptive plasma

	mg/dl	μmol/L
Ketoacids	10	0.1
Free fatty acids	10	0.4
Triglycerides	100	1.2
Cholesterol (Total)	185	4.8
Low density	120	
High density	50	
Very low density	15	

(From Physiology 4 ed R. M. Bern, M. N. Levy, 1998)

The vast majority of food entering the small intestine is cleared by the duodenum and jejunum. Only if there is a problem with normal absorptive processes (such as celiac disease and dumping syndrome) will significant amounts of luminal nutrients reach the large intestine. On the other hand, even in a normal situation, undigestive carbohydrates such as dietary fibers and starch not digested in the upper gut also enter the large intestine. SCFAs, primarily acetate, propionate and butyrate, are organic acids produced within the intestinal lumen by bacterial fermentation of mainly undigested dietary carbohydrates, but also in a minor part



The Large Intestine, J.H. Cummings, Danon Chair Monograph

Fig. 1. Overview of carbohydrate fermentation in the large intestine

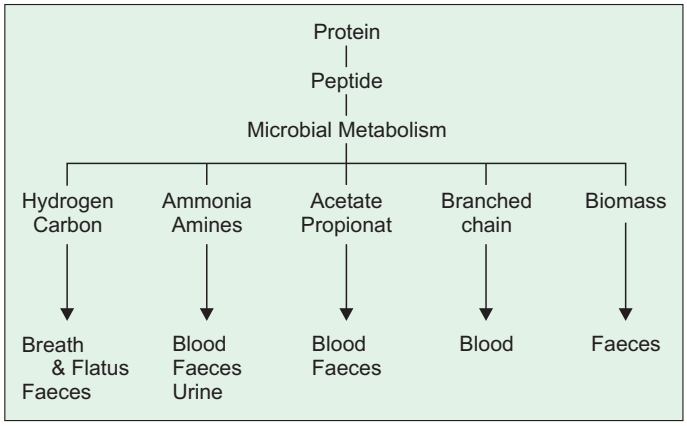


Fig. 2. Overview of protein breakdown and amino acid fermentation in the human large intestine

by dietary and endogenous proteins, such as mucus, and sloughed epithelial cells (57). The principle metabolic pathways in carbohydrate and protein fermentation are summarized in fig. 1 and 2. The production of SCFAs allows the salvage of energy mainly from carbon sources as dietary fiber that is not digested in the small intestine. It has been estimated that SCFAs can contribute to about 5-15% of the total caloric requirements of humans (3). Total and relative molar concentrations of the main SCFAs, acetate, propionate and butyrate produced in the human intestine, depend on the site of fermentation, diet and composition of the intestinal microbiota (15). Absolute concentrations of butyrate in human faeces were found to range from 11 to 25 mM (22, 60) and molar ratios of acetate to propionate to butyrate varied between 48 : 29 : 23 and 70 : 15 : 15, respectively, with mean values of approximately 60 : 20 : 20 (22, 57). However, the *in situ* production of total colonic SCFAs is difficult to determine because more than 90% of the SCFAs are rapidly absorbed and metabolized by the host (57). Recently, Bloemen et al. have done an excellent study to measure SCFA concentrations in humans (11 females; 11 males). They have shown that portal concentrations of acetate, propionate and butyrate in overnight fasting humans were 262.8 ± 31.2, 30.3 ± 5.6 and 30.1 ± 4.8 μmol/l, respectively (4). They also

measured the concentrations of arterial SCFA. Arterial acetate, propionate and butyrate concentrations were 172.9 ± 19.1, 33.6 ± 0.4 and 7.5 ± 12 μmol/l, respectively (n = 22; mean ± SEM) (13). After release SCFA into portal vein, propionate and butyrate are metabolized by the liver and used for gluconeogenesis, whereas acetate is a substrate for lipogenesis, cholesterol synthesis and is taken up by adipose and muscle tissues (61). Micorbiota and diet influence the production of SCFA in the colon. Indeed, Peters et al. reported a rapid increase in SCFA concentrations in portal and peripheral blood within 15-45 min after instillation of lactulose, into the cecum (43). The result indicates that the dietary content of carbohydrates, starches and fibers influence SCFAs concentrations.

Tissue distribution of FFA receptors and their physiological functions

During a search for novel galanin receptor subtypes, a cluster of four GPCR genes, FFA1 (GPR40), FFA2 (GPR43), FFA3 (GPR41) and GPR42 were identified as tandemly encoded genes present on human chromosome 19q13 (46) (fig. 3). FFA1, FFA2 and FFA3 represent a family of receptors because they are more closely related to each other than any other known GPCR. The member of this family shares 30-40% identities with each other. Although FFA3 and the predicted GPR42 protein share near 98% homology, GPR42 is now generally thought to be an open reading frame pseudogene (7). All three FFA receptors remained classified as orphans until 2003 when three articles that identified a range of medium- and long-chain saturated and unsaturated fatty acids as ligands for FFA1 are published (5, 28, 32). At about the same time that long chain fatty acids were reported to be agonists of FFA1, SCFAs with chain length of less than six carbons were described as the potential endogenous agonists for FFA2 and FFA3 (6, 33, 40). Two years later, in addition to FFA1, GPR120 was also found to be a receptor for unsaturated long-chain FFAs (24).

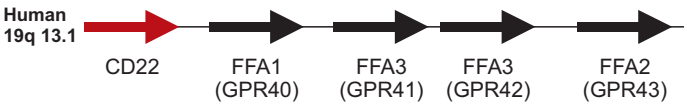


Fig. 3. GPR40 family of receptors
GPR40 family of receptors are tandemly located downstream of CD22 on chromosome 19

FFA1 (GPR40). FFA1, FFA2 and FFA3 show a family of receptor but the family exhibit relatively limited similarity; 43% between FFA2 and FFA3 and 33 and 34% when FFA1 is compared with FFA2 and FFA3, respectively (6). While FFA2 and FFA3 are activated by SCFAs, FFA1 is activated by medium- and long- chain saturated and unsaturated FFAs (5, 28, 32). A variety of fatty acids were found to act as agonists to FFA1 in the micromolar concentration range (5). Interestingly, the potency of the saturated fatty acids was dependent on chain length, with pentadecanoic acid (C15) and plamitic acid (C16) being the most potent, whereas carbon chain length or degree of saturation did not appear to correlate with potency among unsaturated fatty acid (5).

All three initial reports on FFA1 showed high levels of receptor mRNA in the pancreas (5, 28, 32). Expression analysis of FFA1 using RT-PCR, immunohistochemistry, and *in situ* hybridization revealed high expression in insulin-producing pancreatic islets in human and rats (28, 48, 56). FFA1 was found to be enriched 2- to 100-fold in pancreatic islets as compared with whole pancreas (5). These results suggest that FFA1 acts as the receptor for fatty acid-induced insulin secretion. FFA1 expression has also been detailed in various pancreas-derived cell lines, including MIN6, β-TC-3, HIT-T15 and INS-1E (5, 28, 32, 48). Such prominent β cell expression was explained by a recent study on the promoter region of FFA1 that showed several highly conserved regions, one of which, HR2, is known to be a potent β cell-specific enhancer of transcription (2, 45). FFA1 is also reported to present in α-cells (14). Furthermore, FFA1 is expressed in scattered enteroendocrine cells throughout the mouse GI tract including stomach (12). FFA1 expressing enteroendocrine cells are colocalized with gastrin, GIP, GLP-1, ghrelin, CCK, PYY, secretin, serotonin, and substance P (12). Outside the GI tract, FFA1 immunoreactivity was found in the central nervous system of adult monekys (34).

FFAs are known to have pleiotropic effects on pancreatic β-cells. Steneberg et al. showed that FFA1 mediates both acute and chronic effects of FFAs using FFA1 knockout and transgenic mice; 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 activity (52). FFA1 (GPR40)-deficient β-cells secrete less insulin in response to FFAs, and the loss of FFA1 protects mice from obesity-induced hyperinsulinermia, hepatic stenosis, hypertriglyceridemia, increased hepatic glucose output, hyperglycemia, and glucose intolerance (52). Conversely, overexpression of FFA1 in β-cells of mice leads to impaired β-cell function, hyperinsulinemia, and diabetes (52). These results suggest that FFA1 plays an important role in the chain of events linking obesity and type 2 diabetes.

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 (5, 10, 21, 51). Itoh et al. revealed that long-chain fatty acids amplify glucose-stimulated insulin secretion from pancreatic β cells by activating FFA1 (28). When the expression of FFA1 was inhibited by small interfering (si)RNA, the increase in insulin secretion after fatty acid stimulation was eliminated, clearly confirming the involvement of FFA1 in this process.

FFA1 has also been detected in the MCF-7 human breast cell line (64) and has been implicated in control of breast cancer cell growth by fatty acids (23).

FFA2 (GPR43) and FFA3 (GPR41). At the same time of the discovery of FFA1, FFA2 and FFA3 are found as the receptors for SCFAs (6, 33, 40). In original papers, FFA2 mRNA can be detected in a variety of tissues, but the highest expression is found in immune cells including polymorphonuclear (PMN) cells (6, 33, 40). SCFAs are known to exert various cellular effects on PMN cells such as altering cytoplasmic pH, calcium concentration, patghogenesis, cell proliferation, granulocyte motility and Chemotaxis (13, 47). These results suggest that SCFAs might be involved in the activation of leukocytes through FFA2 or FFA3. Recently, Maslowski et al. have shown that (FFA2) GPR43-deficient mice (*Gpr43*$^{-/-}$) show exacerbated or unresolving inflammation in models of colitis and germ-free mice showed a similar certain inflammatory responses (35). Furthermore, they have shown that treatment of germ-free mice with 150 mM acetate in the drinking water markedly improved inflammation. These results indicate that the stimulation of FFA2 by SCFAs is necessary for the normal resolution of certain inflammatory responses, because *Gpr43*$^{-/-}$ mice showed exacerbated or unresolving inflammation in models of colitis, arthritis and asthma as mentioned above.

FFA2 has also been reported in adipose tissue (18, 26), the breast cancer cell line, MCF-7 (64). Ge et al. have recently reported that adipocytes treated with FFA2 natural ligands, acetate and propionate, show a reduction in lipolytic activity and the effect is abolished in adipocytes isolated from FFA2 knockout mouse (18). They further showed that the activation of FFA2 by acetate results in the reduction in plasma free fatty acid levels. These results suggest that FFA2 may implicate in the regulation of lipid homeostasis through the inhibition of lipolysis. FFA2 and FFA3 are coupled to inositol 1,4,5-trisphosphate formation, intracellular Ca^{2+} release, ERK1/2 activation and inhibition of cAMP accumulation (33).

FFA3 has a more widespread expression pattern than FFA2 (33). High levels of expression were observed in adipose tissues, pancreas, spleen, lymph nodes, bone marrow, and peripheral blood mononuclear cells (33). However, there is some debate about the expression of FFA3 in adipocytes; Hong et al were unable to detect FFA3 expression in human adipose tissue, in cultured preadipocytes or adipocytes, or in 3T3-L1 cells, despite using the same probes as original papers for receptor mRNA (26). The discrepancy of FFA3 expression in these studies is yet to be resolved.

As mentioned above, a physiological relevant site of FFA receptor activation is the gut because a large amount of SCFAs is constantly existing in the large intestine. However, the mechanisms by which intraluminal SCFAs are sensed are not known (27, 36, 37, 62, 63). From our and other physiological studies, we hypothesized that FFA2 and FFA3 functioned as chemical sensors to modify colonic function including motility and/or ion transport. Therefore, we started to explore the expression of FFA2 and FFA3 using antisera raised against FFA2 and FFA3. Messenger RNA for FFA2 was detected in extracts of whole wall and separated mucosa from the rat distal ileum and colon (29). By western blotting, FFA2 protein was detected in the mucosa and whole wall but not in muscle plus submucosal layers, both from the rat distal ileum and colon. In the human ascending colon, mRNA for FFA2 was also detected in extracts of whole wall and FFA2 protein was detected in extracts of whole wall and in the separated mucosa but not in extracts of submucosa and muscle layer (30). These results indicate that FFA2 is expressed by cells in the mucosa, but not by enteric neurons or smooth muscle. Until 2009, there have been no reports of FFA3 being expressed in the gut (53). Recently we found that FFA3 protein and mRNA are expressed in human colonic mucosa (55). Protein expression levels were higher in colonic mucosa than submucosa or muscle similar to that of FFA2.

To identify the cellular distribution of FFA2 in rat and human colon, immunohistochemical staining was performed by using anti-FFA2 serum. Immunoreactivity for FFA2 occurred at a low level in enterocytes within mucosa both in rat and human colon. FFA2 immunoreactivity was also found in a population of enteroendocrine cells and small cells in the lamina propria in human ascending colon. FFA2-immunreactive cells in the epithelia had the morphology of enteroendocrine cells as shown in fig. 4. Immunoreactivity for FFA2 in rat ileum and colon showed similar pattern to those of human colon. FFA2 immunoreactive enteroendocrine cells in human ascending colon were open type enteroendocrine cells, which extended their cell body to the luminal surface (fig. 4).

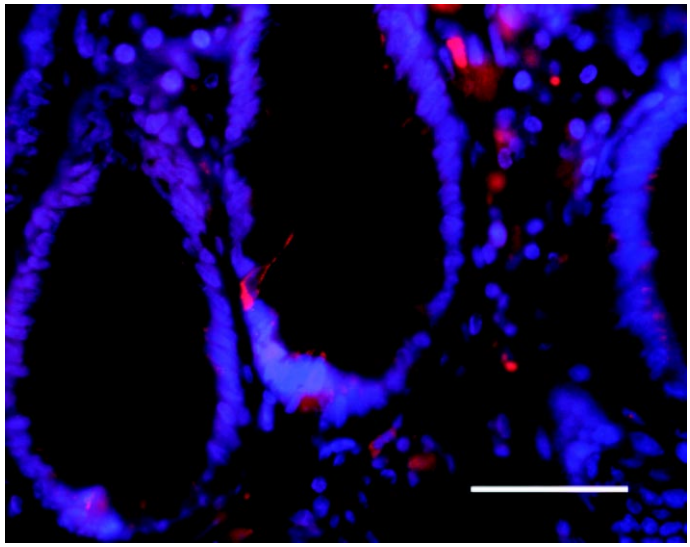


Fig. 4. Immunohistochemistry of FFA2 (GPR43) in the human colon
Bar = 50 μM

Until recently, there was no report for the tissue localization of FFA3 in the intestine as mentioned above, so we examined cellular distribution of FFA3 using human colonic tissues. FFA3 immunoreactivity in human colon was observed as dotted staining in apical cytoplasm of enterocytes, enteroendocrine cells. FFA3-immunoreactive enteroendocrine cells were also open type with thin cell bodies extending to the luminal surface (55). However, number of FFA3 immunoreactive enteroendocrine cells were fewer than those of FFA2 cells. Furthermore, double-immunostaining for FFA2 and FFA3 revealed that these were not colocalized in each other.

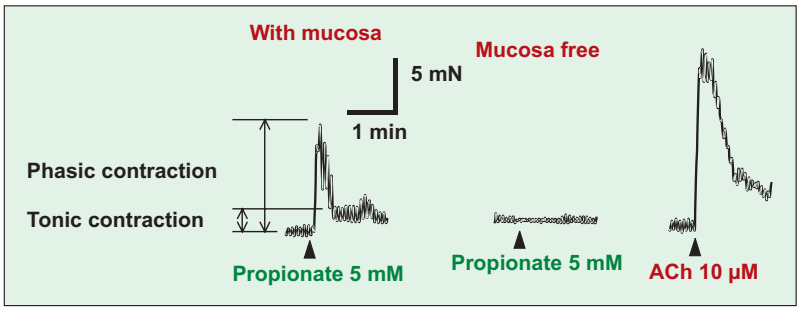


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Fig. 5. Propionate-induced phasic and tonic contractions in rat colonic circular muscle

Previous physiological studies reported that SCFAs modify rat colonic motility through 5-hydroxytryptamine (5-HT) and PYY release (9, 16). Thus, we performed double-staining for FFA2 and 5-HT and FFA2 and PYY, respectively. No FFA2-immunoreactive enteroendocrine cells exhibited 5-HT, whereas FFA2 immunoreactive enteroendocrine cells were colocalized with PYY in both human and rat colon which is consistent with physiological data showing that SCFAs stimulate the release of PYY (9) and 5-HT (16) from rat ileum and colon. We have also performed double-staining for FFA3 and 5-HT or PYY. FFA3 immunoreactive enteroendocrine cells in human colon were also colocalized with PYY similar to those of FFA2 but not 5-HT (55). FFA2 and FFA3 were not colocalized in the same enteroendocrine cells.

In non-ruminant mammals, the physiological importance of SCFAs has been highlighted only recently. A possible direct influence of SCFAs on intestinal motility in monogastric animals was first suggest by Yajima, who recorded a tonic contraction of rat colonic muscle strips in response to propionate, butyrate or valerate *in vitro* (62). The concentration-dependent contractile effect occurred only when SCFAs were applied on the mucosal side and disappeared when the mucosa was removed, suggesting the presence of sensory mechanisms near the epithelium. As indicated above, FFA2 and FFA3-immunoreactive intestinal cells were found both in rat and human colon (29, 30, 55). Therefore, we investigated the contribution of FFA2 and FFA3 on the regulation of colonic motility using *in vitro* animal models.

Circular muscle. When recorded intestinal motility mechanically, *in vitro* and *in vivo* colonic motor activity in most species, including mice and rats, is characterized by two distinct types of contraction: (1) rhythmic phasic contractions, and (2) spontaneous contractions, which are also termed giant contractions (GCs) by Gonzalez and Sarna (20). We have shown that propionate increases the frequency and decreases the mean amplitude of spontaneous GCs (38). However, acetate and butyrate had no such effects. GCs of colonic circular muscle layer are thought to enhance the propulsion of luminal contents because at least part of these contractions propagates in anal direction (44). Thus the stimulatory effect of propionate on the frequency of GCs seems to be important for the propulsion of feces in the colon.

SCFAs also affect basal circular muscle activity; propionate evoked phasic and tonic muscle contractions in rat distal colon (36-38, 41, 42). Propionate and butyrate concentration-dependently (10 μM-10 mM) induced rapid, large amplitude phasic contractions followed by tonic contraction in strips of the circular muscle in rat distal colon. However, acetate itself had no effect on basal muscle activity. The propionate-induced phasic and tonic contractions were not observed in the mucosal-free preparations as shown in fig. 5. The results suggest that propionate does not directly act on circular muscle. We have further analyzed the propionate-induced circular muscle contractions then found that the propionate-induced circular muscle contraction was attenuated by atropine, tetrodotoxin (TTX) and 5-HT_4 receptor antagonist, SB204070 (37). Taken together, these results suggest that propionate acts on SCFA receptors, FFA2 or FFA3 expressed in the mucosa causing release of 5-HT from the enterochromaffin cells containing 5-HT. Then, released 5-HT may act 5-HT_4 receptors on the endings of intrinsic primary afferent neurons that in turn activate cholinergic motor neurons that contract the circular muscle. On the other hand, the tonic contraction was attenuated by the non-selective COX inhibitor, piroxicam or COX-1 inhibitor, SC-560 (37). Therefore, propionate probably induces release of COX products to cause the tonic contractions. For the involvement of FFAs to SCFAs-induced circular muscle contractions, the rank order potency of the SCFAs correspond to that seen for activation of FFA3. Thus, propionate-induced circular muscle contractions might be involved in FFA3.

Since SCFAs are produced by bacterial fermentation of the carbohydrates of dietary fiber in the large intestinal lumen as mentioned above, the presence of SCFAs or individual ratio of SCFAs in the colonic lumen reflect the activity of luminal bacterial flora and SCFA receptors possibly monitor the activity of bacteria to maintain the colonic health. Indeed, FFA2

and FFA3 have been reported to highly express by cells of the immune defense system including PMN cells (FFA2 and FFA3), monocytes (FFA2) and dendritic cells (FFA3). Our previous study also showed that the mucosal mast cells expressed FFA2 (29). PMN cells, moncytes (macrophages in the tissue), and dendritic cells are the phagocytes for non-selective antigens involved in the innate immunity. Therefore, the evidence seems to support a hypothesis that one of the role of FFA2 and FFA3 in the intestinal mucosa may be concerned with host defense mechanisms including innate immunity.

Longitudinal muscle. In comparison with circular muscle, the contribution of longitudinal smooth muscle to colonic propulsion has been less studied. However, longitudinal as well as circular muscle layers are also important during peristalsis because GCs are observed not only in circular muscle layer, but also in longitudinal muscle layer of the colon (44). Therefore, we have investigated the spontaneous longitudinal muscle contractions induced by SCFAs. At more than 1 mM of SCFAs (mixture of acetate, propionate and butyrate), they concentration-dependently decreased the frequency of spontaneous longitudinal muscle contractions and reached a maximum at 5 mM (41). However, the SCFAs did not affect the amplitude or duration of spontaneous longitudinal muscle contractions. Among individual SCFAs, only acetate decreases the frequency of spontaneous contractions in longitudinal strips of the rat distal colon. Thus, it is suggested that acetate appears to be a substantial stimulus of SCFA-induced inhibitory response in rat distal colon. TTX and the combination of nicotinic receptor blockade, hexamethonium and 5-HT_3 receptor antagonist, granisetron abolished SCFA-induced inhibitory response (41). The results suggest that the inhibitory response induced by acetate is mediated by enteric nervous system including nicotinic and 5-HT_3 receptors. For the involvement of FFA2 or FFA3 receptors on acetate-induced inhibitory response, FFA2 receptor seems to be involved in the response because the potency orders of each SCFA for FFA3 is propionate > butyrate >> acetate, whereas FFA2 is equal sensitive to SCFA and acetate is more selective for FFA2.

GPR84 and GPR120. GPR84 and GPR120 have recently been shown to be activated by FFAs (24, 58, 59). The GPR84 responds to medium-chain FFA (C9--C14) and is expressed in the leukocytes (59). The functional analysis using GPR84-deficient mice revealed that GPR84 has a functional role in the regulation of early IL-4 gene expression in activated T cells (56). Like FFA1, GPR120 is a receptor for both saturated (C14-C18) and unsaturated (C16-C22) FFAs (24). GPR120 is highly expressed in the human and mouse intestinal tract and mouse enteroendocrine cell line, STC-1 (24, 39, 54). STC-1 cell line is known to release of CCK and GLP-1 by FFAs (24, 49). GPR120 promotes the secretion of the GLP-1 from enteroendocrine L cells (24); unsaturated long-chain FFAs (such as α-linolenic acid) evoke the GLP-secretion via GPR120 in STC-1 enteroendocrine cells (1, 24). In addition, FFAs were found to inhibit serum-deprivation-induced apoptosis through GPR120 in the murine enteroendocrine cell line STC-1 (31). Oral administration of FFA and direct administration to the colon also increase circulating GLP-1 and insulin levels in mice (24).

Immunoreactivity for GPR120 was abundant in the mouse large intestine, lung, and adipose tissue (39). In the lung, the GPR120 protein was expressed in the areas that were positive for the Clara cell marker protein CC10. This observation may indicate that the FFA receptor GPR120 is involved in the function of Clara cells. In adipose tissues, GPR120 was found to be highly and widely expressed on the plasma membrane surface of mature mouse adipocytes (39).

Conclusion

Recently, multiple GPCR have been deorphanized as FFA receptors of FFAs. FFA receptors belong to the nutrient-sensing receptors, which directly monitor the levels of nutrients in the gut lumen and mediate the secretion or production of gut hormones. We have briefly reviewed the role of FFA receptors and the SCFAs-induced muscle contractions based on our recent studies and others. SCFA receptors, FFA2 and FFA3 are located in mucosal enteroendocrine cells containing PYY related to energy balance. Thus, FFA2 and FFA3 are important molecular devices to monitor the chemical composition in colonic lumen. For the local function of SCFAs, it should be stressed that individual SCFA have different mode of actions on colonic smooth muscles. These different effects may be due to the relative contribution of FFA2 and FFA3 on the control of intestinal muscle activity. For the remote effects of FFA2 or FFA3 on the whole body energy balance, FFA2 or FFA3 may be contributed through the release of gastrointestinal hormones related to feeding and satiety control including PYY and GLP-1. Although further studies are needed to identify the precise roles of FFA receptors, the functional analysis of these FFA receptors should be valuable for understanding nutrient metabolism in the body.

References

1. *Adachi T., Tanaka T., Takemoto K., Koshimizu T.-A., Hirasawa A., Tsujimoto G.*: Free fatty acids administered into the colon promote the secretion of glucagon-like peptide-1 and insulin. Biochem. Biophys. Res. Commun. 2006, 340, 332-337.
2. *Bartoov-Shifman R., Rinder G., Bahar K., Rubins N., Walker M. D.*: Regulation of the gene encoding GPR40, a fatty acid receptor expressed selectively in pancreatic β cells. J. Biol. Chem. 2003, 282, 23561-23571.
3. *Bergman E. N.*: Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 1990, 70, 567-590.
4. *Bloemen J. G., Venema K., van de Poll M. C., Olde Damink S. W.*: Short chain fatty acids exchange across the gut and liver in humans measured at surgery. Clinical Nutri. 2009, 28, 657-661.
5. *Briscoe C. P., Tadayyon M., Andrews J. L., Benson W. G., Chambers J. K., Eilert M. M., Ellis C., Elshourbagy N. A., Goetz A. S., Minnick D. T. et al.*: The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. J. Biol. Chem. 2003, 278, 11303-11311.

6. *Brown A. J., Goldsworthy S. M., Barnes A. A., Eilert M. M., Tcheang L., Daniels D., Muir A. I., Wigglesworth M. J., Kinghorn I., Fraser N. J., Pike N. B., Strum J. C., Steplewski K. M., Murdock P. R., Holder J. C., Marshall F. H., Szekeres P. G., Wilson S., Ignar D. M., Foord S. M., Wose A., Dowell S. J.*: The orphan G protein-coupled receptor GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J. Biol. Chem. 2003, 278, 11312-11219.
7. *Brown A. J., Jupe S., Briscoe C. P.*: A family of fatty acid binding receptors. DNA Cell Biol. 2005, 24, 54-61.
8. *Buchan A. M.*: Nutrient tasting and signaling mechanism in the gut. III. Endocrine cell recognition of luminal nutrients. Am. J. Physiol. 1999, 277, G1103-G1107.
9. *Cherbut C., Ferrier L., Roze C., Anini Y., Blottiere H., Lecannu G., Galmiche J. P.*: Short-chain fatty acids modify colonic motility through nerves and popypeptide YY release in the rat. Am. J. Physiol. 1998, 275, G1415-G1422.
10. *Dobbins R. L., Chester M. W., Stevenson B. E., Daniels M. B., Stein D. T., Garry J. D.*: A fatty acid-dependent step is critically important for both glucose- and non-glucose-stimulated insulin secretion. J. Clin. Invest. 1998, 101, 2370-2376.
11. *Dockary G. J.*: Luminal sensing in the gut: an over view. J. Physiol. Pharmacol. 2003, 54, (Suppl. 4), 9-17.
12. *Edfalk S., Steneberg P., Edlund H.*: Gpr40 is expressed enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. Diabetes 2008, 57, 2280-2287.
13. *Eftimiadi C., Buzzi E., Tonetti M., Buffa P., Buffa D., van Steenbergen M. T., de Graaff J., Botta G. A.*: Short-chain fatty acids produced by anaerobic bacrteria alter the physiological responses of human neutrophils to chemotactic peptide. J. Infect. 1987, 14, 43-53.
14. *Flodgren E., Olde B., Meidute-Abaraviciene S., Winzell M. S., Ahren B., Salehi A.*: GPR40 is expressed in glucagon producing cells and affects glucagon secretion. Biochem. Biophys Res. Commun. 2007, 354, 240-245.
15. *Fredstrom S. B., Lampe J. W., Jung H. J. et al.*: Apparent fiber digestibility and fecal short-chain fatty acid concentrations with ingestion of two types of dietary fiber. JPEN J. Parebter. Enteral Nutr. 1994, 18 (1), 14-19.
16. *Fukumoto S., Takewaki M., Yamada T., Fujimiya M., Manthy C., Voss M., Eubanks S., Harris M., Pappas T. N., Takahashi T.*: Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. Am. J. Physiol. 2003, 284, R1269-R1276.
17. *Furness J. B., Kunze W. A., Clerc N.*: Nutrient tasting and signaling mechanisms in the gut. II. The intestine as sensory organ: neural, endocrine, and immune responses. Am. J. Physiol. 1999, 277, G922-G928.
18. *Ge H., Li X., Weiszmann J., Wang P., Baribault H., Chen J. L., Tian H., Li Y.*: Activation of GPR43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. Endocrinology 2008, 149, 4519-4526.
19. *Genuth S. M.*: Whole body Metabolism. Physiology 4th ed. Berne R. M. and Levy M. N. eds. Mosby, Inc. 1998, p. 810.
20. *Gonzalez A., Sarna S. K.*: Neural regulation of in vitro giant contractions in the rat. Am. J. Physiol. 2001, 281, G275-G282.
21. *Gravena C., Mathias P. C., Ashcroft S. J.*: Acute effects of fatty acids on insulin secretion from rat and human islets of Langerhans. J. Endocrinol. 2002, 173, 73-80.
22. *Hallert C., Bjorck I., Nyman M. et al.*: Increasing fecal butyrate in ulcerative colitis patients by diet: controlled pilot study. Inflamm. Bowel Dis. 2003, 9 (2), 116-121.
23. *Hardy S., St-Onge G. G., Joly E., Langelier Y., Prentki M.*: Oleate promotes the proliferation of breast cancer cells via the G protein-coupled receptor GPR40. J. Biol. Chem. 2005, 280, 13285-13291.
24. *Hirasawa A., Tsumaya K., Awaji T., Katsuma S., Adachi T., Yamada M., Sugimoto Y., Miyazaki S., Tsujimoto G.*: Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat. Med. 2005, 11, 90-94.
25. *Hofer D., Asan E., Drenckhahn D.*: Chemosensory perception in the gut. News Physiol. Sci. 1999, 14, 18-23.
26. *Hong Y. H., Nishimura Y., Hishikawa D., Tsuzuki H., Miyahara H., Gotoh C., Choi K. C., Feng D. D., Chen C., Lee H. G., Katoh K., Roh S. G., Sasaki S.*: Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. Endocrinology 2005, 146, 5092-5099.
27. *Hubel K. A., Russ L.*: Mechanisms of the secretory response to luminal propionate in rat descending colon in vitro. J. Auton. Nerv. Sys. 1993, 43, 219-230.
28. *Itoh Y., Kawamata Y., Harada M., Kobayashi M., Fujii R., Fukushima S., Ogi K., Hosoya M., Tanaka Y., Uejima H., et al.*: Free fatty acids regulate insulin secretion from pancreatic b cells through GPR40. Nature 2003, 322, 173-176.
29. *Karaki S.-I., Mitsui R., Hayashi H., Kato I., Sugiya H., Iwanaga T., Furness J. B., Kuwahara A.*: Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. Cell Tissue Res. 2006, 324, 353-360.
30. *Karaki S.-I., Tazoe H., Hayashi H., Kashiwabara H., Tooyama K., Suzuki Y., Kuwahara A.*: Expression of the short-chain fatty acid receptor, GPR43, in the human colon. J. Mol. Histol. 2008, 39, 135-142.
31. *Katsuma S., Hatase N., Yano T., Ruike Y., Kimura M., Hirasawa A., Tsujimoto G.*: Free fatty acids inhibit serum deprivation-induced apoptosis through GPR120 in a murine enteroendocrine cell line STC-1. J. Biol. Chem. 2005, 280, 19507-19515.
32. *Kotarsky K., Nilsson N. E., Flodgren E., Owman C., Olde B.*: A human cell surface receptor activated by free fatty acids and thiazolidinedione drugs. Biochem. Biophys. Res. Res. Commun. 2003, 301, 406-410.
33. *Le poul E., Loison C., Struyf S., Springael J.-Y., Lannoy V., Decobecq M.-E., Brezillon S., Dupriez V., Vassart G., Van Damme J., Parmentier M., Detheux M.*: Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. J. Biol. Chem. 2003, 278, 25481-25489.
34. *Ma D., Tao B., Warashina S., Kotani S., Lu L., Kaplamadzhiev D. B., Mori Y., Tonchev A. B., Yamashima T.*: Expression of the free fatty acid receptor GPR40 in the central nervous system of adult monkeys. Neurosci. Res. 2007, 58, 394-401.
35. *Maslowski K. M., Vieira A. T., Ng A., Kranich J., Sierro F., Yu D., Schilter H. C., Rolph M. S., Mackay F., Artis D., Xavier R. J., Teixeira M. M., Mackay C. R.*: Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Natrue 2009, 461, 1282-1287.
36. *Mitsui R., Karaki S.-I., Kubo Y., Sigiura Y., Kuwahara A.*: Fibre-free diet leads to impairment of neuronally mediated muscle contractile response in rat distal colon. Neurogastroenterol. Motil. 2006, 18, 1093-1101.
37. *Mitsui R., Ono S., Karaki S.-I., Kuwahara A.*: Neural and non-neural mediation of propionate-induced contractile responses in the rat distal colon. Neurogastroenterol. Motil. 2005, 17, 585-594.
38. *Mitsui R., Ono S., Karaki S.-I., Kuwahara A.*: Propionate modulates spontaneous contractions via enteric nerves and prostglandin in the rat distal colon. Jpn. J. Physiol. 2005, 55, 331-338.
39. *Miyauchi S., Hirasawa A., Iga T., Liu N., Itsubo C., Sadakane K., Hara T., Tsujimoto G.*: Distribution and regulation of protein expression of the free fatty acid receptor GPR120. Naunyn Schmmiedebergs Arch Pharmacol. 2009, 379, 427-433.
40. *Nilsson N. E., Kotarsky K., Owman C., Olde B.*: Identification of a free fatty acid receptor, FFRA2, expressed on leukocytes and activated by short-chain fatty acids. Biochem. Biophys. Res. Commun. 2003, 303, 1047-1052.
41. *Ono S., Karaki S.-I., Kuwahara A.*: Short-chain fatty acids decrease in the frequency of spontaneous contractions of longitudinal muscle via enteric nerves in rat distal colon. Jpn. J. Physiol. 2004, 54, 483-493.
42. *Ono S., Mitsu R., Karaki S.-I., Kuwahara A.*: Muscarinic and 5-HT4 receptors participate in the regulation of the frequency of spontaneous contractions of the longitudinal muscle in rat distal colon. Bomedical Res. 2005, 26, 173-177.
43. *Peters S. G., Pomare E. W., Fisher C. A.*: Portal and peripheral blood short chain fatty acid concentrations after caecal lactulose instillation at surgery. Gut 1992, 33 (9), 1249-1252.
44. *Powel A. K., Bywater R. A. R.*: Murine intestinal migrating motor complexes: longitudinal components. Neurogastroenterol.&Mitil. 2003, 15, 245-256.
45. *Rinder G., Bartoov-Shifman R., Zalogan T., Avnit-Sagi T., Bahar K., Sharivkin R., Kantorovich L., Weiss S., Walker M. D.*: Regulation of GPR40 locus: towards a molecular understanding. Biochem. Soc. Trans. 2008, 36, 360-362.
46. *Sawzdarogo M., George S. R., Nguyen T., Xu S., Kolakowski Jr. L. F., O'Dowd B. F.*: A cluster of four novel human G proteing-coupled receptor genes occurring in close proximity to CD22 gene on chromosome 19q13.1 Biochem. Biophys. Res. Commun. 1997, 239, 543-547.
47. *Senga T., Iwamoto S., Yoshida T., Yokota T., Adachi K., Azuma E., Hamaguchi M., Iwamoto T.*: LSSIG is a novel murine leukocyte-specific GPCR that is induced by the activation of STAT3. Blood 2003, 101, 1185-1187.
48. *Shapiro H., Shachar S., Sekler I., Hershfinkel M., Walker M. D.*: Role of GPR40 in fatty acid action on the b-cell line INF-1E. Biochem. Biophys. Res. Commun. 2005, 335, 97-104.
49. *Sidhu S. S., Thompson D. G., Warhurst G., Case R. M., Benson R. S.*: Fatty acid-induced cholecystokinin secretion and changes in intracellular Ca^{2+} in two enteroendocrine cell lines, STC-1 and GLUTag. J. Physiol. 2000, 528, 165-176.
50. *Spector A. A., Hoak J. C.*: Letter: Fatty acids, platelets, and microcirculatory obstruction. Science 1975, 190, (4213), 490-492.
51. *Stein D. T., Esser V., Stevenson B. E., Lane K. E., Whiteside J. H., Daniels M. B., Chen S., McGarry J. D.*: Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. J. Clin. Invest. 1996, 97, 2728-2735.

52. *Stenberg P., Rubins N., Bartoov-Shifman R., Walker M. D., Edlund H.*: The FFA receptor GPR40 links hyperinsulinemia, hepatic stetosis, and impaired glucose homeostasis in mouse. Cell Metab. 2005, 1, 245-258.
53. *Stoddart L. A., Smith N. J., Milligan G.*: International union of pharmacology. LXXI. Free fatty acid receptors, FFA1,-2, and -3: pharmacology and pathophysiological functions. Pharmacol. Rev. 2008, 60, 405-417.
54. *Tanaka T., Katsuma S., Adachi T., Koshimuzu T.-A., Hirasawa A., Tsujimoto G.*: Free fatty acids induce cholecystokinin secretion through GPR120. Naunyn Schmmiedebergs Arch. Pharmacol. 2008, 377, 523-527.
55. *Tazoe H., Otomo Y., Karaki S.-I., Kato I., Fukami Y., Terasaki M., Kuwahara A.*: Expression of short-chain fatty acid recptor GPR41 in the human colon. Biomedical Res. 2009, 30, 149-156.
56. *Tomita T., Masuzaki H., Noguchi M., Iwakura H., Fujikura J., Tanaka T., Ebihara K., Kawamura J., Komoto I., Kawaguchi Y. et al.*: GPR40 gene expression in human pancreas and insulinoma. Biochem. Biophys. Res. Commun. 2005, 338, 1788-1790.
57. *Topping D. L., Clifton P. M.*: Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol. Rev. 2001, 81 (3), 1031-1064.
58. *Venkataraman C., Kuo F.*: The G-protein coupled receptor, GPR84 regulates IL-4 production by T lymphocytes in response to CD3 crosslinking. Immunol. Lett. 2005, 101, 144-153.
59. *Wang J., Wu X., Simonavicius N., Tian H., Ling L.*: Medium-chain fatty acids as ligands fro orphan G protein-coupled receptor GPR84. J. Biol. Chem. 2006, 281, 34457-34464.
60. *Weaver G. A., Tangel C. T., Krause J. A. et al.*: Acarbose enhances human colonic butyrate production. J. Nutr. 1997, 127 (5), 717-723.
61. *Wong J. M., de Souza R., Kendall C. W., Eman A., Jenkins D. J.*: Colonic health: fermentation and short chain fatty acids. J. Clin. Gastroenterol. 2006, 40 (3), 235-243.
62. *Yajima T.*: Contractile effect of short-chain fatty acids on the isolated colon of the rat. J. Physiol. 1985, 368, 667-678.
63. *Yajima T.*: Luminal propionate-induced secretory response in the rat distal colon in vitro. J. Physiol. 1988, 403, 559-575.
64. *Yonezawa T., Katoh K., Obara Y.*: Existence of GPR40 functioning in a human brest cancer cell line, MCF-7. Biochem. Biophys. Res. Commun. 2004, 314, 806-809.
65. *Yuli I., Oplatka A.*: Cytosolic acidification as an early transductory signal of human neutrophil Chemotaxis. Science 1987, 235, 340-342.

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Receptory wolnych kwasów tłuszczowych i ich rola w okrężnicy

Streszczenie

Wolne kwasy tłuszczowe (FFA) są nie tylko ważnym źródłem energii, ale również monitorują skład chemiczny światła okrężnicy. FFA działają jako ligandy dla receptorów sprzężonych z białkami G (RSBG), takich jak: FFA2, FFA3, GPR84 i GPR120. Spośród wymienionych receptorów kwasy tłuszczowe średnio- (MCFA) i długołańcuchowe (LCFA) aktywują receptory FFA1 i GPR120. Receptory GPR84 pobudzane są wyłącznie przez MCFA. Natomiast zarówno receptory FFA2, jak i FFA3 występujące w okrężnicy, aktywowane są przez krótkołańcuchowe kwasy tłuszczowe (SCFA) i uczestniczą w regulacji motoryki mięśniówki okrężnej. Propionian i maślan, w sposób zależny od stężenia wzbudzają fazowe (okresowe) i toniczne (ciągłe) skurcze mięśniówki okrężnej okrężnicy szczura. Każdy SCFA odmiennie odbierany jest przez te receptory i odmiennie działa na wzory motoryczne okrężnicy.

Pierwszym poziomem integracji informacji pochodzącej ze światła jelit są komórki enteroendokrynne i wyspecjalizowane komórki nabłonka enterocytów. Na ich błonach komórkowych są zarówno receptory FFA1 (początkowo określane jako GPR40) i GPR120 aktywowane przez średnio- (MCFT) i długo- (LCFA) łańcuchowe kwasy tłuszczowe, jak i receptory FFA2 (GPR43) i FFA3 (GPR41) aktywowane przez SCFA. W opracowaniu sumarycznie przybliżono wyniki badań własnych oraz aktualną wiedzę nt. specyficznej roli w regulacji motoryki okrężnicy różnych form receptorów kwasów tłuszczowych (FFAs), szczególnie zaś receptorów FFA2 i FFA3, dotychczas uważanych za „sieroce", tj. pozbawione specyficznego działania w przekaźnictwie nerwowym.

Źródłem SCFA w jelicie grubym są procesy fermentacyjne niestrawionych węglowodanów, głównie włókna i skrobi, a także, przy zaburzeniach wchłaniania (choroba trzewna, zespół poposiłkowy – dumping syndrom), węglowodany wymykające się z jelit cienkich. Powstają one również w niewielkim zakresie z bakteryjnej fermentacji białek endogennych (złuszczony nabłonek, śluz) i paszowych (ryc. 1 i 2). Całościowe i relatywne stężenia molarne głównych SCFA w jelitach człowieka: octanu, propionianu i maślanu przeciętnie wynoszą 60:20:20, zależą jednak od miejsca fermentacji, rodzaju pokarmu i składu jelitowego mikrobiotu. Ze względu na szybkie wchłanianie około 90% SCFA i metabolizację trudno jest uchwycić tempo ich wytwarzania.

Receptory FFAR zostały odkryte podczas poszukiwań nowych podtypów receptora galaninowego, jako zbiorowisko czterech tandemowych genów GPCR: FFA1 (GPR40), FFA2 (GPR43), FFA3 (GPR41) i GPR42, rozmieszczonych na ludzkim chromosomie 19q13 (ryc. 3). GPR42 jest pseudogenem z otwartą ramką odczytu. Agonistami receptorów FFA1 są LCFA, a SCFA o długości łańcucha poniżej sześciu węgli są endogennymi agonistami receptorów FFA2 i FFA3. Nienasycone kwasy tłuszczowe (NSFA) pobudzają receptor GPR120.

Receptor FFA1 aktywowany jest przez nasycone (SFA) i nienasycone (NSFA) kwasy MCFA i LCFA. Siła pobudzania tego receptora zależy od długości łańcucha nasyconych kwasów tłuszczowych (SFA), z najsilniejszym działaniem kwasu pentadekanowego (C15) i palmitynowego (C16). W przypadku NSFA ani długość łańcucha, ani też stopień nasycenia nie korelują z ich siłą pobudzającą. Największa ekspresja FFA1 u ludzi i szczurów występuje zarówno na poziomie mRNA, jak i białka, w insulinowych wysepkach trzustkowych. W stanie głodzenia u ludzi i gryzoni FFAs odgrywają ważną rolę w zachowaniu zarówno podstawowego, jak i pobudzanego glukozą wydzielania insuliny.

Receptory FFA2 (GPR43) i FFA3 (GPR41) są pobudzane przez SCFA. Największa ekspresja FFA2 znajduje się na komórkach immunologicznych, w tym na komórkach z polimorficznym jądrem (PMN). Wykazano, że stymulacja FFA2 przez SCFA konieczna jest do normalnego rozwiązania pewnych odpowiedzi zapalnych. Występowanie receptorów FFA2 w tkance tłuszczowej powoduje, że w obecności ich ligandów (octanu i propionianu) obniża się aktywność lipolityczna. Wysoki poziom ekspresji receptora FFA3 rozpoznano w trzustce, śledzionie, węzłach chłonnych, szpiku kostnym, obwodowych komórkach mononuklearnych krwi oraz przypuszczalnie w tkance tłuszczowej. Ze względu na dużą ilość SCFA w jelicie grubym, fizjologicznie właściwym miejscem aktywacji receptorów FFA jest jelito. Autorzy tego opracowania po przygotowaniu przeciwciał przeciw FFA2 i FFA3 prześledzili ekspresję FFA2 i FFA3 w okrężnicy. Immunoreaktywność w stosunku do receptorów FFA2 wykazano w enterocytach śluzówki okrężnicy szczurów i ludzi, w komórkach enteroendokrynnych oraz małych komórkach blaszki właściwej (ryc. 4). FFA3 występują w ilości mniejszej od FFA2 w okrężnicy człowieka jako punktowe plamki w szczytowej części enterocytów i komórek enteroendokrynnych (KEE). Ponadto oba typy receptorów nie występują w kolokalizacji. Okazało się, że SCFA modyfikują motorykę okrężnicy przez wpływ na uwalnianie 5 -hydroksytryptaminy (5-HT, serotoniny) i PYY.

Mięśniówka okrężnicy, tak *in vivo,* jak i *in vitro,* u większości gatunków odznacza się dwoma oddzielnymi typami skurczów: rytmicznymi skurczami fazowymi i skurczami spontanicznymi, określanymi jako skurcze masowe (giant contractions, GCs). Według Kuwahary i wsp., tylko propionian zwiększa częstość i zmniejsza średnią amplitudę skurczów GCs. Stymulujący efekt propionianu odnośnie do częstości GCs ważny jest więc dla przesuwania mas kałowych w okrężnicy.

SCFA oddziałują również na podstawową aktywność skurczową mięśniówki okrężnej. Propionian i maślan w sposób zależny od stężenia (10 μM – 10 mM) wzbudzają fazowe skurcze o dużej amplitudzie, po czym pojawiają się skurcze toniczne. Toniczne i fazowe skurcze nie występowały w skrawkach okrężnicy pozbawionych śluzówki (ryc. 5). Propionian nie działa więc bezpośrednio na mięśniówkę okrężną. Pobudzający, skurczowy efekt w mięśniówce okrężnej propionianu atenuuje atropina, tetrodotoksyna (TTX) i antagonista receptora 5-HT_4 (SB204070). Propionian działa na śluzówkowe receptory FFA2 albo FFA3, powodując uwalnianie 5-HT z komórek enterochromochłonnych. Aktywacja przez 5-HT receptorów 5-HT_4 znajdujących się na zakończeniach pierwotnych neuronów aferentnych, zwrotnie aktywuje motoryczne neurony cholinergiczne kurczące mięśniówkę okrężną. Atenuacja skurczów tonicznych zarówno przez nieselektywny inhibitor COX, piroksikam, jak i selektywny inhibitor COX-1 (SC-560) najwyraźniej wskazuje, że propionian wzbudza również uwalnianie produktów COX powodujących skurcze toniczne. Wydaje się, że skurcze mięśniówki okrężnej wzbudzane propionianem odbywają się z udziałem receptorów FFA3. Ponadto receptory FFAs są ekspresjonowane przez komórki obronne układu immunologicznego i są przez to zaangażowane w mechanizmy obronne związane z odpornością wrodzoną.

Mięśniówka podłużna ma małe znaczenie w przesuwaniu treści pokarmowej. Pojedynczo tylko octan, uważany za znaczący bodziec, obniża częstość skurczów spontanicznych w mięśniówce podłużnej okrężnicy dalszej szczura. Ta odpowiedź hamująca znoszona jest przez TTX i kombinacyjną blokadę receptora nikotynowego przez heksametonium i antagonistę receptora 5-HT_3, granisetron. Odpowiedź hamująca wzbudzana octanem zachodzi zatem za pośrednictwem neuronów enterycznego układu nerwowego (EUN) i receptorów nikotynowych oraz 5-HT_3. Wydaje się, że odpowiedzi hamujące octanu zachodzą przy udziale receptorów FFA2.

Białko GPR84 jest wprawdzie receptorem dla MCFA, ale większą rolę niż w przewodzie pokarmowym odgrywa w sygnalizacji aktywującej komórek T do wytwarzania IL-4. Natomiast zbliżone do FFA1 białko GPR120 odbiera sygnalizację pochodzącą od SFA (C14-C18) i USFA (C16-C22), występujących w przewodzie pokarmowym.

Z pewnej części dotychczas sierocych receptorów sprzężonych z białkami G wyodrębniono w okrężnicy grupę receptorów FFAs aktywowanych przez wolne kwasy tłuszczowe (FFA). Receptory FFAs, jako sensory składników pokarmowych w jelitach, bezpośrednio monitorują ich poziom w świetle jelit i pośredniczą, z jednej strony, w wytwarzaniu oraz sekrecji hormonów jelitowych, z drugiej – w aktywności motorycznej. Poszczególne SCFA różnie aktywują FFAs, a odpowiedzi motoryczne uzależnione są od relatywnego poziomu receptorów FFA2 i FFA3.

Słowa kluczowe: G receptor sprzężony z białkiem G, krótkołańcuchowy kwas tłuszczowy, FFA2, FFA3, okrężnica człowieka, okrężnica szczura, motoryka okrężnicy