Functional Characterization of Human Receptors for Short Chain Fatty Acids and Their Role in Polymorphonuclear Cell Activation*

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Emmanuel Le Pouli‡, Cécile Loison‡, Sofie Struyf§, Jean-Yves Springael, Vincent Lannoy‡, Marie-Eve Decobecq, Stéphane Brezillon‡, Vincent Dupriez‡, Gilbert Vassart***, Jo Van Damme‡, Marc Parmentier‡‡, and Michel Detheux‡

From ‡Euroscreen, rue Adrienne Bolland 47, 5041 Gossele, §Laboratory of Molecular Immunology, Rega Institute for Medical Research, Catholic University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, and Institut de Recherche Interdisciplinaire en Biologie Humaine et Nucléaire and **Service de Génétique Médicale, Université Libre de Bruxelles, Campus Erasme, 808 route de Lennik, 1070 Brussels, Belgium

Short chain fatty acids (SCFAs), including acetate, propionate, and butyrate, are produced at high concentrations by bacteria in the gut and subsequently released in the bloodstream. Basal acetate concentrations in the blood (about 100 μM) can further increase to millimolar concentrations following alcohol intake. It was known previously that SCFAs can activate leukocytes, particularly neutrophils. In the present work, we have identified two previously orphan G protein-coupled receptors, GPR41 and GPR43, as receptors for SCFAs. Propionate was the most potent agonist for both GPR41 and GPR43. Acetate was more selective for GPR43, whereas butyrate and isobutyrate were more active on GPR41. The two receptors were coupled to inositol 1,4,5-trisphosphate formation, intracellular Ca2+ release, ERK1/2 activation, and inhibition of cAMP accumulation. They exhibited, however, a differential coupling to G proteins; GPR41 coupled exclusively through the Pertussis toxin-sensitive Gβγ family, whereas GPR43 displayed a dual coupling through Gβγ and Pertussis toxin-insensitive Gq protein families. The broad expression profile of GPR41 in a number of tissues does not allow us to infer clear hypotheses regarding its biological functions. In contrast, the highly selective expression of GPR43 in leukocytes, particularly polymorphonuclear cells, suggests a role in the recruitment of these cell populations toward sites of bacterial infection. The pharmacology of GPR43 matches indeed the effects of SCFAs on neutrophils, in terms of intracellular Ca2+ release and chemotaxis. Such a neutrophil-specific SCFA receptor is potentially involved in the development of a variety of diseases characterized by either excessive or inefficient neutrophil recruitment and activation, such as inflammatory bowel diseases or alcoholism-associated immune depression. GPR43 might therefore constitute a target allowing us to modulate immune responses in these pathological situations.

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‡ To whom correspondence should be addressed. Tel.: 32-2-555-41-71; Fax: 32-2-555-46-55; E-mail: mparment@ulb.ac.be.

†† Contributed equally to this work.

§§ The abbreviations used are: GPCR, G protein-coupled receptor; FBS, fetal bovine serum; MLF, formyl-Met-Leu-Ph; PBMC, peripheral blood mononuclear cells; PTX, Pertussis toxin; SCFA, short chain fatty acid; PMN, polymorphonuclear cells; RT, reverse transcription; [35S]GTPγS, [35S]-labeled guanosine 5’-O-(thiotriphosphate); CHO, Chinese hamster ovary; ERK, extracellular signal-regulated kinase; BisTris, 2-(bis(2-hydroxyethyl) amino)-2-hydroxyethyl)propane-1,3-diol; MOPS, 3-(N-morpholino)propane sulfonic acid.
SCFAs act as specific activators of GPR41 and GPR43. An extensive pharmacological study revealed differences in the rank order of potency of SCFA toward each receptor, as well as in the G protein coupling leading to intracellular cascade activation. Pharmacological data obtained on human polymorphonuclear cells involved GPR43 as the main functional SCFA receptor on these cells.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Culture media, antibiotics, fetal bovine serum (FBS), and trypsin were from Bio-Whittaker. FuGENE 6, restriction, and DNA-modifying enzymes were from Roche Diagnostics. \[^{35}\text{S}\]GTP/H9253 and myo-D-[2-\(^{3}H\)]inositol (17.7 Ci/mmol) was from Amersham Biosciences. Dowex AG1X8 (formate form) was from Bio-Rad. Short chain fatty acids (SCFAs) and Pertussis toxin were from Sigma. Forskolin and isobutylmethylxanthine were from Eurobiochem (Louvain la Neuve, Belgium). Oligonucleotide primers were from Eurogentec (Liège, Belgium). Total and messenger RNAs were from Ambion (Huntingdon, Great Britain).

**Cloning and Sequencing of Human GPR43 and Human GPR41**—Oligonucleotide primers were synthesized on the basis of the sequence of the human GPR41 and GPR43 receptors (GenBank™ accession numbers AF024688 and AF024690, respectively). For GPR43 cloning, sense primer 5’-CCGGAGTTCCATCTGTCCGACTGGAAAG-3’ and antisense primer 5’-CTTGTTCTAGAATCTGTGGGAATGC-3’ were used in a PCR amplification using human genomic DNA as template and \(\text{Pfu}\) DNA polymerase (Stratagene) under the following conditions: 1 min at 94 °C, 30 s at 56 °C, 1 min at 72 °C, 3 cycles; 1 min at 94 °C, 30 s at 56 °C, 1 min at 72 °C, 30 cycles. A fragment of 1 kb containing the entire coding sequence of human GPR43 gene was amplified, digested by EcoRI and XbaI, and cloned in the bicistronic pEFIN5 expression vector. In the bicistronic vector, designated pEFIN5, both the recombinant receptor and the neomycin phosphotransferase selection marker are transcribed from a single promoter element.

For GPR41 cloning, sense primer 5’-CCGGAGATTCCATCTGTCCGACTGGAAAG-3’ and antisense primer 5’-CTTGTTCTAGAATCTGTGGGAATGC-3’ were used in a similar strategy, except that the coding sequence was cloned using EcoRV and XbaI, and cloned in the bicistronic pEFIN5 expression vector. In the bicistronic vector, designated pEFIN5, both the recombinant receptor and the neomycin phosphotransferase selection marker are transcribed from a single promoter element.

**Fig. 1.** Structure and clustering of SCFA and related receptors. A, amino acid alignment of human GPR40, GPR41, GPR42, and GPR43. Residues identical in all receptors are shaded in black, whereas residues shared by a subset of the family are shaded in gray. Putative transmembrane domains are boxed. B, dendrogram representing sequence similarities among GPR43, GPR41, and a set of structurally related receptors. Multiple alignment and clustering was performed using the ClustalX algorithm, whereas the dendrogram was constructed using TreeView. Accession numbers are as follows: human GPR92, CAC03715.1; human GPR40, O14842; human GPR43, AF024690; human GPR41, AF024688; human GPR42, O15529; human protease-activated receptor (PAR) 1, P25116; human PAR2, P55085; human P2Y1, P51582; human P2Y2, P41231; human P2Y11, NP_00257; human HM74, P49101; and human eicosanoid receptor, NP_683765. The scale indicates the difference in the percentage of homology between receptors.
Receptors for Short Chain Fatty Acids

Twelve Distribution of GPR41 and GPR43—Reverse transcription (RT)-PCR experiments were carried out using a panel of total RNA (peripheral blood mononuclear cells (PBMC), dendritic cells, monocytes, T lymphocytes, small intestine) and polyclonal RNA (thymus, spleen, lymph node, bone marrow, lung, stomach, adipose, breast). The total RNA from neutrophils was prepared from venous blood of healthy donors using TriPure (Roche Diagnostics). Approximately 50 ng of polyclonal RNA or 500 ng of total RNA was reverse-transcribed with SuperScript II (Invitrogen) and used for PCR. Human GPR43 and GPR41 receptors transcripts were detected by PCR using the following primers: 5'-TCTTACAGCACATCTCTG-3' (GPR43 forward), 5'-GAGGCACACAGGAAATTGA-3' (GPR43 reverse), 5'-TACCTCATAGAA-11032AGGAG-5' (GPR41 forward), and 5'-TCTTCTAGGTCTTCTTCTT-3' (GPR41 reverse). The expected size of the amplified bands was 439 and 508 bp for GPR43 and GPR41, respectively. PCR was performed using the Taq polymerase under the following conditions: 94 °C for 5 min; 30 cycles at 94 °C for 1 min, 53 °C for 1 min 30 s, and 72 °C for 40 s (for GPR43); or 94 °C for 5 min, 30 cycles at 94 °C for 1 min, 52 °C for 1.5 min, and 72 °C for 35 s (for GPR41). A control was performed with glyceraldehyde-3-phosphate dehydrogenase cDNA fragment (509 bp) as described previously (16). Aliquots of the PCR were analyzed by 1% agarose gel electrophoresis.

Cell Culture and Transfection—The recombinant ePFIN-GPR41, ePFIN-GPR43 plasmids, and the empty pEFIN5 vector were transfected into CHO-K1 cells (PCR-9618, ATCC, Manassas, VA), WTA11 cells (a CHO-K1 cell line coexpressing mitochondrial aequorin and Gαt), or human embryonic kidney 293 cells (ATCC CRL-1573), using FuGENE 6. The transfected cells were selected with 400 μg/ml G418 in Nutrient Ham’s F-12 medium supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin, from 2 days after transfection. The medium of WTA11 cells contained, in addition, 25 μg/ml kanamycin. The resistant clones were selected by RT-PCR and sequencing. COS-7 and human embryonic kidney 293 cells were grown in Dulbecco’s modified Eagle’s medium containing 10% FBS, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 μg/ml streptomycin and transfected using LipofectAMINE 2000 (Invitrogen), with the ePFIN5 plasmids encoding GPR41 or GPR43. The cells were used in functional assays as described below.

Aequorin Assay—The functional response to SCFAs was analyzed by measuring the luminescence of aequorin as described previously (17). For all assays, data were analyzed with the PRISM software (GraphPad Prism Software, San Diego, CA) using nonlinear regression applied measuring the luminescence of aequorin as described previously (17).

Results were labeled overnight with 10 μCi/ml [3H]inositol in culture medium. Cells were then washed three times and incubated in Kreb’s buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 4.2 mM KCl, 1 mM CaCl2, 0.5 mM MgCl2, 5.6 mM d-glucose) for 15 min in medium containing 1 mM LiCl. SCFA agonists were then added for 30 min. The reaction was stopped by replacing the incubation medium with 1 ml of 5% HClO4. The total inositol phosphate pool was then extracted and purified on Dowex columns as described by Joly et al. (18). Results were expressed as the ratio between the radioactivity collected in the inositol phosphate fraction over the radioactivity recovered from the cellular membranes solubilized in 10% Triton and 0.1 mM NaOH and used as standard. The use of this ratio allows for greater homogeneity in the data, as it reduces variations resulting from differences in cell numbers from fragments of the amplified bands.

Mitogen-activated Protein Kinase Assay—ERK1/2 activation was assayed by Western blotting, using an anti-phospho-p42/44 monoclonal antibody. Briefly, cells serum-starved for 24 h were collected and suspended in serum-free Dulbecco’s modified Eagle’s medium. After stimulation of cells at the indicated time with 10 μM propionate or acetate, in the presence or not of 100 ng/ml Pertussis toxin, cells were collected by centrifugation (12,000 rpm, 3 min) and heated to 100 °C for 5 min in lysis buffer (100 μM Tris-HCl, pH 6.8, 4 mM EDTA, 4% SDS, 20% glycerol, and 0.02% β-mercaptoethanol). For Western blot analysis, solubilized proteins corresponding to ~6 × 106 cells were loaded onto Nupage 10% BisTris gel (Invitrogen) in a Nupage MOPS SDS running buffer. After incubated in nitrocellulose membranes, proteins were probed with mouse anti-phospho-p42/p44 (1:1000) antibody (Cell Signaling Technology).

Polymorphonuclear Cell Pharmacology: Intracellular Calcium Release and Chemotaxis Assays—Polymorphonuclear cells were purified from buffy coats of healthy volunteers. PMN chemotaxis was performed in Boyden microchambers (Neuro Probe, Gaithersburg, MD) with polyvinylpyrrolidone-free polycarbonate membranes (5-μm pore size; Corning Separations Division, Acton, MA) as described previously (19). For intracellular Ca2+ assays, cells were incubated in Hank’s balanced salt medium containing 0.1% bovine serum albumin and 2.8 mM Na2-ATP for 37 °C for 45 min. Cells were washed, resuspended at 5 × 106 cells/ml in serum-free Hank’s balanced salt medium containing 0.1% bovine serum albumin and allowed to rest for 10 min at 37 °C. Cells were then transferred to cuvettes, and calcium transients were monitored through fluorescence measurements using an LS 50B spectrophotometer (PerkinElmer Life Sciences).

RESULTS

Identification of GPR43 and GPR41 as Receptors for Short Chain Fatty Acids—In the frame of a general strategy for characterizing ligands for orphan G protein-coupled receptors, a CHO-K1 cell line coexpressing GPR43, Gαt, and aequorin (GPR43-WTA11) was established and screened in an aequorin-based functional assay against a large collection of reference compounds comprising peptides, lipids, carbohydrates, and small chemical compounds. A biological activity specific for GPR43-expressing cells was observed for a number of peptide solutions containing acetate as the counter ion. Control tests revealed that the acetate ion itself, and not the peptides, was responsible for the agonist activity on GPR43. A pH effect could rapidly be excluded, as neutral acetate buffers displayed the same activity as acetic acid. Furthermore, different salts of acetate (Na+, NH4+, and K+) confirmed the activity of the acetate anion. We also excluded that the effect of acetate on intracellular calcium release was subsequent to the acidification of the cell cytosol, as several organic acids with a similar pKa were not active on GPR43-expressing cells. This specificity of the GPR43 response to acetate was further demonstrated by testing in the same assay a set of about 60 orphan and characterized receptors belonging to different GPCR classes and subclasses, in addition to the wild-type cell lines WTA11. None of these responded to acetate. The determination of concentration-action curves allowed us to estimate the EC50 of GPR43 for sodium acetate at 268 ± 26 μM (Fig. 2A). Additional SCFAs were tested at this stage and were found to activate GPR43, as well, in the aequorin-based assay (data not shown).

We also screened the receptors structurally related to GPR43 and found that GPR41, which shares 42% amino acid identity

[1]
with GPR43, was also activated by acetate, although to a lower extent (Fig. 2B). Indeed, the EC$_{50}$ of GPR41 for acetate was estimated at 1390 ± 1100 μM, and the other SCFA propionate was found to display a higher potency on this receptor, with an EC$_{50}$ of 11.6 ± 1.4 μM (Fig. 2B).

Intracellular Coupling of GPR41 and GPR43 — The natural coupling properties and the intracellular signaling pathways activated by GPR43 and GPR41, upon stimulation by acetate or propionate, were investigated in CHO-K1 cells expressing the human receptors but devoid of aequorin or additional coupling proteins such as Go$_{16}$ (CHO/GPR43 and CHO/GPR41 cells). We first demonstrated that both receptors coupled negatively to adenylyl cyclase through a Pertussis toxin-sensitive G protein (Gi/o class), while being unable to promote accumulation of cAMP in the absence of forskolin (not shown). This cAMP accumulation assay was used to characterize further the detailed pharmacology of the receptors (see Fig. 3, A and B, and see below). A [$^{35}$S]GTPγS binding assay was used alternatively as a functional test to monitor Gi/o coupling of these receptors in a cell-free assay, to exclude further the possibility that cell activation would result from nonspecific actions on the cell metabolism or cytoplasmic components. The activity of acetate and propionate on GPR43 and GPR41 was confirmed in this assay (Fig. 4, A and B). Propionate was equipotent on GPR43 (EC$_{50}$ = 259 ± 67 μM) and GPR41 (EC$_{50}$ = 274 ± 75 μM), whereas acetate was more potent on GPR43 (EC$_{50}$ = 537 ± 31 μM) than on GPR41 (EC$_{50}$ = 1299 ± 65 μM). PTX treatment inhibited the response to SCFAs for both receptors. Stimulation of GPR41 and GPR43 also resulted in the release of intracellular calcium, with a similar rank order of potency (see Fig. 4, C and D and Table I). Although the EC$_{50}$ values observed in both assays were similar for GPR41, they were higher in the Ca$^{2+}$ assay as compared with the cAMP assay for GPR43. Furthermore, PTX abolished the response of GPR41 but not of GPR43 (Fig. 4, C and D). This suggested a unique Gi/o coupling for GPR41 and a dual coupling through the Gi/o and Gq families for GPR43.

The activity of SCFAs was confirmed following expression of GPR43 and GPR41 in other cell lines (COS-7 and human embryonic kidney 293), with similar EC$_{50}$ values observed for acetate and propionate. Moreover, transient expression of GPR43 in COS-7 cells led to the accumulation of inositol phosphates in a PTX-independent mechanism with an EC$_{50}$ of 325 ± 36 μM for propionate and 132 ± 40 μM for acetate (Fig. 4E). Stimulation of GPR41 expressed in COS-7 cells resulted in the accumulation of inositol phosphates, only following the cotransfection of the chimeric G protein Go$_{5}$ (Fig. 4F). Furthermore, the cotransfection of GPR43 and Go$_{5}$ increased significantly the basal level of inositol phosphates, as compared with control conditions, or the expression of one of the plasmids alone, suggesting that GPR43 is endowed with constitutive activity (data not shown).

Stimulation of GPR43 and GPR41 expressed in CHO-K1 cells with 10 mM propionate induced a time-dependent phosphorylation of p42 and p44 mitogen-activated protein kinases.
FIG. 4. Intracellular cascades activated by GPR41 and GPR43. A and B, the binding of [35S]GTP<sup>S</sup> to membranes of CHO-K1 cells expressing GPR43 (A) or GPR41 (B) was measured following stimulation by acetate or propionate. C and D, intracellular calcium mobilization was measured in CHO-K1 cells expressing GPR43 (C) or GPR41 (D), after culturing the cells in the presence or absence of Pertussis toxin. The data represent the mean ± S.E. for triplicate data points, and the displayed curves are representative of at least three independent experiments. E and F, inositol phosphate accumulation was determined in COS-7 cells transiently expressing GPR43 (E) or cotransfected with GPR43 or GPR41 and the chimeric G<sub>qi5</sub> protein (F). G and H, phosphorylation of ERK1/2 mitogen-activated protein kinases following stimulation of GPR43 (G) and GPR41 (H) expressed in CHO-K1 cells by acetate or propionate for 5 min. The cellular extract (20-µg proteins) was separated by SDS-polyacrylamide gel electrophoresis, transferred to nylon membranes, and labeled with antibodies specific for the phosphorylated forms of ERK1 and ERK2.
The functional parameters of GPR43 and GPR41 activation by SCFAs and related molecules were determined using a cAMP accumulation assay (in the presence of forskolin) and a fluorescent-based calcium mobilization assay. Values are the mean $\mathrm{pEC}_{50}$ and S.E. for at least three independent determinations. The number in parentheses indicates the potency order for each assay and each receptor. ND, not determined.

| SCFA       | GPR43 $\mathrm{pEC}_{50}$ | GPR43 $\mathrm{Ca}^{2+}$ | GPR41 $\mathrm{pEC}_{50}$ | GPR41 $\mathrm{Ca}^{2+}$ |
|------------|---------------------------|--------------------------|---------------------------|--------------------------|
| Formate    | 2.61 ± 0.06 (8)           | 1.99 ± 0.03 (8)          | >10 mM (10)               | >10 mM (10)              |
| Acetate    | 4.46 ± 0.05 (3)           | 3.99 ± 0.02 (2)          | 2.90 ± 0.04 (8)           | 2.97 ± 0.02 (8)          |
| Propionate | 4.85 ± 0.07 (1)           | 4.10 ± 0.02 (1)          | 5.21 ± 0.07 (1)           | 4.70 ± 0.10 (1)          |
| Butyrate   | 4.55 ± 0.12 (2)           | 3.47 ± 0.03 (3)          | 4.38 ± 0.10 (3)           | 4.24 ± 0.06 (3)          |
| Isobutyrate| 3.84 ± 0.05 (4)           | 3.23 ± 0.08 (4)          | 4.52 ± 0.07 (2)           | 4.31 ± 0.01 (2)          |
| L-OH-butyrate| 2.32 ± 0.02 (10)       | 1.92 ± 0.08 (9)          | 2.29 ± 0.10 (9)           | 2.06 ± 0.16 (9)          |
| Pivalate   | 2.59 ± 0.14 (9)           | 2.34 ± 0.10 (7)          | 3.63 ± 0.16 (7)           | 3.19 ± 0.01 (7)          |
| Valerate   | 2.78 ± 0.15 (7)           | 2.72 ± 0.08 (5)          | 4.38 ± 0.14 (4)           | 4.11 ± 0.06 (4)          |
| Isovalerate| 2.67 ± 0.06 (6)           | 2.51 ± 0.06 (6)          | 4.24 ± 0.14 (5)           | 3.91 ± 0.02 (5)          |
| Caproate   | 2.86 ± 0.06 (5)           | >5 mM (10)               | 3.99 ± 0.07 (6)           | 3.87 ± 0.06 (6)          |
| Heptanoate | >10 mM (11)               | >5 mM (10)               | >100 mM (11)              | ND                       |
| Caprylate  | >10 mM (11)               | >5 mM (11)               | >100 mM (11)              | ND                       |

SCFAs have been described as displaying activities on leukocyte populations, particularly PMNs (19–22). Given the identification of specific receptors for these molecules and the characterization of their expression profile we investigated the effects of SCFAs on human leukocytes. PMN cells were particularly investigated, provided the strong and specific expression of GPR43 in this population and the previously described activity of SCFAs on these cells. Stimulation of PMN cells by propionate or acetate resulted in a transient increase of intracellular calcium (Fig. 6, A and B), as described before the identification of specific receptors for these agents (23–25). The $\mathrm{Ca}^{2+}$ response was similar to that obtained with the bacterial peptide formyl-Met-Leu-Phe (fMLP), the prototypical agonist of the formyl peptide receptor. Prior stimulation of the cells with acetate or propionate inhibited subsequent responses to propionate or acetate (Fig. 6B), suggesting a homologous desensitization of a common receptor for the two agonists. Acetate or propionate did not affect, however, the functional responses to fMLP, interleukin-8, or leukotriene B4 (Fig. 6A). Conversely, a first stimulation of PMNs with fMLP, interleukin-8, or leukotriene B4 reduced or abolished, in a concentration-dependant manner, the subsequent response to acetate or propionate (Fig. 6C). Concentration-action curves were established for propionate (Fig. 6D) and acetate (Fig. 6E), demonstrating that both molecules were equally active on PMNs, with $\mathrm{EC}_{50}$ values of 537 ± 36 $\mu$M for acetate and 540 ± 36 $\mu$M for propionate. This calcium-mobilizing effect was not sensitive to PTX and not abolished in the presence of EGTA in extracellular medium, indicating the mobilization of calcium from intracellular pools (data not shown).
PMN cells were also tested for their chemotactic response to sodium acetate and sodium propionate. Both SCFAs resulted in a classical bell-shaped dose-response curve with an optimal concentration of 1 mM (Fig. 6F). The potency of SCFAs in neutrophil chemotaxis was significantly lower than that of fMLP, which was still fully active at concentrations of 1 nM (data not shown). Furthermore, the efficacy of fMLP was also higher than that of SCFAs, as the maximal chemotactic index obtained with fMLP was on average at least 3-fold higher than with SCFAs. Additional experiments indicated that, at optimal agonistic concentrations (1 mM), SCFAs could inhibit neither spontaneous neutrophil migration nor chemotaxis induced by 10 nM fMLP (data not shown).

**DISCUSSION**

GPR43 and GPR41 belong to a cluster of four orphan GPCRs, together with GPR40 and GPR42, which share little structural similarities with other subfamilies of G protein-coupled receptors. GPR43 is mainly expressed on leukocyte populations, particularly neutrophils, whereas GPR41 expression is more widely distributed in tissues. The involvement of GPR43 in leukocyte function and host defense is supported by the induction of its mRNA during the differentiation and activation of monocytes and PMN cells (14).

The screening of a library of reference bioactive molecules allowed us to identify acetate and then other short chain fatty acids.
acids as specific agonists for GPR43. The SCFAs were then used to screen the structurally related receptors, and we found that GPR41 was also activated by SCFAs with a different rank order of potency, although its amino acid identity with GPR43 was somehow limited (39%). A cell line expressing GPR40 did not respond to SCFAs in our hands, whereas GPR42, which shares 98% amino acid identity with GPR41, has not been tested in this study. Acetate was 100-fold less potent on GPR41 as compared with propionate and butyrate, whereas the three ligands activated GPR43 with a similar potency. A number of observations in various cell environments and functional assays suggested that the two receptors are coupled differently to intracellular signaling cascades. GPR41 appears to be coupled exclusively to the Gs family of proteins, whereas a dual coupling through Gi and Gq families is observed for GPR43. In both cases, the resulting intracellular pathways activated by the receptors included inositol 1,4,5-trisphosphate generation, intracellular Ca2+ release, ERK1/2 activation, and inhibition of cAMP accumulation.

Among the compounds tested, a set of ten carboxylic acids and related molecules were found to be active on both GPR41 and GPR43 in CAMP accumulation and intracellular Ca2+ release assays. Structure-activity relationships of the active compounds showed that the carboxylic moiety is required for activity. Indeed, aldehydes, ketones, or alcohols with similar carbon chains were totally inactive, as well as ester derivatives of the active compounds. A carboxylic moiety has to be located at the end of aliphatic chain comprising one to six carbon atoms, linear or branched. The optimal length is two to three carbon atoms for activity on GPR43 and three to five carbon atoms for GPR41. The presence of two (or more) carboxyl groups is, however, not tolerated as a number of di-acids were inactive, whatever the length of the carbon chain. These inactive compounds included oxalate (C2), malonate (C3), succinate (C4), aspartate (C4), glutamate (C5), and citrate (C6).

In vivo relevant SCFAs include, but are not limited to, acetate, propionate, and butyrate. These molecules are produced in considerable amounts by microbial fermentation in the hindgut, where they reach local concentrations as high as 70 to 100 mM (26). SCFAs are also produced as metabolic by-products of anaerobic bacteria present in the periodontal pocket. SCFAs are rapidly transferred from these compartments to the bloodstream, and the usual concentration in peripheral blood is around 100 to 150 μM for acetate, 4 to 5 μM for propionate, and 1 to 3 μM for butyrate (27). Moreover, the plasmatic concentration of acetate can increase up to 10-fold as compared with basal values, following ethanol administration (28, 29). A few inherited diseases, caused by specific enzyme defects, result in the accumulation of propionate and butyrate (30, 31).

The high and specific expression of GPR43 in neutrophils can be related to the well established effects of SCFAs on these cells. Indeed, the observed calcium mobilization and chemotaxis of neutrophils, in response to SCFAs, confirms an abundant literature describing activation of PMN by SCFAs, which includes morphological changes and cell polarization, Ca2+ release, actin cytoskeleton remodeling, and cytoplasmic pH oscillations (20, 25). Our pharmacological data suggest that GPR43 is the functional receptor responsible for these previously reported actions of SCFAs on neutrophils.

The identification of functional receptors for SCFAs, expressed on leukocytes, opens new perspectives in the modulation of immune system functions in various pathophysiological situations. The average concentrations of propionate and butyrate in blood are too low to activate GPR41 or GPR43, but the blood concentrations reached by acetate are well within the active range for GPR43. Moreover, the main sources of SCFAs are bacteria in the gut, where their local concentrations are known to be much higher than in the blood. It has been established that products of the gut commensal flora can promote the activation of the mucosal immune system in the presence of an impaired or injured mucosal barrier, contributing to the development of inflammatory bowel diseases (32). Leukocytes also infiltrate diffusely the intestinal wall, in the absence of obvious morphological, clinical, or endoscopic evidence of inflammatory processes. Neutrophils play a key role in the early steps of a number of inflammatory processes of the gastrointestinal tract, including gastritis, enterocolitis, ulcerative colitis, and ischemia reperfusion injury. Following migration from the systemic circulation into the mucosal interstitial space, neutrophils undergo activation to produce reactive oxygen intermediates and chemokines, leading to the perpetuation of the inflammatory response, as well as contributing to the ultimate mucosal injury. The development of therapeutic strategies to block neutrophil recruitment and activation might be highly beneficial in a number of diseases characterized by an inflammatory component. Cyclosporin A, which inhibits neutrophil and T cell recruitment, is an efficient treatment for inflammatory bowel diseases. The identification of GPR43 as a functional receptor for SCFA could potentially lead to new therapeutic strategies.

Acetate concentration can increase up to 10-fold and reach millimolar concentrations after alcohol ingestion, as a consequence of the conversion of 60 to 75% of alcohol to acetate (29). It has been established that acute and chronic alcohol intake increases the susceptibility to infections caused by bacterial and viral pathogens (33, 34). The impaired host defense associated with alcohol is the consequence of a defective inflammatory response, with altered cytokine production and decreased neutrophil function (35, 36). Chronic ethanol intake was shown to affect MLLP-induced chemotactic activity, superoxide production by neutrophils, and their bactericidal activity (37, 38). Some of these effects might involve the desensitization of neutrophil SCFA receptors by high blood concentrations of acetate, resulting in a reduction of their recruitment to sites of bacterial infection. Leukocyte infiltration is also an important component of alcoholic liver disease. In acute alcoholic hepatitis, PMN are selectively recruited to the liver where alcohol is metabolized into acetate (37, 39).

As a conclusion, we have identified two specific receptors of SCFAs. These receptors could play a role similar to that of the fMLP receptor (40), by recognizing a bacterial metabolite and promoting leukocyte recruitment to the site of infection, as well as their activation. The pharmacological profile of GPR43 and its specific expression in neutrophils suggest, therefore, an early role in the induction of immune and inflammatory responses, with a possible involvement in inflammatory bowel diseases, as well as in alcoholism-associated disease susceptibility. The precise function of GPR41, given its broader distribution, is less clear. Additional studies, including in vivo pharmacology and the generation of knockout models, will be necessary for identifying further the precise roles of these two receptors and their potential applications as therapeutic targets. During the completion of the present work, two publications describing the independent identification of GPR40 as a receptor for medium fatty acids and of GPR41 and GPR43 as receptors for SCFAs became available on-line (41, 42). These authors describe a pharmacological profile of SCFAs for GPR41 and GPR43 that is similar to that reported here.

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