Cell Tissue Res (2006) 324: 353–360
DOI 10.1007/s00441-005-0140-x

REGULAR ARTICLE

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Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine

Received: 18 October 2005 / Accepted: 28 November 2005 / Published online: 1 February 2006

Abstract Short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, are the major anions in the large intestinal lumen. They are produced from dietary fiber by bacterial fermentation and are known to have a variety of physiological and pathophysiological effects on the intestine. In the present study, we investigated the expression of the SCFA receptor, GPR43, in the rat distal ileum and colon. Expression of GPR43 was detected by reverse transcriptase/polymerase chain reaction (RT-PCR), Western blotting, and immunohistochemistry. mRNA for GPR43 was detected, by RT-PCR, in extracts of the whole wall and separated mucosa from the ileum and colon and from muscle plus submucosa from the ileum, but not from muscle plus submucosa preparations from the colon. We raised a rabbit antiserum against a synthesized fragment of rat GPR43; this was specific for rat GPR43. GPR43 protein was detected by Western blot analysis in extracts of whole wall and separated mucosa, but not in muscle plus submucosa extracts. By immunohistochemistry, GPR43 immunoreactivity was localized to enteroendocrine cells expressing peptide YY (PYY), whereas 5-hydroxytryptamine (5-HT)-immunoreactive (IR) enteroendocrine cells were not immunoreactive for GPR43. Mast cells of the lamina propria expressing 5-HT were also GPR43-IR. The results of the present study suggest that the PYY-containing enteroendocrine cells and 5-HT-containing mucosal mast cells sense SCFAs via the GPR43 receptor. This is consistent with physiological data showing that SCFAs stimulate the release of PYY and 5-HT from the ileum and colon.

Keywords Short-chain fatty acids · GPR43 · Enteroendocrine cell · Mucosal mast cell · Peptide YY · 5-Hydroxytryptamine · Intestine · Rat (Wistar, male)

This study was supported by the Promotion of Health and Nutrition from the Danone Institute, by an AstraZeneca Research Grant, and by the Australian Research Council (grant #DP0557307).

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Introduction

Short-chain fatty acids (SCFAs) are the major anions, present at about 100 mM, in the lumen of the non-ruminant mammalian large intestine. They are produced by bacterial fermentation of undigested carbohydrate from ingested dietary fiber. They are 2-carbon to 5-carbon weak acids, including acetate (C2), propionate (C3), butyrate (C4), and valerate (C5). The ratio of SCFA concentrations in the colonic lumen is about 60% acetate, 25% propionate, and 15% butyrate. Luminal SCFAs not only are absorbed as

nutrients across the intestinal epithelium, but also influence various functions of the gastrointestinal tract (Cummings et al. 1995). For example, SCFAs are known to affect colonic motility and ion transport (Yajima 1985, 1988; Fukumoto et al. 2003) and inhibit transit in regions more proximal to their luminal application (Cherbut et al. 1997).

Yajima (1985, 1988) first reported that propionate, butyrate, and valerate, but not acetate, induce transient contraction and chloride (Cl^-) secretion in the rat distal colon. Moreover, Fukumoto et al. (2003) have reported that luminal application of SCFAs induces intraluminal 5-hydroxytryptamine (5-HT) release, provokes contractions, and stimulates transit in the rat proximal colon. The propionate-induced contraction is mediated neuronally via 5-HT and acetylcholine and is non-neuronally mediated through prostaglandin release (Mitsui et al. 2005). SCFAs stimulate sodium (Na^+) and Cl^- absorption, and bicarbonate secretion in the rat colon (Umesaki et al. 1979). The inhibition of Cl^- secretion in the human colonic epithelial cell line, T84, is associated with the inhibition of adenylate cyclase expression (Resta Lenert et al. 2001).

Although these reports indicate that luminal SCFAs activate colonic motility and ion transport, there is opposing evidence that SCFAs inhibit intestinal motility and ion transport. Squires et al. (1992) have reported that luminal infusion of SCFAs inhibits contractile activity in the rat colon. Cherbut et al. (1998) have also reported a similar inhibitory effect of SCFAs mediated through a neuroendocrine mechanism involving peptide YY (PYY). Moreover, we have observed that acetate decreases the frequency of spontaneous contractions in the longitudinal muscle of the rat distal colon (Ono et al. 2004).

In addition to local effects, luminal SCFAs in the ileum and colon reduce the motility of more proximal gut regions. These inhibitory effects induced by luminal fat are known as the ileal or colonic brake (Lin et al. 1990; Cherbut et al. 1997). The brake is a physiological mechanism to slow the transit of contents from the upper small intestine to the distal ileum and colon, presumably to allow time for the metabolism of fats. The inhibition of more proximal regions by luminal fats is dependent on the release of PYY (Lin et al. 1996; Cuche et al. 2000). PYY is a product of enteroendocrine cells that are abundant in the distal ileum and colon (Lundberg et al. 1982; El-Salhy et al. 1983). Lin et al. (2003, 2004) have reported that the slowing of intestinal transit by PYY is dependent on β-adrenergic, serotonergic, and opioid pathways.

The mechanisms by which intraluminal SCFAs are sensed are not known. In 2003, two orphan G-protein coupled receptors, GPR41 and GPR43, were discovered to be receptors for SCFAs (Brown et al. 2003; Le Poul et al. 2003). The potency orders of each SCFA for GPR41 is propionate>butyrate>>acetate, whereas GPR43 is equally sensitive to each SCFA. Acetate is more selective for GPR43 than GPR41. Neither of the SCFA receptors has been localized in the gastrointestinal tract. We have been successful in raising antibodies to GPR43 and now report its presence in the intestine as revealed by reverse transcriptase/polymerase chain reaction (RT-PCR), Western blotting, and immunohistochemistry.

Materials and methods

Animal preparations

The methods used in the present study were approved by the National Institute for Physiological Sciences of Japan, according to "The Guide for Animal Experimentation" of the National Institute. For RT-PCR and Western blotting analyses, male Wister rats (SLC; Hamamatsu, Shizuoka, Japan) were anesthetized with diethylether and decapitated with a guillotine. For immunohistochemistry, rats were anesthetized by injection (i.p.) with pentobarbital (Nembutal Injection; Dainippon Pharmaceutical, Osaka, Japan) at 1 ml/kg. Phosphate-buffered saline (PBS; 200–300 ml) was perfused into the left ventricle of the heart to flush out the blood, followed by perfusion of 200–300 ml Zamboni's fixative [2% formaldehyde and 15% saturated picric acid in 0.1 M phosphate buffer pH 7.4).

RT-PCR analysis

The distal ileum and colon were taken, immersed in PBS at 4°C, and cut open along the mesenteric border. Whole tissue samples (about 5 mg) were removed and were immersed immediately in the RNAlater RNA Stabilization Reagent (Qiagen, Tokyo, Japan). Remaining tissues were placed on an acrylic board (mucosal side up), and the mucosa was removed by scraping with the edge of a glass slide. The mucosa samples were immersed immediately in RNAlater RNA Stabilization Reagent. After removal of the mucosa, the remaining gut wall was put in a silicon-rubber-lined dish (mucosal side down, in PBS at 4°C), and the muscle and submucosal layer, including the myenteric and submucosal plexuses, were removed by using micro-scissors. The muscle plus submucosa preparations were also immersed immediately in RNAlater RNA Stabilization Reagent. All samples were then transferred to new 2.0-ml Eppendorf tubes and freeze-ground by using a grinding mill (SK-100; Tokken, Kashiwa, Chiba, Japan). Total RNAs from whole wall, mucosa, and muscle plus submucosa samples were isolated by RNeasy Micro Kit (Qiagen).

Primers for RT-PCR of GPR43 (Table 1) were based on the rat GPR43 mRNA sequence (GenBank: NM_001005877). Primers for β-actin (Table 1) were used to provide a positive control. RT-PCR was performed by using the Qiagen OneStep RT-PCR Kit (Qiagen) and GeneAmp PCR System 2400 (Perkin-Elmer, Norwalk, Conn., USA). Reaction mixtures of isolated RNA samples from whole wall, mucosa, and muscle plus submucosa were incubated at 50°C for 30 min for reverse transcription and then incubated at 95°C for 15 min to activate the HotStarTaq DNA polymerase and to denature the reverse transcriptases. PCR cycles consisted of denaturing at 94°C

Table 1 Primers for rat GPR43 and β-actin

Protein	Primer	
GPR43	Forward	5′–GGCTTCGGCTTCTACAGCAGTATCT–3′
	Reverse	5′–TCCAAAGCACACCAGGAAATTAAGA–3′
β-Actin	Forward	5′–GACTACCTCATGAAGATCCT–3′
	Reverse	5′–CCACATCTGCTGGAAGGTGG–3′

Table 3 Secondary antibodies (*FITC* fluorescein isothiocyanate)

Antibody	Dilution	Source
Donkey anti-rabbit IgG-Cy3	1:200	Jackson
Donkey anti-goat IgG-Alexa488	1:200	Molecular Probes
Donkey anti-chicken IgY-FITC	1:100	Chemicon

for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, and the reactions were repeated for 30 cycles, followed by extension at 72°C for 10 min. Finally, amplification products were stored at 4°C.

The amplification products were separated by electrophoresis on 1.7% agarose gel in 0.5× TRIS-borate-EDTA buffer and stained with ethidium bromide. Bands of the amplification products were viewed by ultraviolet light, and the images were taken by GelDoc2000 (Bio-Rad Laboratories, Hercules, Calif., USA).

Peptide synthesis

Rat GPR43 (246–259)-Cys (FHLRQSPSWRVEAVC) was synthesized by a solid-phase methodology with the Fmoc-strategy by using an automated peptide synthesizer (Pioneer, Applied Biosystems, Foster, Calif., USA). Crude peptide was liberated from the resin by trifluoroacetic acid and was extensively purified by using a Delta 600 high-performance liquid chromatography System (Waters, Milford, Mass., USA) equipped with a 2×25 cm reverse-phase column (Develosil ODS-HG-5; Nomura Chemical, Seto, Aichi, Japan). Elution was carried out on a gradient of 0.01 N HCl/CH_3CN. The purity of the resultant peptide was over 95%.

Production of anti-GPR43 antiserum

The *N*-(6-maleimidocaproyloxy)-sulfosuccinimide sodium salt (Dojindo Laboratories, Kumamoto, Japan; 72.3 mg) and 80.0 mg keyhole limpet hemocyanin (KLH; Wako Pure Chemical, Osaka Japan) were dissolved in 0.1 M HEPES buffer (6 ml, pH 8.1). The mixture was stirred for 2 h at room temperature and purified on an Econo-Pac 10DG column (Bio-Rad Laboratories). The purified maleimido activated KLH in 0.1 M phosphate buffer (PB; 6 ml, pH 7.2) was combined with 16.0 mg synthetic rat GPR43 (246–259) in 0.1 M PB (2 ml, pH 7.2), and the mixture was stirred for 18 h at 4°C. The ensuing conjugate (1.0 ml) was emulsified with 1.0 ml Freund's complete adjuvant (Calbiochem-Boehringer, San Diego, Calif., USA) and mixed for 45 min in an ice bath. The emulsion was injected intradermally into multiple sites of three Japanese white female rabbits (2.0–2.5 kg). For primary immunization, each rabbit received a portion of the emulsion containing approximately 1.33 mg peptide. Immunization was performed at 2-week intervals by using half the dose of the immunogen used for the primary immunization. The rabbits were bled from the marginal ear vein 10 days after each immunization. After the sixth immunization, one of the three rabbits gave a high titer antiserum (RY1505) against rat GPR43 (246–259). The anti-rat GPR43 antiserum (RY1505) was used in the present study.

Table 2 Primary antibodies

Antigen	Host	Dilution	Source
GPR43	Rabbit	1:5,000	Own (RY1505)
5-HT	Goat	1:4,000	ImmunoSter
PYY	Chicken	1:200	GenWay Biotech

Western blot analysis

The distal ileum and colon were taken, immersed in PBS at 4°C, and cut open along the mesenteric border. Samples of whole wall (about 5 mg) were removed, placed in 2.0-ml Eppendorf tubes, and frozen in liquid nitrogen. The remaining tissues were put on an acrylic board (mucosal side up). The mucosa was scraped off with the edge of a glass slide, placed in 2.0-ml Eppendorf tubes, and frozen in liquid nitrogen. The remaining tissue layers were placed in a dish lined with silicon-rubber (mucosal side down) in PBS at 4°C, and the muscle plus submucosal layer, including myenteric and submucosal plexuses, were removed by using micro-scissors. The muscle plus submucosa preparations were also placed in 2.0-ml Eppendorf tubes and frozen in

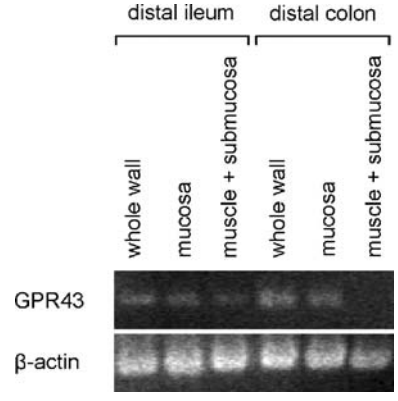

Fig. 1 Analysis of expression of GPR43 mRNA in the rat distal ileum and colon by RT-PCR. All samples in the distal ileum expressed GPR43 mRNA, but no expression was detected in the combined external muscle plus submucosa layers in the distal colon. RT-PCR for β-actin message was used as a control

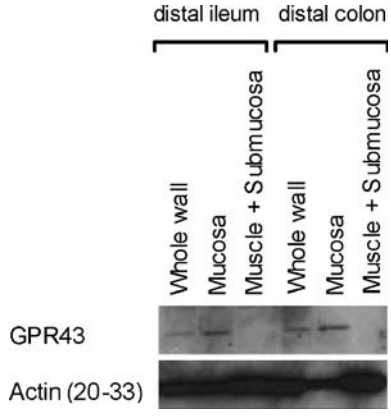

Fig. 2 Analysis of expression of GPR43 protein in the rat distal ileum and colon by Western blots probed with anti-GPR43 antiserum. GPR43-immunoreactive protein was most abundant in the mucosa but was undetectable in the muscle plus submucosa layers. The same volumes of extracted proteins were separated by SDS-PAGE. Westerns blots probed with anti-actin (20–33) antibody were used as a control

liquid nitrogen. All samples were then freeze-ground with a cold grinding mill (SK-100; Tokken) and dissolved in a lysate buffer consisting of 50 mM TRIS-HCl pH 7.4, 150 mM NaCl, 0.05% Triton X-100, 1% protease inhibitor cocktail (P8340, Sigma, St. Louis, Mo., USA). Samples were centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant was taken. Protein concentrations were measured by a Protein Assay kit (Bio-Rad Laboratories). The same amount of 2× Laemmli sample buffer consisting of 2% sodium dodecyl sulfate (SDS), 6% β-mercaptoethanol, and 10% glycerol in 50 mM TRIS-HCl, pH 6.8, with a small amount of bromophenol blue was added to each sample. The samples were incubated at 65°C for 15 min and were stocked at −20°C.

Equal amounts of protein and pre-stained standards (Bio-Rad Laboratories) were separated by electrophoresis in 10% SDS-polyacrylamide gels. Separated proteins were transferred onto nitrocellulose membranes and stained with 1% Ponceau in 7% acetic acid in distilled water. Nitrocellulose membranes were incubated with 5% skim-milk and 0.1% Triton X-100 in PBS for 1 h to suppress non-specific binding of immunoglobulins. The pre-blocked nitrocellulose membranes were incubated with anti-rat GPR43 (RY1505) antibody diluted 1:5,000 or in anti-actin (20–33) diluted 1:10,000 (Sigma) as a positive control in PBS containing 5% skim milk and 0.1% Triton X-100, at 4°C overnight, washed in PBS (3×10 min), incubated with horseradish-peroxidase (HRP)-labeled secondary antibody (1:5,000; Santa Cruz, Calif., USA) in PBS containing 5% skim-milk and 0.1% Triton X-100 for 1 h at room temperature, and washed in PBS (3×10 min). Bands of GPR43

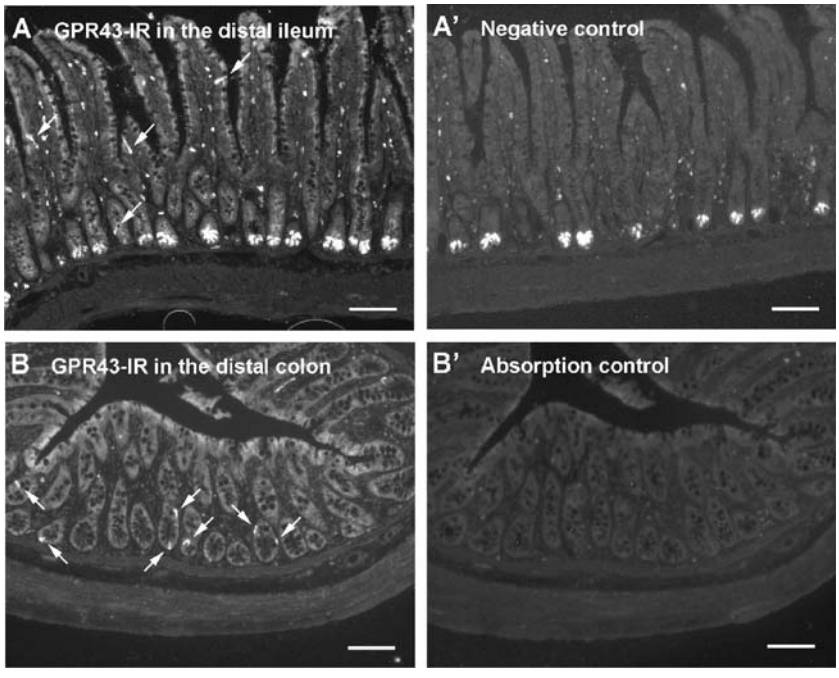

Fig. 3 Immunohistochemistry for GPR43 in the rat distal ileum and colon. Sections (10 μm thick) of the rat distal colon were stained with rabbit anti-rat GPR43 antiserum as the primary antibody and Cy3-labeled anti-rabbit IgG antibody as the secondary antibody. **a** In the ileum, GPR43 immunoreactivity occurred in enteroendocrine cells (*arrows*), small cells in the lamina propria, and the columnar epithelium of the mucosa. **a'** Negative control without GPR43 antiserum. Paneth cells in the bottom of crypts were stained non-specifically only by the secondary antibody. **b** GPR43 immunoreactivity occurred in enteroendocrine cells (*arrows*) and the columnar epithelium of the mucosa of the distal colon. **b'** All immunoreactivity was abolished when the antiserum was equilibrated with the peptide against which the antiserum was raised. *Bar* 100 μm

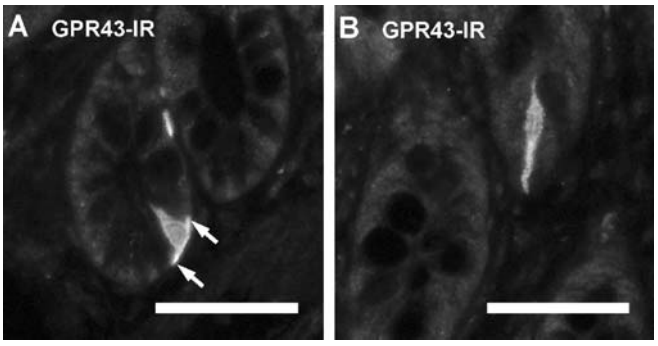

Fig. 4 Morphology of GPR43-IR enteroendocrine cells. Two typical GPR43-IR enteroendocrine cells are shown in the epithelium. **a** GPR43-IR enteroendocrine cell at the base of a colonic crypt. Processes are seen at the base of the cell (*arrows*). **b** Immunoreactive cell that extends across the epithelium (open-type enteroendocrine cell). *Bar* 50 μm

and actin proteins were detected by an analysis system (Amersham Biosciences, Piscataway, N.J., USA) with enhanced chemiluminescence (ECL) Western blotting detection reagents and exposed to X-ray films.

Immunohistochemistry

The distal ileum and colon were taken from rats perfused with Zamboni's fixative and were furthermore immersed in Zamboni's fixative at 4°C overnight. Fixed tissues were washed in PBS (3×10 min) and stored in PBS containing 0.1% sodium azide at 4°C, the PBS being changed each day for 3 days. After being washed, the tissues were stored in PBS containing 30% sucrose and 0.1% sodium azide at 4°C. Cryoprotected tissues were rapidly frozen with optimal cutting temperature (OCT) compound (Tissue-Tek, Sakura Finetechnical, Tokyo, Japan) in a freezer at −80°C and cut into 10-μm-thick sections in a cryostat. Sections on glass slides were dried, washed in PBS (3×10 min) to remove OCT compound, incubated with 5% skim milk and 0.3% Triton X-100 in PBS at room temperature for 30 min to suppress non-specific binding of antibodies, incubated with primary antibodies (Table 2) with 0.3% Triton X-100 at 4°C overnight, washed in PBS (3×10 min), incubated with secondary antibodies (Table 3) for 1 h at room temperature, washed in PBS (3×10 min), and coverslipped with mounting medium (DakoCytomation, Glostrup, Denmark). Immunoreactivity was then visualized by using a fluorescence microscope (IX70, Olympus, Tokyo, Japan), and images were captured by means of a cooled charge-coupled device digital camera system (AxioVision 135; Zeiss, Munich-Hallbergmoos, Germany).

To check the specificity of secondary antibody, a section of distal ileum incubated without primary antibody was stained by secondary antibodies as a negative control. Moreover, to check the specificity of antiserum for GPR43, absorption tests were performed in the distal colon. The diluted (1:1,000) antigen solution, viz., 2 mg rat GPR43 (246–259) plus 8 mg KLH conjugate in 2 ml H_2O, was mixed with the primary antiserum at the final concentration used for immunohistochemistry, and the antigen and diluted antibodies were allowed to equilibrate overnight at 4°C. Sections were incubated with this mixture of antibody and antigen in the immunohistochemistry procedure.

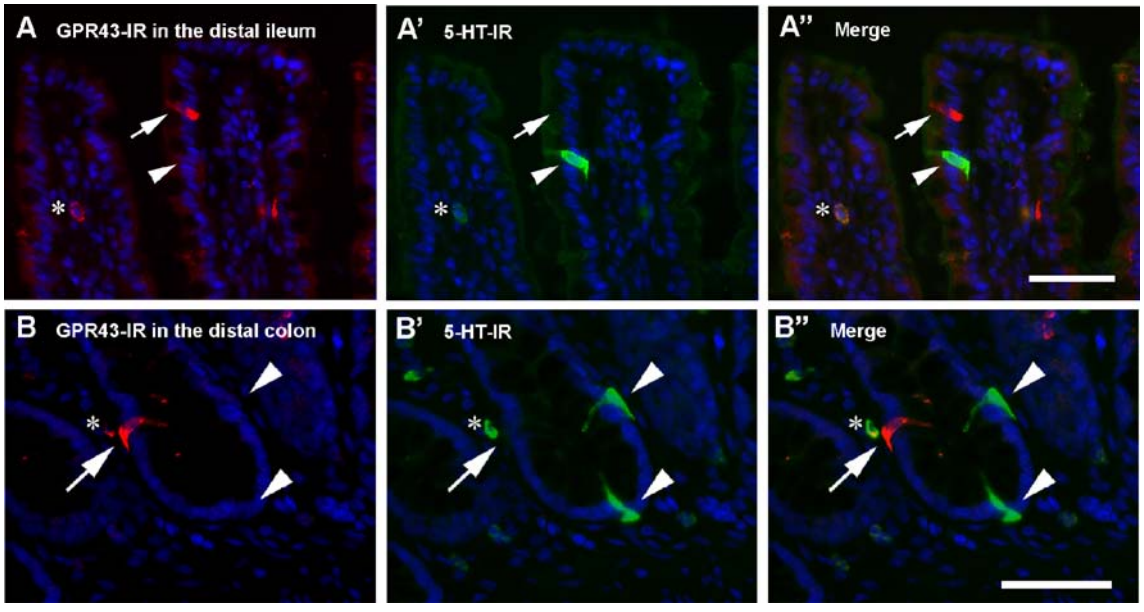

Fig. 5 Lack of colocalization of GPR43 and 5-HT in enteroendocrine cells. Triple-staining for GPR43 (*red*), 5-HT (*green*), and DAPI (4,6-diamidino-2-phenylindole; *blue*) in the mucosa of the distal ileum (**a**) and colon (**b**). Colocalization would appear *yellow*, but there is no colocalization between GPR43 and 5-HT in enteroendocrine cells (*arrows* GPR43-IR enteroendocrine cells, *arrowheads* 5-HT-IR enteroendocrine cells, *asterisk* mucosal mast cells immunoreactive for both GPR43 and 5-HT in the lamina propria). *Bar* 50 μm

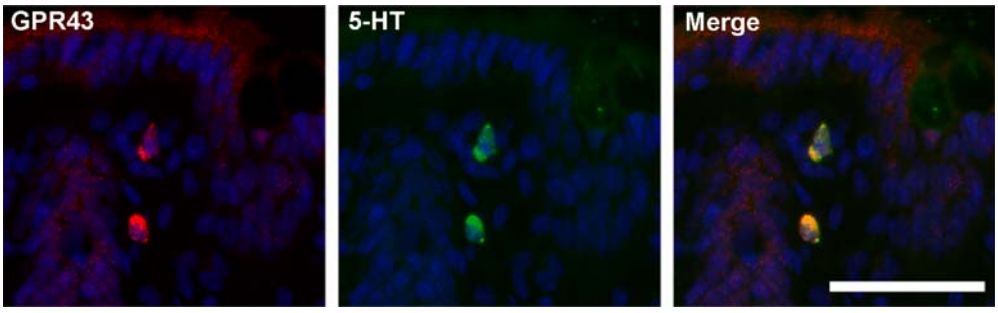

Fig. 6 Colocalization of GPR43 and 5-HT in lamina proprial cells. Triple-staining for GPR43 (*red*), 5-HT (*green*), and DAPI (*blue*) in the lamina propria of the mucosa of the distal colon (*yellow* colocalization). *Bar* 50 μm

Results

RT-PCR analysis

Messenger RNA for GPR43 was detected in extracts of whole wall and separated mucosa from the rat distal ileum and colon and of muscle plus submucosa from the distal ileum, but not in extracts of muscle plus submucosa from the distal colon (Fig. 1). The mRNA of β-actin was detected in all the samples (Fig. 1). RT-PCR analysis for GPR43 was conducted on extracted total RNA samples from three animals; the same results were obtained in all cases.

Western blot analysis

GPR43 were detected in extracts of whole wall and in the separated mucosa in the rat distal ileum and colon, but not in extracts of muscle plus submucosa, whereas actin protein was detected in all the samples (Fig. 2). Although the same amounts of proteins were applied among samples, the band density of the separated mucosa sample was clearly higher than that of whole tissue samples both in the distal ileum and colon. We carried out Western blot analysis for GPR43 on the extracted protein samples from three animals and obtained the same results in each case.

Immunohistochemistry

Immunoreactivity for GPR43 occurred at a low level in all enterocytes within the epithelium of the mucosa in 10-μm-thick sections in the distal ileum and colon (Fig. 3a,b). Immunoreaction also occurred in a population of enteroendocrine cells and in small cells in the lamina propria. In negative controls incubated without the anti-GPR43 antiserum, Paneth cells in the ileum were observed to be nonspecifically stained only by the secondary antibody (Fig. 3a). The absorption test showed that the immunoreactivity was abolished by equilibration of the antiserum with the peptide antigen (Fig. 3b). GPR43-immunoreactive (IR) cells in the epithelium had the morphology of enteroendocrine cells (Fig. 4a,b). GPR43-IR enteroendocrine cells were open-type enteroendocrine cells, which extended their cell bodies to the luminal surface and often

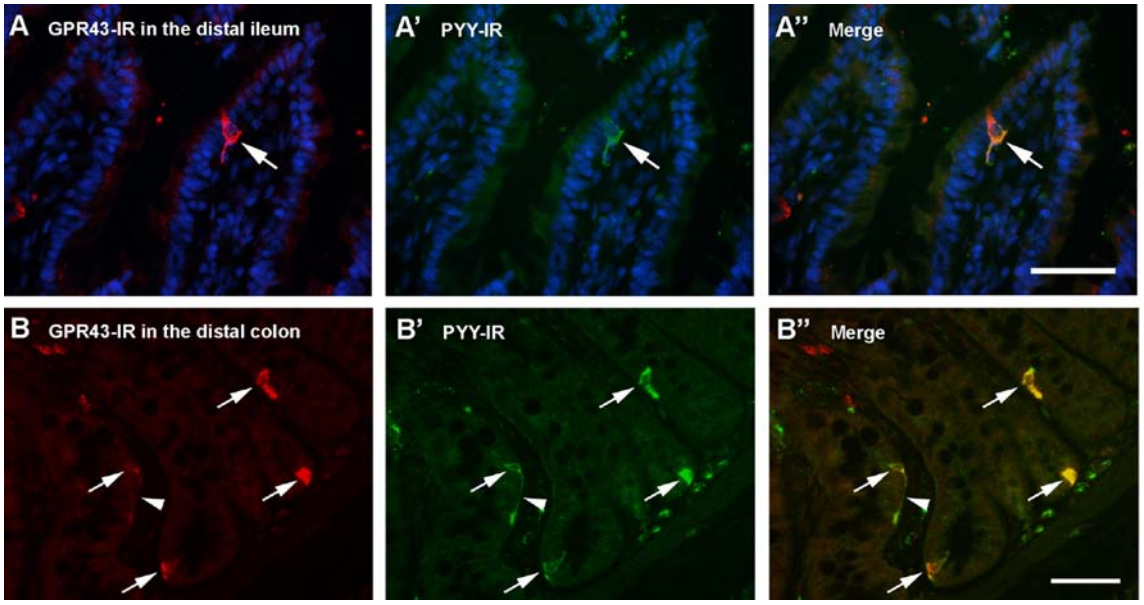

Fig. 7 Colocalization of GPR43 and PYY in enteroendocrine cells. Triple- or double-staining for GPR43 (*red*), PYY (*green*), and DAPI (*blue*) in the epithelium of the distal ileum (**a**) and colon (**b**). Colocalization appears *yellow*. Note that immunoreactivity for GPR43 and PYY is colocalized in enteroendocrine cells (*arrows*). Processes are seen at the base of the cell (*arrowheads*). *Bar* 50 μm

had processes that extended from their bases and ran beneath adjacent epithelial cells (Fig. 4).

Previous physiological studies reported that SCFAs caused 5-HT and PYY release (see Introduction). Thus, we performed double-staining for GPR43 and 5-HT, and GPR43 and PYY. No GPR43-IR enteroendocrine cells also exhibited 5-HT immunoreactivity (Fig. 5), whereas, GPR43 immunoreactivity was colocalized with 5-HT immunoreactivity in small round cells of the lamina propria in the distal ileum and colon (Fig. 6). These were mucosal mast cells (see Discussion).

There was complete co-localization of immunoreactivity for GPR43 and PYY in enteroendocrine cells in the distal ileum and colon (Fig. 7), i.e., all GPR43-IR cells were PYY-IR, and vice-versa.

Discussion

The present study is the first to demonstrate the expression of the mRNA and protein for the SCFA receptor, GPR43, and to localize it in the intestine. GPR43 mRNA was detected in the mucosa and in the whole wall from the rat distal ileum and colon, and in muscle plus submucosal layers from the distal ileum, but not from the distal colon by RT-PCR (Fig. 1). By Western blot, GPR43 protein was detected in the mucosa and whole wall, but not in muscle plus submucosal layers, both from the distal ileum and colon (Fig. 2). In the distal colon, these results indicated that GPR43 was expressed by cells in the mucosa, but not by enteric neurons or smooth muscle. In the ileum, mRNA was detected in muscle plus submucosal layers by RT-PCR, but no protein signal was detected by Western blot analysis. These results indicated that the mRNA in muscle plus submucosal layer in the distal ileum did not cause sufficient protein expression for the protein to be detected. By immunohistochemistry, no GPR43 signal was detected in muscle plus submucosa of the ileum. Therefore, GPR43 protein was expressed only in the mucosa of the distal ileum at a detectable level. Immunoreactivity for GPR43 was observed in PYY-IR enteroendocrine cells in the mucosal epithelium and in mast cells of the lamina propria of the distal ileum and colon. These data are consistent with previous physiological and pharmacological studies and suggest that SCFA directly affects 5-HT and PYY release by cells containing these mediators, and that this occurs via the GPR43 receptor type.

SCFAs have been reported to induce enhancement of colonic motility via the release of 5-HT (Fukumoto et al. 2003; Mitsui et al. 2005). These are two possible sources of 5-HT: enteroendocrine cells that are numerous throughout the gastrointestinal tract (Facer et al. 1979) and 5-HT-containing mast cells that occur in the mucosal lamina propria of the rat intestine (Yu et al. 1999). However, the present results show that GPR43 immunoreactivity does not occur in 5-HT-IR enteroendocrine cells, whereas 5-HT-IR mast cells of the lamina propria co-express GPR43. Therefore, the GPR43-IR lamina proprial cells are the source of 5-HT that is released by SCFAs. Ono et al. (2004) have reported that the acetate-induced decrease in the frequency of spontaneous contractions of longitudinal muscle in the rat distal colon is abolished by pre-treatment with nicotinic and 5-HT_3 receptor antagonists. Thus, the acetate-induced inhibitory response might be attributable to the 5-HT released from lamina proprial mast cells via GPR43 acting on adjacent nerve endings through 5-HT_3 receptors, and the stimulation of a nerve circuit involving nicotinic synapses.

Fats, including SCFAs, induce an inhibition of upper gastrointestinal motility, the so-called ileal and colonic brakes, via PYY release into the blood circulation (Lin et al. 1996, 2003, 2004; Cuche et al. 2000). Consistent with this, the present results have shown that all PYY-IR enteroendocrine cells of the distal ileum and colon are immunoreactive for GPR43. Therefore, the majority of PYY-containing enteroendocrine cells probably expresses GPR43 as a SCFA receptor, and SCFAs might stimulate PYY-containing enteroendocrine cells directly, via GPR43, to release PYY. Moreover, acetate, which is selective for GPR43, is reported to cause an increase in the release of PYY (Longo et al. 1991). Other SCFA receptors might also occur on the PYY cells. Processes extend from the basal parts of the PYY/GPR43 endocrine cells. These processes are close to the bases of adjacent epithelial cells, suggesting that PYY has a local paracrine role, in addition to its hormonal role. These processes have been previously described by Lundberg et al. (1982).

In the rat distal colon, SCFAs, including propionate and butyrate, but not acetate, are reported to induce a phasic muscle contraction and transient Cl^- secretion (Yajima 1985, 1988). According to the potency order of SCFAs, these effects of SCFAs seem to be attributable to GPR41 rather than GPR43. Thus, investigation of GPR41 expression in the intestine is also necessary to clarify the sensing mechanism mediating the SCFA-induced responses.

SCFAs are produced by bacterial fermentation of the carbohydrates of dietary fiber in the large intestinal lumen. Therefore, the presence of SCFAs in the lumen reflects the activity of the luminal bacterial flora. Thus, SCFA receptors possibly monitor the bacteria for host defense. Indeed, two types of putative SCFA receptor, GPR41 and GPR43, have been reported to be expressed by cells of the immune defense system, including polymorphonuclear cells (GPR41 and GPR43), monocytes (GPR43), and dendritic cells (GPR41; Le Poul et al. 2003). The present study indicates that the mucosal mast cells also express GPR43. Polymorphonuclear cells, monocytes (macrophages in the tissue), and dendritic cells are the phagocytes for non-selective antigens involved in the innate immune system. Le Poul et al. (2003) have reported that SCFAs induce chemotaxis of neutrophils. The evidence seems to support our hypothesis that one of the roles of SCFA receptors in the intestinal mucosa is the luminal surveillance of bacterial flora, but further study is necessary to clarify the roles of SCFA-receptor-mediated host defense mechanisms in the intestine. One of the causes of inflammatory bowel diseases (IBD) is considered to be the collapse of the mucosal immune system. Therefore,

GPR43 might have the potential of being a pharmacological target to modify IBD.

GPR43 immunoreactivity has been found in the enterocytes of the mucosa. Thus, these absorptive/secretory cells probably detect the presence of SCFAs in the lumen through GPR43, but the way that the cells react to this stimulus needs to be resolved.

In conclusion, the present study has shown that a putative SCFA receptor, GPR43, is expressed by 5-HT-containing mast cells and PYY-containing enteroendocrine cells in the rat distal colon. We hypothesize that the SCFA-induced excitatory and inhibitory physiological effects on colonic motility and secretion might be attributable to the activation of GPR43 on 5-HT-containing mast cells and that the ileo-colonic brake might be the result of direct stimulation of PYY-containing enteroendocrine cells.

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