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Acetate and Propionate Short Chain Fatty Acids Stimulate Adipogenesis via GPCR43

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It has recently been discovered that G protein-coupled receptors (GPCR) 41 and 43 are characterized by having the short chain fatty acids acetate and propionate as their ligands. The objective of this study was to investigate the involvement of GPCR41, GPCR43, and their ligands in the process of adipogenesis. We measured the levels of GPCR41 and GPCR43 mRNA in both adipose and other tissues of the mouse. GRP43 mRNA expression was higher in four types of adipose tissue than in other tissues, whereas GPCR41 mRNA was not detected in any adipose tissues. A high level of GPCR43 expression was found in isolated adipocytes, but expression level was very low in stromal-vascular cells. Expression of GPCR43 was up-regulated in adipose tissues of mice fed a high-fat diet compared with those fed a normal-fat diet. GPCR43 mRNA could not be detected in confluent and undifferentiated 3T3-L1 adipocytes; however, the levels rose with time after the initiation of differentiation. GPCR41 expression was not detected in confluent and differentiated adipocytes. Acetate and propionate treatments increased lipids present as multiple droplets in 3T3-L1 adipocytes. Propionate significantly elevated the level of GPCR43 expression during adipose differentiation, with up-regulation of PPAR-γ2. Small interfering RNA mediated a reduction of GPCR43 mRNA in 3T3-L1 cells and blocked the process of adipocyte differentiation. In addition, both acetate and propionate inhibited isoproterenol-induced lipolysis in a dose-dependent manner. We conclude that acetate and propionate short chain fatty acids may have important physiological roles in adipogenesis through GPCR43, but not through GPCR41. (*Endocrinology* 146: 5092–5099, 2005)

WHITE ADIPOSE TISSUE is indispensable to the maintenance of life because it accumulates triacylglycerols during excess energy intake and releases them during fasting periods. Adipose tissue is known to secrete a variety of factors, some of which are thought to be involved in the modulation of adipose mass. So far, it has been reported that adipogenesis, and the development of adipose tissue, is regulated by several factors secreted by adipose tissue, including leptin and adiponectin. In addition, adipocyte development in different fatty tissues is affected and regulated by several genes and membrane proteins variably expressed in fat depots. Several transcription factors play key roles in adipocyte differentiation. PPARγ2 is a member of the peroxosome proliferator-activated receptor subfamily of nuclear hormone receptors that is induced very early in adipocyte differentiation (1). CCAAT-enhancer-binding protein α (C/EBPα) is a transcription factor expressed relatively late in adipogenesis. As with PPARγ2, C/EBPα activates adipocyte-specific genes such as the adipocyte P2 (*aP2*) gene, which promotes synthesis of an intracellular fatty acid-binding protein (2, 3).

G protein-coupled receptors (GPCRs) are important receptors in cellular function; they are seventh transmembrane receptors and can activate heterotrimeric G proteins, for example Gs, Gi, and Gq. Each ligand (or ligands) of the GPCR binds specifically. The GPCR family transduces extracellular signals across the plasma membrane, activating cellular responses through a variety of second messenger cascades, for example, the cAMP-dependent protein kinase A and cAMP-dependent protein kinase C signaling pathways. These receptors provide rapid responses to a variety of stimuli, and their signaling is often rapidly attenuated. GPCR7 and its endogenous ligands, neuropeptide B, and neuropeptide W, play a biologically important role in regulating food intake, energy expenditure, and body weight; they act in a sexually dimorphic manner, independently of leptin and melanocortin signaling (4). Recently, the endogenous ligands for these receptors were reported to be carboxylic acid anions, including long-chain free fatty acids for GPCR40 (5–7) and short-chain carboxylic acids for GPCR41 and GPCR43 (8, 9). Propionate, the ligand of GPCR41, stimulated leptin expression

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Abbreviations: aP2, Adipocyte fatty acid binding protein; C/EBPα, CCAAT-enhancer-binding protein α; GPCR, G protein-coupled receptor; KRB, Krebs-Ringer bicarbonate; PAC, cAMP-dependent protein kinase C; PPAR-γ2, peroxisome proliferators-activated receptor γ; SCFAs, short chain fatty acids; siRNA, small interfering RNA.

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in both a mouse adipocyte cell line and mouse adipose tissue in primary culture, and acute oral administration increased circulating leptin levels in mice (10). However, the involvement of GPCR43, another receptor of propionic acid, in adipogenesis has not been demonstrated. These observations suggest that there must also be a GPCR subfamily involved in the physiological functions of adipocytes, for example cell response, clonal expansion, differentiation, metamorphosis, and so on, which works through the activation of G proteins by binding their various ligands. To date, the functions of GPCR43 in adipogenesis have not yet been clearly established. In the present report, we show that GPCR43 has an important role in adipogenesis and the development of adipose tissues.

Materials and Methods

Animals for dietary fat comparison

Three-week-old male C57BL/6J mice were obtained from Charles River Japan (Tokyo, Japan). They were housed individually in cages with wire-mesh bottoms at a constant temperature (20 C to ~22 C) and humidity (50–60%) and under a 12-h light/12-h dark cycle. The animals had free access to water and chow (Oriental Yeast, Chiba, Japan) containing 8.5% (wt/wt) fat, 43.7% carbohydrate, and 29.7% protein, with an energy content of 3.69 kcal/g, for an acclimatization period of 1 wk. The mice were then weighed and divided into two groups of six with approximately equal mean body weights. One group was fed the standard diet and the other received a high-fat diet for 11 wk (4–15 wk of age). The high-fat diet was obtained from Research Diet and contained 41% fat, 36% carbohydrate, and 23% protein, with an energy content of 4.33 kcal/g; its fat source was the same as that of the standard diet and it contained the same absolute amounts of protein and fiber as the standard diet. The animals were weighed every week. At the end of the experimental period, the mice were killed by decapitation. White adipose tissues were rapidly separated from sc, perirenal, mesenteric, epididymal, and parametrial fat sites, immediately frozen in liquid nitrogen, and stored at −80 C until RNA extraction. A range of nonadipose tissues were also collected and stored: brain, pituitary, heart, kidney, liver, lung, stomach, muscle, colon, spleen, small intestine, and pancreas. All experiments were conducted in accordance with the Shinshu University Guide for the Care and Use of Experimental Animals.

Total RNA extraction from tissues and cells

Total RNA was extracted from adipose and nonadipose tissues and from adipocytes and stromal-vascular cells (see below), by the acid guanidium-thiocyanate-phenol-chloroform method (11). Total RNA was extracted from confluent 3T3-L1 cells and differentiated adipocytes using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA).

Isolation of adipocytes and stromal-vascular cells from adipose tissues

White adipose tissue from the parametrial regions of 26-wk-old female mice was dissected from connective tissue and blood vessels. The adipose tissue was divided into stromal-vascular cell and adipocyte fractions. Briefly, freshly excised fat pads from 26-wk-old female mice were rinsed in Krebs-Ringer bicarbonate (KRB), minced, and digested for 40 min at 37 C in KRB (pH 7.4) with 3.5% BSA and 0.5 mg/ml type I collagenase (Worthington, Freehold, NJ). The digested tissue was filtered through a 250-μm nylon mesh to remove undigested tissue and centrifuged at 500 $\times$ *g* for 5 min. The floating adipocyte fraction was removed, washed in buffer, and recentrifuged to isolate free adipocytes. The stromal-vascular pellet was resuspended in an erythrocyte lysis buffer (154 mM NH_4Cl, 10 mM $KHCO_3$), filtered through a 28-μm nylon mesh to remove endothelial cells, and pelleted at 1500 $\times$ *g* for 5 min. Isolated adipocytes and stromal-vascular cells were used for the measurement of GPCR43 and GPCR41 mRNA.

Cell culture of 3T3-L1 cells and differentiation

Low passage number 3T3-L1 cells were obtained from ATCC (Manassas, VA). The cells were plated and maintained to 2 d after confluence in DMEM containing 10% fetal bovine serum. Medium was replenished every 48 h. The cells were induced to differentiate by the addition of DMEM containing 10% fetal bovine serum, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 1.7 μM insulin. After 48 h, medium was replaced with DMEM supplemented with 10% fetal bovine serum, and cells were maintained in this medium for at least 7 d after the induction of differentiation. Acetate and propionate were obtained from Nacali Tesque (Kyoto, Japan) as sodium salts and were prepared as 1 M stock solutions and adjusted to pH 7.0. After the culture medium was changed to the differentiation-inducing medium, acetate and propionate were added at 24-h intervals. In another set of experiments, cells were treated with troglitazone (5 μM) and all-*trans*-retinoic acid (0.1 μM) during differentiation.

Oil red O staining

Cytoplasmic lipid droplets were stained with oil red O. Briefly, cells were rinsed three times in PBS and then fixed in 10% (vol/vol) formaldehyde for 10 min. After being washed twice with PBS, cells were stained for 30 min at 37 C in freshly diluted oil red O (Sigma Chemical Co., St. Louis, MO) solution (six parts oil red O stock and four parts H_2O; oil red O stock solution is 0.5% oil red O in isopropanol), followed by further washing with PBS.

Semiquantitative RT-PCR analysis of GPCR43, GPCR41, leptin, and PPAR-γ2 mRNA

Total RNA was extracted from freshly dissociated adipocytes, from primary cultured preadipocytes, and from differentiated adipocytes in six-well culture plates. Semiquantitative RT-PCR was performed as previously described (12) to measure levels of mouse GPCR43, GPCR41, PPAR-γ2, leptin, aP2 and β-actin mRNA. The primer pairs used were: GPCR41, forward primer (5′-CTGCTCCTGCTCCTCTTC-3′), reverse primer (5′-CCAGGCGACTGTAGCAGTA-3′); GPCR43, forward primer (5′-GGCTTCTACAGCAGCATCTA-3′), reverse primer (5′-AAGCACACCAGGAAATTAAG-3′); PPAR-γ2, forward primer (5′-TGGGTGAAACTCTGGGAGAT-3′), reverse primer (5′-CCATAGTGGAAGCCTGATGC-3′); leptin, forward primer (5′-TGACACCAAAACCCTCATCA-3′), reverse primer (5′-CTCAAAGCCACCACCTCTGT-3′); aP2, forward primer (5′-TCTCCAGTGAAAACTTCGAT-3′), reverse primer (5′-CTCATGCCCTTTCATAAACT-3′); and β-actin forward primer (5′-AGGTCATCACTATTGGCAAC-3′), reverse primer (5′-ACTCATCGTACTCCTGCTTG-3′). Preliminary experiments established that the exponential phase of PCR amplification could be sampled using 25 to approximately 35 amplification cycles. We performed PCR for 28 cycles with a 55 C annealing temperature for the GPCR43 (product size of 443 bp), 28 cycles with a 58 C annealing temperature for the PPAR-γ2 (product size of 454 bp), 30 cycles with a 55 C annealing temperature for the leptin (product size of 357 bp), and 28 cycles with a 60 C annealing temperature for the aP2 (product size of 366 bp). β-Actin (product size of 363 bp), the housekeeping gene, was amplified as an internal control with 28 cycles and 55 C annealing temperature. PCR products were resolved on a 1.2% agarose gel. The DNA was visualized by ethidium bromide staining and analyzed using NIH image software. The mRNA levels of GPCR43 were corrected using the transcription level of the β-actin gene as an internal standard. The data in Figs. 1–7 were expressed as fold (n-fold) over an appropriate control value, as described in the figure legends. The amplified cDNAs were subcloned into the pGEM-T easy vector (Promega, Madison, WI), and the sequences were confirmed using an automated DNA sequencer (ABI 310).

Small interfering RNA (siRNA) construction and transfection

siRNA oligonucleotides were designed to interact with GPCR43 mRNA using the siRNA design tool provided by Dharmacon Research (Lafayette, CO). Oligonucleotide sequences were: 5′-AAUCGUCAUCAUCGUUCAGUA-3′ (GPCR43 siRNA), and 5′-AAUCAACUGACUCGACCACUA-3′ (scrambled siRNA). The scrambled siRNA was used as

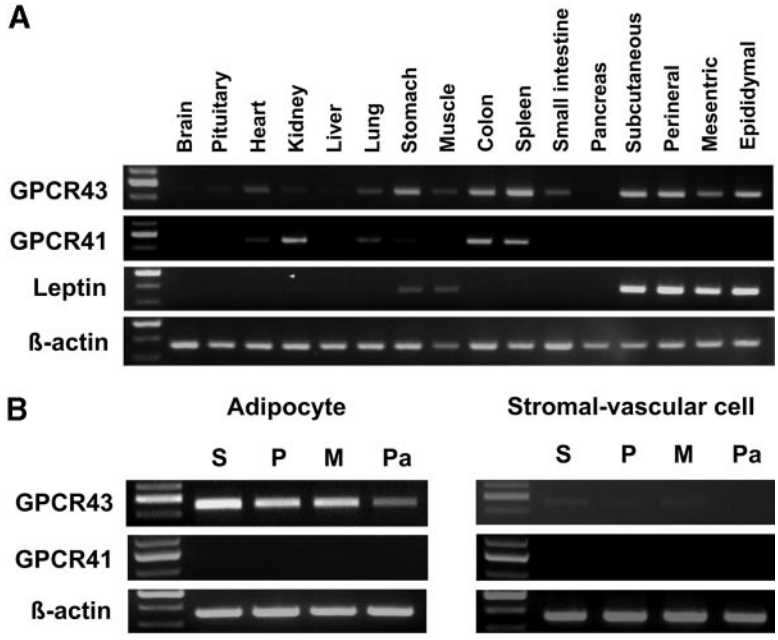

FIG. 1. A, The levels of GPCR43, GPCR41 and leptin mRNAs in various tissues of 15-wk-old male mice. RT-PCR analysis was performed on total RNA extracted from various tissues of six mice. The RT-PCR results shown are representative of six separate experiments with the same protocol. B, The levels of GPCR43 and GPCR41 mRNA expression in adipocyte and stromal-vascular cells. RT-PCR analysis was performed on total RNA extracted from adipocyte and stromal-vascular cells isolated from four different adipose tissues of 26-wk-old female mice. The RT-PCR results shown are representative of four separate experiments with the same protocol. β-actin was used as the internal standard. P, Perirenal; S, sc; M, mesenteric; Pa, parametrial.

a negative control. The siRNAs were constructed employing the Silencer siRNA construction kit (Ambion, Austin, TX) according to the protocol provided by the manufacturer. Upon confluence, the 3T3-L1 cell media were changed to growth media without antibiotics. Two days later, cells were transfected with siRNAs (50 nM) using the siPORT lipid transfection reagent (Ambion) according to the manufacturer's instructions. Five volumes of differentiation medium without antibiotics were added 4 h after transfection, and the cells were maintained at normal growing conditions and induced to differentiate as described above. The down-regulation of the GPCR43 targeted by siRNA was confirmed by analysis of its levels of expression using RT-PCR.

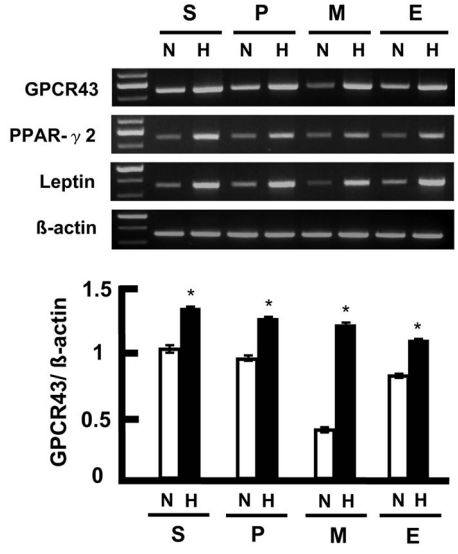

FIG. 2. The levels of GPCR43 mRNA in four different adipose tissues of male mice fed either a normal fat or a high-fat diet for 15 wk. RT-PCR analysis was performed on total RNA extracted from pooled adipose tissues of normal and high-fat-fed mice tissue. Data were normalized using β-actin mRNA, and expressed relative to sc adipose tissue of mice fed the normal diet. The RT-PCR results shown are representative of six separate experiments with the same protocol. The data represent the means ± SEM of the six experiments. *, $P < 0.05$ *vs.* control. N, Normal-fat-fed mice; H, high-fat-fed mice; S, sc; P, perirenal; M, mesenteric; E, epididymal.

Incubation experiment for lipolysis on 3T3-L1 adipocytes

Differentiated 3T3-L1 adipocytes were used to investigate the effects of acetic acid and propionic acid on lipolysis. Adipocytes were incubated with isoproterenol, acetate, and propionate for 30 min. The rate of lipolysis was determined by measuring the level of released free fatty acid in the incubation medium. The medium was frozen at −20 C until assay of free fatty acid was performed.

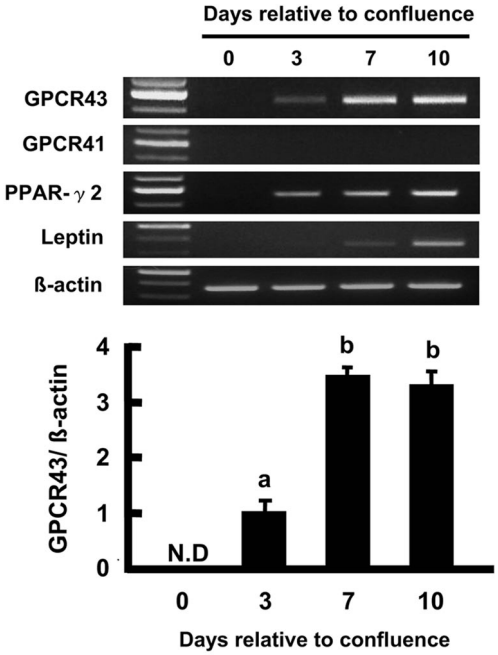

FIG. 3. The levels of GPCR43 and GPCR41 mRNA during differentiation of 3T3-L1 adipocytes. RT-PCR analysis was performed on confluent preadipocytes (0 d) and adipocytes that had differentiated for 3, 7, and 10 d. Data were normalized using β-actin mRNA and expressed as number of times the value obtained at d 3. The RT-PCR results shown are representative of three separate experiments with the same protocol. The data represent the means ± SEM of the three experiments. a and b, Mean values with *different superscripts* are significantly different ($P < 0.05$).

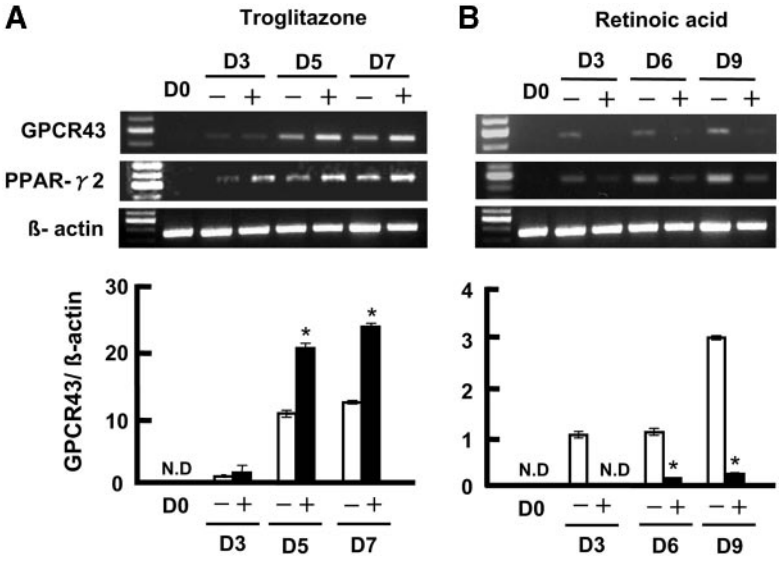

FIG. 4. A, The levels of GPCR43 and PPAR-γ2 mRNA in 3T3-L1 adipocytes treated with troglitazone during differentiation. The 3T3-L1 cells were allowed to proliferate to confluence and then to differentiate to adipocytes in differentiation medium with or without troglitazone (5 μM) for up to 7 d. *Upper panel*, Representative ethidium bromide-stained agarose gel showing amplified GPCR43, PPAR-γ2 and β-actin with molecular markers on different days of differentiation indicated above. *Lower panel*, Data were normalized using β-actin mRNA and expressed as number of times the value obtained at d 3 with no treatment. B, The levels of GPCR43 and PPAR-γ2 mRNA in 3T3-L1 adipocytes treated with retinoic acid during differentiation. The 3T3-L1 cells were allowed to proliferate to confluence and then to differentiate to adipocytes in differentiation medium with or without all-*trans*-retinoic acid (RA, 0.1 μM) for up to 9 d. *Upper panel*, Representative ethidium bromide-stained agarose gel showing amplified GPCR43, PPAR-γ2, and β-actin with molecular markers on different days of differentiation indicated above. *Lower panel*, Data were normalized using β-actin mRNA and expressed as number of times the value obtained at d 3 with no treatment. All data represent means ± SEM of three independent experiments. ND, Not detected. *, $P < 0.05$ *vs.* no treatment.

Statistical analysis

The data in Fig. 2 are presented as means ± SEM of six animals treated with the same protocol. The data in Fig. 3, 6, 8, and 9 are presented as means ± SEM of three experiments with the same protocol. Differences in mean values in Fig. 3, 6, 8, and 9 were assessed statistically using Duncan's multiple range test followed by a one-way ANOVA. The data in Fig. 2 were analyzed using Student's *t* test.

Results

Levels of GPCR41 and GPCR43 mRNA in tissues, adipocytes and stromal-vascular cells

We investigated the expression levels of GPCR41 and GPCR43 in adipose and nonadipose tissues. In a subsequent experiment to validate our results, we determined the expression levels of leptin and PPAR-γ2. The expression of GPCR43 in four adipose tissues was higher than in nine of the nonadipose tissues examined but not in the stomach, colon, and spleen (Fig. 1A). GPCR41 mRNA was not detected in four adipose tissues. Furthermore, to clarify whether GPCR43 and GPCR41 were expressed in isolated adipocytes, their mRNA levels were analyzed in adipocytes and stromal-vascular cells isolated from four different adipose tissues. GPCR43 was highly expressed in adipocytes, with a much lower expression in stromal-vascular cells (Fig. 1B). GPCR41 mRNA was not detected in adipocytes or stromal-vascular cells.

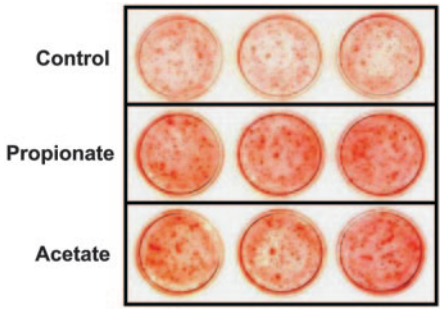

FIG. 5. Functional role of acetate and propionate on differentiation of 3T3-L1 cells. The 3T3-L1 cells were allowed to proliferate to confluence and then to differentiate to adipocytes in differentiation medium with or without acetate (10^{-7} M) or propionate (10^{-7} M) for 7 d. Oil red O staining was done on d 7.

Level of GPCR43 mRNA in adipose tissue of mice fed normal and high-fat diets

Differential gene expression of GPCR43 in response to changes in nutritional status appears to be an essential feature of the biological function of this receptor. Accordingly, we next examined GPCR43 gene expression in adipose tissues from mice fed high-fat and normal diets. RT-PCR analysis showed that the levels of GPCR43, PPAR-γ2, and leptin mRNA were up-regulated at different rates in the four adipose tissues screened (Fig. 2).

Level of GPCR43 mRNA during differentiation of 3T3-L1 preadipocytes

To investigate the involvement of GPCR43 in adipocyte differentiation *in vitro*, GPCR43 mRNA levels were analyzed during differentiation of 3T3-L1 cells. The levels of GPCR43 mRNA increased after adipocyte differentiation with a peak at d 7 (Fig. 3). However, GPCR41 mRNA was not detected

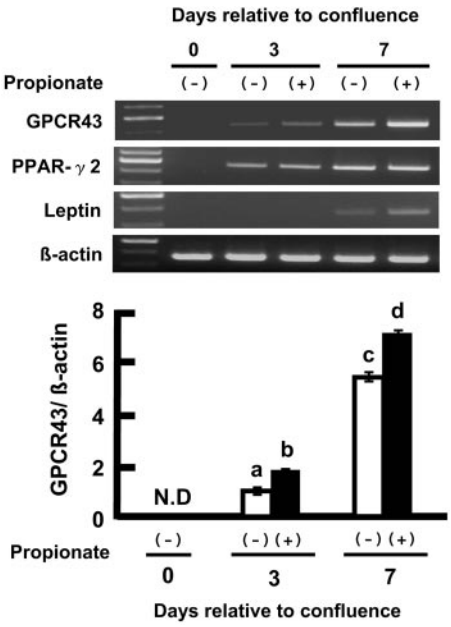

FIG. 6. The effect of propionate on the expression of GPCR43, PPAR-γ2, and leptin during the differentiation of 3T3-L1 cells. The 3T3-L1 cells were allowed to proliferate to confluence and then to differentiate to adipocytes in differentiation medium with or without propionate (10^{-7} M) for up to 7 d. Data were normalized using β-actin mRNA, and expressed as number of times the value obtained at control of d 3. The RT-PCR results shown are representative of three separate experiments with the same protocol. The data represent the means ± SEM of the three experiments. ND, Not detected. a–d, Mean values with *different superscripts* are significantly different ($P < 0.05$).

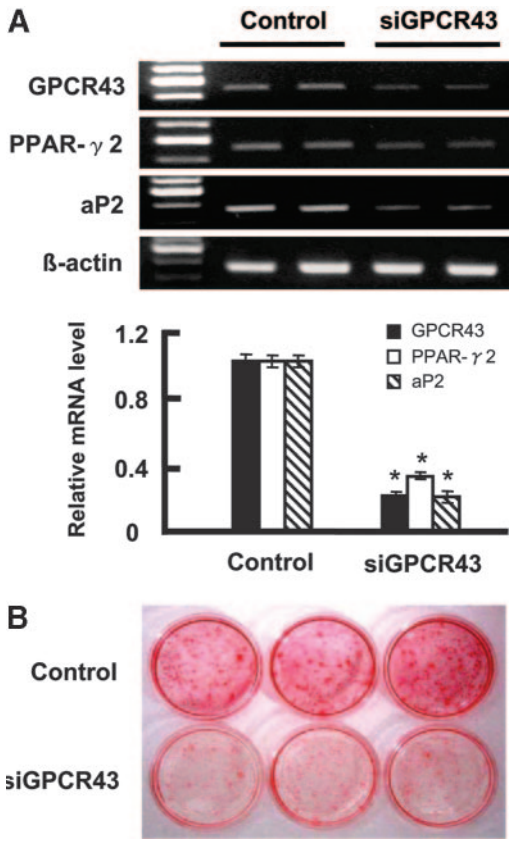

FIG. 7. Adipocyte differentiation in siRNA-transfected 3T3-L1 cells. A, The levels of GPCR43, PPAR-γ2 and aP2 mRNA in 3T3-L1 cells transfected with siGPCR43 and scrambled siRNAs during differentiation for 7 d. *Upper panel*, Representative ethidium bromide-stained agarose gel showing amplified GPCR43, PPAR-γ2, aP2, and β-actin with molecular markers. Lower panel: data were normalized using β-actin mRNA and expressed as number of times the value obtained for controls. All data represent means ± SEM of three independent experiments. *, $P < 0.05$ *vs.* control. B, 3T3-L1 cells were cultured and transfected with 50 nM scrambled siRNA (control) and GPCR43 siRNA (siGPCR43) before induction of differentiation. After 7 d, the cells were fixed and stained with oil red O to detect oil droplets.

during adipocyte differentiation. The expression of PPAR-γ2 and leptin increased after differentiation. PPAR-γ2, an adipocyte differentiation marker, has been widely used as an index of preadipocyte differentiation. To investigate whether up- and down-regulation of PPAR-γ2 is related to the expression of GPCR43 mRNA, 3T3-L1 cells were treated by troglitazone and retinoic acid during adipocyte differentiation. Troglitazone treatments stimulated PPAR-γ2 mRNA expression during differentiation for 7 d, during which time the level of GPCR43 mRNA was up-regulated (Fig. 4A). However, retinoic acid inhibited PPAR-γ2 expression and down-regulated the expression of GPCR43 (Fig. 4B).

Functional roles of acetic acid and propionic acid on adipogenesis of 3T3-L1 cells

To confirm the effects of ligands on adipocyte differentiation, 3T3-L1 cells were treated with acetate or propionate (each 10^{-7} M). Oil red O staining showed that treatment with propionate or acetate for 7 d increased the degree of adipocyte differentiation compared with the control (Fig. 5). RT-PCR analysis showed that propionic acid treatment increased the levels of GPCR43 mRNA (Fig. 6). Furthermore, the levels of PPAR-γ2 and leptin mRNA were elevated by treatment with propionate.

Inhibitory effect of GPCR43 siRNA during adipocyte differentiation

We next examined how the down-regulation of GPCR43 affects the process of adipogenesis. GPCR43 siRNA silenced its target mRNA specifically and effectively in 3T3-L1 cells (Fig. 7A). To determine whether the reduction of GPCR43 involves alteration in the expression of genes encoding a key adipogenic transcription factor, we examined the expression of PPAR-γ2 and adipocyte fatty acid binding protein (aP2) during the differentiation of cells that were transfected with GPCR43 siRNA. PPAR-γ2 and aP2 mRNA levels declined in the transfected cells compared with the control cells. In addition, the number of oil red O staining cells was dramatically less in GPCR43 siRNA-transfected cells (Fig. 7B).

The antilipolysis activity of acetate and propionate in 3T3-L1 adipocytes

Finally, we investigated the effect of acetate and propionate on adipocytes by isoproterenol-induced lipolysis. In this experiment, we used 3T3-L1 differentiated adipocytes 8 d after confluence. As shown in Fig. 8, treatment differentiated adipocytes with either acetate or propionate reduced the rates of isoproterenol-induced lipolysis. Acetate and propionate blocked isoproterenol-induced lipolysis in a

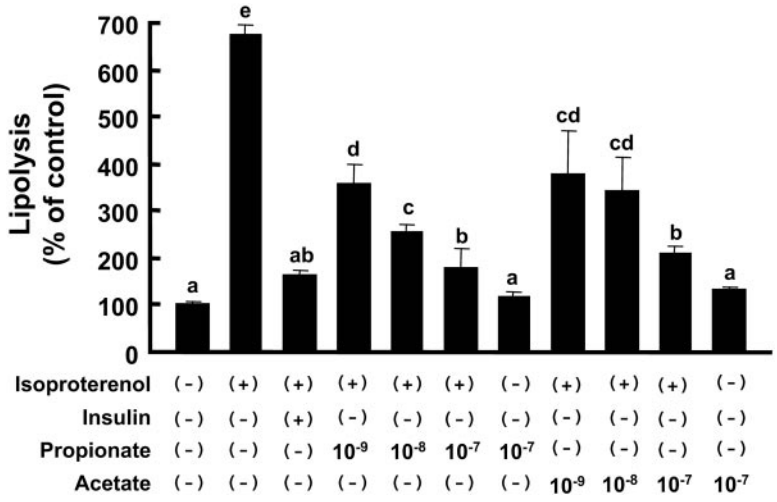

FIG. 8. Effect of acetate and propionate on isoproterenol-induced lipolysis in 3T3-L1 adipocytes. Confluent 3T3-L1 preadipocytes were induced to differentiate cultured for 7 d. On d 8, the 3T3-L1 adipocytes were incubated for 30 min with isopreterenol (10^{-8} M), insulin (10^{-7} M) and various concentrations of acetate or propionate. Lipolysis was determined by free fatty acid release into the medium. All data are expressed as percentages of the basal rate of 100% in the control group without isoproterenol, acetate or propionate. The data represent the means ± SEM of four experiments. a–e, Mean values with *different superscripts* are significantly different ($P < 0.05$).

dose-dependent manner (from 10^{-7} to 10^{-9} M). However, there was no difference between acetate and propionate in their abilities to protect against induced lipolysis. In addition, Fig. 9 shows the antilipolytic activity of acetate and propionate in 3T3-L1 adipocytes transfected with scramble and GPCR43 siRNA. Acetate (10^{-7} M) and propionate (10^{-7} M) did not significantly block the isoproterenol-induced lipolysis in GPCR43 siRNA-transfected 3T3-L1 cells compared with scramble siRNA-transfected cells (Fig. 9).

Discussion

The present study demonstrates that acetate and propionate act on lipid accumulation and inhibition of lipolysis mainly through GPCR43, not GPCR41. GPCR43 is known to be highly expressed in immune cells such as monocytes and neutrophils, and the transcripts of GPCR41 are higher in adipose tissues (8, 9). However, the results of these two papers contradict our data. We found that the level of GPCR43 mRNA was high in four different adipose tissues, whereas GPCR41 was not detected in adipose tissues. Xiong *et al.* (10) reported that propionate-stimulated gene expression and leptin secretion via the GPCR41 pathway. GPCR41 mRNA was not detected in differentiated adipocytes of 3T3-L1 in our experiment, even though we used the same PCR primers as Xiong *et al.* (10). In addition, Le Poul *et al.* (13) showed that both GPCR41 and GPCR43 was expressed in human adipose tissue. However, we did not detect expression of GPCR41 and GPCR43 in the human adipose tissues and cultured human preadipocyte and adipocyte samples tested (data not shown). We do not know the reason for this discrepancy. Interestingly, the expression of GPCR43 was up-regulated in all adipose tissues of mice fed the high-fat diet, indicating that GPCR43 is on the lipid accumulation pathway. Moreover, GPCR43 mRNA was highly expressed as a result of treatment with troglitazone. However, GPCR43 was not detected in confluent 3T3-L1 cells. Acetate and propionate stimulated the expression of GPCR43 and PPAR-γ2 mRNA in differentiated adipocytes, with stimulation of fat accumulation shown by oil red O staining. Although there was no evidence that acetate and propionate stimulated PPAR-γ2 mRNA, these two short fatty acids increased fat accumulation. This suggests that GPCR43 and its ligands function as regulators of adipogenesis in adipocyte development and differentiation.

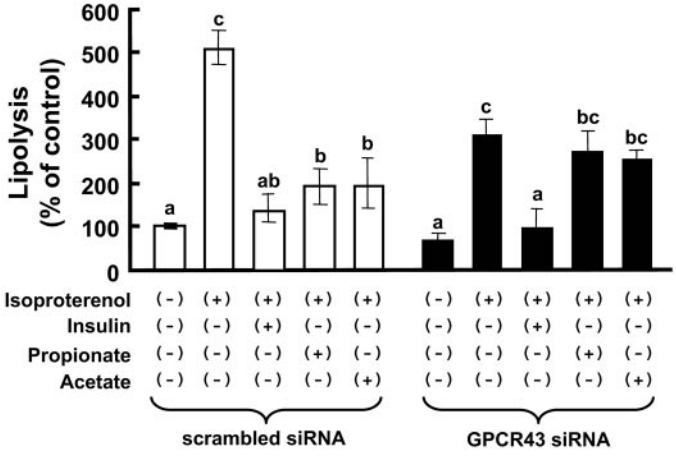

FIG. 9. Effect of acetate and propionate on isoproterenol-induced lipolysis in scrambled and GPCR43 siRNA-transfected 3T3-L1 adipocytes. 3T3-L1 cells were cultured and transfected with 50 nM scrambled and GPCR43 siRNA before induction of differentiation. On d 8, the 3T3-L1 adipocytes were incubated for 30 min with isopreterenol (10^{-8} M), insulin (10^{-7} M), acetate (10^{-7} M), and/or propionate (10^{-7} M). Lipolysis was determined by free fatty acid release into the medium. All data are expressed as percentages of the basal rate of 100% in the control group without isoproterenol, acetate or propionate. The data represent the means ± SEM of three experiments. a–c, Mean values with *different superscripts* in each siRNA-transfected cells are significantly different ($P < 0.05$).

To date, there have been few investigations of factors that regulate the concentration of acetate and propionate in the blood. In addition, the roles of acetate and propionate are still controversial. Short chain fatty acids (SCFAs) involved *in vivo* 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 70–100 mM (14). 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 100–150 μM for acetate, 4–5 μM for propionate, and

1–3 μM for butyrate (15). Moreover, the plasma concentration of acetate can increase up to 10-fold compared with basal values after ethanol administration (16, 17). Some inherited diseases, caused by specific enzyme defects, result in the accumulation of propionate and butyrate (18, 19). Accumulation of propionate in the blood is a characteristic feature of the disease propionic acidemia. This rare inherited disorder is caused by deficient activity of propionyl-coenzyme A carboxylase. Remarkable deposition of fat was observed in the liver of mice lacking propionyl-coenzyme A carboxylase at 24 h after birth (20). However, this fat deposition was not detected at the late embryonic stage (embryonic d 18.5), suggesting that the deposition appeared to be rapidly generated in proportion to the progression of acidosis after birth. This also indirectly suggests that increments of propionate may be involved in fat accumulation in adipose tissues. Our data showed that acetate and propionate stimulated fat accumulation in 3T3-L1 adipocytes, with up-regulation of PPAR-γ2. Moreover, siRNA-mediated reduction of GPCR43 mRNA in 3T3-L1 cells inhibited differentiation. When GPCR43 expression was knocked down, the accumulation of oil droplets decreased and further expression of PPAR-γ2, an adipocyte differentiation marker, declined during adipogenesis. These results clearly demonstrate that short chain fatty acids stimulate fat accumulation by the same pathway that stimulates PPAR-γ2 in adipogenesis.

Isoproterenol-stimulated lipolysis was reduced in a dose-dependent manner by acetate or propionate treatment *in vitro*. One of the most important metabolic actions of insulin is the inhibition of lipolytic activity in fat cells (21). Activation by insulin of the adipocyte cGMP-inhibited cAMP phosphodiesterase is believed to be the major mechanism whereby insulin reduces cellular cAMP. Reduction in cAMP leads in turn to inactivation of the cAMP-dependent protein kinase, with net dephosphorylation of hormone-sensitive lipase and antilipolysis (22–24). The antilipolytic effect of acetate and propionate is very similar to that of insulin, which inhibits lipolytic activity in fat cells. Moreover, acetate and propionate did not show the antilipolytic activity in GPCR43 siRNA-transfected 3T3-L1 cells. GPCR43 belongs to a cluster of four orphan GPCRs, the subfamily GPCR40–43. Each GPCR has many divergent functions, but GPCR41 has been found to share 38% identity with GPCR43 in amino acid sequence (25). Propionate was the most potent agonist for both GPCR41 and GPCR43. Acetate was more selective for GPCR43, whereas butyrate and isobutyrate were more active on GPCR41. The two receptors were coupled to inositol 1,4,5-trisphosphate formation, intracellular [Ca^{2+}] release, ERK1/2 activation, and inhibition of cAMP accumulation. They exhibit, however, a differential coupling to G proteins: GPCR41 coupled exclusively through the pertussis toxin-sensitive Gi/o family, whereas GPCR43 displayed a dual coupling through Gi/o and pertussis toxin-insensitive Gq protein families. Our present results showed that this antilipolytic activity of acetate and propionate was through GPCR43 because GPCR41 was not expressed in 3T3-L1 adipocytes.

In conclusion, the present study demonstrates that GPCR43 plays an important role in adipocyte differentiation and adipocyte development and further, that acetate and propionate stimulate lipid accumulation and have antilipolysis activity. Although clearly speculation at this point, the expression pattern of GPCR43 suggests the possibility that its rapid and transient up-regulation during differentiation may relieve transcriptional repression on other adipogenic genes. Further studies are required to clarify the mechanisms of action of short chain fatty acids in adipogenesis.

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