Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41)

Ikuo Kimura[a], Daisuke Inoue[a], Takeshi Maeda[a], Takafumi Hara[a], Atsuhiko Ichimura[a], Satoshi Miyauchi[a], Makio Kobayashi[b], Akira Hirasawa[a], and Gozoh Tsujimoto[a,1]

[a]Department of Genomic Drug Discovery Science, Kyoto University Graduate School of Pharmaceutical Sciences, Sakyo-ku, Kyoto 606-8501, Japan; and [b]Department of Pathology, Tokyo Women's Medical University, Shinjuku-ku, Tokyo 162-8666, Japan

The maintenance of energy homeostasis is essential for life, and its dysregulation leads to a variety of metabolic disorders. Under a fed condition, mammals use glucose as the main metabolic fuel, and short-chain fatty acids (SCFAs) produced by the colonic bacterial fermentation of dietary fiber also contribute a significant proportion of daily energy requirement. Under ketogenic conditions such as starvation and diabetes, ketone bodies produced in the liver from fatty acids are used as the main energy sources. To balance energy intake, dietary excess and starvation trigger an increase or a decrease in energy expenditure, respectively, by regulating the activity of the sympathetic nervous system (SNS). The regulation of metabolic homeostasis by glucose is well recognized; however, the roles of SCFAs and ketone bodies in maintaining energy balance remain unclear. Here, we show that SCFAs and ketone bodies directly regulate SNS activity via GPR41, a Gi/o protein-coupled receptor for SCFAs, at the level of the sympathetic ganglion. GPR41 was most abundantly expressed in sympathetic ganglia in mouse and humans. SCFA propionate promoted sympathetic outflow via GPR41. On the other hand, a ketone body, β-hydroxybutyrate, produced during starvation or diabetes, suppressed SNS activity by antagonizing GPR41. Pharmacological and siRNA experiments indicated that GPR41-mediated activation of sympathetic neurons involves Gβγ-PLCβ-MAPK signaling. Sympathetic regulation by SCFAs and ketone bodies correlated well with their respective effects on energy consumption. These findings establish that SCFAs and ketone bodies directly regulate GPR41-mediated SNS activity and thereby control body energy expenditure in maintaining metabolic homeostasis.

microbiota | superior cervical ganglion | FFAR3 | probiotics | fasting

To balance energy intake, dietary excess and fasting triggers an increase or a decrease in energy expenditure, respectively, by regulating activity of the sympathetic nervous system (SNS) (1–3), and its dysregulation leads to metabolic disorders such as obesity and diabetes (4, 5). In feeding, excessive energy is consumed by the enhancement of sympathetic function, resulting in increases in heart rate and diet-induced thermogenesis (2, 6), whereas in fasting, energy use is saved by the suppression of the sympathetic function as a survival mechanism, resulting in the reduction in heart rate and activity (3, 6). Under a fed condition, mammals use glucose as the main metabolic fuel, and short-chain fatty acids (SCFAs) produced by the colonic bacterial fermentation of dietary fiber also contribute a significant proportion of the daily energy requirement (7, 8). Under ketogenic conditions such as fasting and diabetes, ketone bodies produced in the liver from fatty acids are used as the main energy sources (9, 10). However, the effect of monocarboxylic metabolites, as SCFAs and ketone bodies, on the regulation of SNS activity remains unclear.

Free fatty acids (FFA) not only are essential nutrients but also act as signaling molecules in various cellular processes. Recently, several groups reported that five orphan G protein-coupled (GPR) receptors—GPR40, GPR41, GPR43, GPR84, and GPR120—can be activated by FFAs. Long-chain fatty acids are specific agonists for GPR40 and GPR120 (11, 12) and medium-chain fatty acids are for GPR84 (13). Short-chain fatty acids can activate GPR41 (14) and GPR43 (15). Stimulation of GPR41 by FFAs resulted in inhibiting cAMP production and activation of the ERK cascade, which suggests interactions with the Gα(i/o) family of G proteins (16, 17). GPR41 has been reported to be expressed in adipose tissue and to promote secretion of leptin (18, 19). Other physiological functions of GPR41 still remain to be explored.

In the present study we show that SCFAs and ketone bodies, major energy sources in the body, directly regulate sympathetic activity via GPR41. By examining the cardiac and metabolic sympathetic responses in vitro as well as in mice lacking GPR41, we found that propionate (a major SCFA) and β-hydroxybutyrate (a major ketone body) promotes or depresses GPR41-mediated SNS activation, respectively.

Results

Abundant *Gpr41* Expression in Sympathetic Ganglia and Reduced Sympathetic Nerve Activity in *Gpr41*^{−/−} Mice. Examining *Gpr41* and *Gpr43* expression in tissues, we found that *Gpr41* was most abundantly expressed in the sympathetic ganglia such as the superior cervical ganglion (SCG) in adult mouse (Fig. 1*A*). SCG is an extension of the cervical sympathetic chain and a mass of sympathetic neurons. Compared with *Gpr41*, *Gpr43* was scarcely expressed in the SCG of either wild-type or *Gpr41*^{−/−} mice (Fig. S1 *A* and *B*). In situ hybridization revealed that *Gpr41* was most abundantly expressed in sympathetic ganglia and trunks during embryonic (E13.5 and E15.5) and postnatal (P1) stages (Fig. 1 *B* and *C*) to adulthood in mice and also in the sympathetic ganglia of human adults (Fig. S1 *C* and *D*).

To determine the effect of GPR41 on the SNS, we generated *Gpr41*^{−/−} mice (Fig. S2). *Gpr41*^{−/−} mice exhibited normal growth and no major morphological abnormalities. Body weight, heart-weight/body-weight ratio and metabolic parameters and hormones (plasma concentrations of glucose, triglycerides, free fatty acids, leptin, and insulin) were comparable between wild-type and *Gpr41*^{−/−} mice (Fig. S3 *A–G*). During development (P1), the SCG volume was significantly smaller in *Gpr41*^{−/−} than in wild-type mice (Fig. 1 *D* and *E*). *Gpr41*^{−/−} mice exhibited significantly reduced density of sympathetic innervations and tyrosine hydroxylase (TH) protein in the heart (Fig.1 *F–H*), indicating that GPR41 may be involved in sympathetic nerve growth. TH is the rate-limiting enzyme for catecholamine biosynthesis and plays an im-
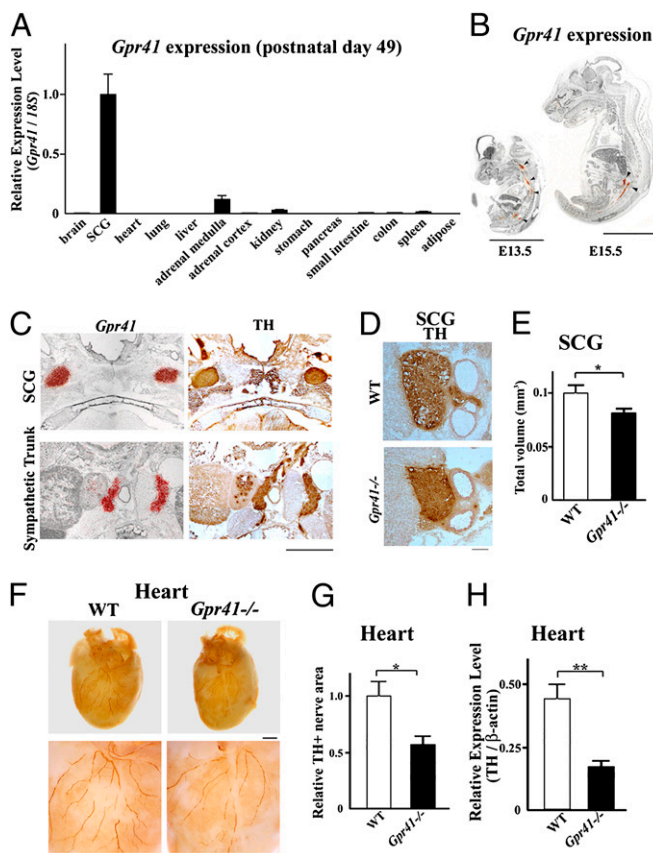
Fig. 1. Abundant *Gpr41* expression in sympathetic ganglia and reduced sympathetic nerve activity in GPR41-deficient mice. (*A*) *Gpr41* expression in postnatal mouse tissues (P49) measured by qRT-PCR (*n* = 3). SCG, superior cervical ganglion. Internal control: *18S* rRNA expression. (*B*) *Gpr41* mRNA localization in mouse embryos (E13.5 and E15.5) as determined by in situ hybridization using an ^{35}S-labeled antisense *Gpr41* RNA probe. Red grains (arrowheads) superimposed on a hematoxylin–eosin-stained section indicate *Gpr41* mRNA localization. (Scale bar: 5 mm.) (*C*) *Gpr41* mRNA localization in postnatal day 1 (P1) mice (*Left*). Anti-tyrosine hydroxylase (TH) immunostaining (brown) (*Right*). (Scale bar: 1 mm.) (*D* and *E*) Anti-TH antibody immunostaining (brown) in SCG at P1. Total volume was measured by quantifying the TH-positive area (*n* = 6). (*F*) (*Upper*) Whole-mount immunostaining of the heart with anti-TH antibodies (brown). (*Lower*) High magnification view. (Scale bar: 1 mm.) (*G*) Quantitative analysis of TH+ nerve areas (*n* = 5). (*H*) TH protein expression (*n* = 6). β-Actin (loading control). Mice were analyzed at 12 wk of age (*F*–*H*). *$P < 0.05$; **$P < 0.005$.

portant role in the sympathetic regulation of the heart function. Corresponding with the retarded growth of sympathetic innervations, resting heart rate was significantly reduced in *Gpr41$^{-/-}$* mice (682 ± 10 beat/min and 610 ± 16 beat/min in wild-type and *Gpr41$^{-/-}$*; *n* = 12 each, respectively) (Fig. 2*B*). In *Gpr41$^{-/-}$* mice, on the other hand, cardiac noradrenaline (NA) content was significantly increased, whereas plasma NA level was decreased (Fig. 2*A*). The reduction in heart rate associated with treatment using the β-adrenoreceptor blocker propranolol was more marked in wild-type than in *Gpr41$^{-/-}$* mice, and the resultant heart rates were similar to those of both wild-type and *Gpr41$^{-/-}$* mice (Fig. 2*B*). Treatment with tyramine, an agent causing release of stored NA from sympathetic nerve terminals, reduced heart rate and plasma and cardiac NA concentrations in both groups of mice, and the between-group differences in the parameters before tyramine treatment were not found (Fig. 2*C* and Fig. S3*H*). Results suggested that the lower resting heart rate in *Gpr41$^{-/-}$* mice is due to the reduced SNS activity. Furthermore, enhanced cardiac NA

stores and the reduced circulating NA levels in *Gpr41$^{-/-}$* mice indicated that GPR41 may contribute to NA release from SNS.

Effects of SCFA on Sympathetic Activity. To demonstrate that SCFAs are relevant for the effects of GPR41 on SNS, we examined the effect of propionate, an SCFA found to have the most potent agonistic effect in the heterologous expression system (Fig. S4). Administration of propionate (1 g/kg, i.p.), but not of a middle-chain fatty acid octanoate (1 g/kg, i.p.), caused a significant increase in heart rate in wild-type and *Gpr43$^{-/-}$* mice, whereas neither propionate nor octanoate caused any change in heart rate in *Gpr41$^{-/-}$* mice (Fig. 2 *D* and *E* and Fig. S5 *A* and *B*). Also, metabolic parameters and hormones (plasma concentrations of glucose, triglycerides, free fatty acids, and leptin) following administration of propionate were comparable between wild-type and *Gpr41$^{-/-}$* mice (Fig. S5*C*). Treatment with a ganglion blocker hexamethonium (20 mg/kg i.p.) had little effect on this propionate-induced increase in heart rate, whereas propranolol (4 mg/kg i.p.) abolished the response (Fig. 2*F*). To confirm that the treatments with hexamethonium and propranolol have respective ganglion blocking and β-blocking effects, we examined their effects on heart rate responses to a cholinergic agonist carbachol and β-adrenoreceptor agonist isoproterenol. Carbachol- and isoproterenol-induced increases in heart rate were suppressed by hexamethonium and by propranolol, respectively (Fig. 2*F*). Thus, propionate may modulate the SNS activity at the level of sympathetic ganglion via GPR41 but not via GPR43.

SCFA Propionate Induced Sympathetic Activation via GPR41-Gβγ in Sympathetic Neurons. We further examined whether this propionate-induced positive chronotropism is due to the GPR41-mediated SNS activation or not, by using coculture of primary-cultured fetal isolated cardiomyocytes and sympathetic neurons. RT-PCR assays confirmed that primary-cultured sympathetic neurons obtained from fetal SCG highly express *Gpr41*, but that fetal isolated cardiomyocytes lack *Gpr41* expression (Fig. S6*A*). In primary-cultured sympathetic neurons from wild-type and *Gpr43$^{-/-}$* mice, propionate evoked extracellular action potentials (Fig. 3*A* and Fig. S6*B*). However, this propionate-activated response was not observed in sympathetic neurons obtained from *Gpr41$^{-/-}$* mice (Fig. 3*A*). Propionate reduced intracellular cAMP concentrations and promoted ERK1/2 phosphorylation in primary-cultured sympathetic neurons (Fig. S6 *C* and *D*). These propionate-induced responses were not observed in sympathetic neurons from *Gpr41$^{-/-}$* mice, and they were abolished by pertussis toxin (PTX) (Fig. S6 *C–E*), indicating that propionate-activated GPR41 signaling is required for Gi/o. We further examined the effects of SCFAs on cardiac SNS activity by using the coculture of fetal isolated cardiomyocytes with primary-cultured sympathetic neurons. Isoproterenol significantly increased beat rate in either monocultured or cocultured cardiomyocytes. Propionate, on the other hand, reduced beat rate in monocultured cardiomyocytes, whereas in cocultured cardiomyocytes propionate did not reduce, but rather increased, beat rate, which was abolished by propranolol (Fig. 3*B*). Therefore, propionate significantly ($P < 0.005$) increased the beat rate of the cardiomyocytes cocultured with SCG neurons compared with that of the monocultured myocytes (Fig. 3*B*). As with wild-type mice, isoproterenol increased and propionate reduced beat rate, respectively, in monocultured cardiomyocytes isolated from *Gpr41$^{-/-}$* mice (Fig. S6*F*). However, in coculture of isolated cardiomyocytes with sympathetic neurons obtained from *Gpr41$^{-/-}$* mice, propionate did not elicit a rise in beat rate (Fig. 3*C*). Rather, propionate reduced beat rate, as was observed in experiments with monocultured cardiomyocytes (Fig. 3*C*). Results showed that an SCFA propionate evokes action potentials in sympathetic neurons via GPR41 and thereby directly enhances SNS outflow. We further examined the GPR41-mediated signaling in SCG neurons. As G protein signaling has been recently well characterized by using selective pharmacological tools (such as inhibitors for Gα and Gβγ proteins), we adopted NF023 [Gα(i/o) blocker] and

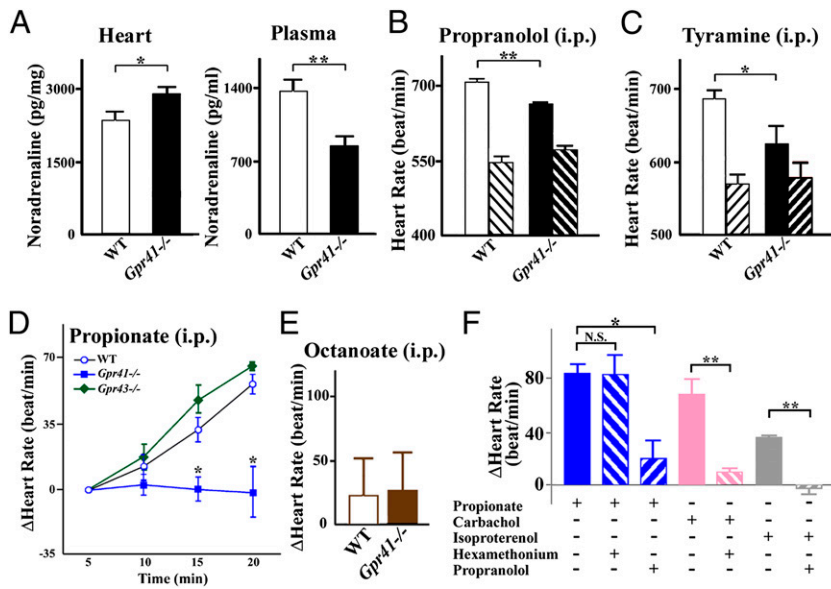PHYSIOLOGY

Fig. 2. Effects of SCFA on sympathetic activity via GPR41 in GPR41-deficient mice. (*A*) Noradrenaline (NA) concentrations in hearts (*n* = 10) and plasma (*n* = 6). (*B*) Effects of propranolol on heart rate in *Gpr41*^{−/−} mice. Measurement of heart rate at 10 min after propranolol administration (i.p.; *n* = 7–9). Both hatched bars, propranolol-treated. (*C*) Effects of tyramine on heart rate. Measurement of heart rate (*n* = 9) at 24 h following tyramine injection (i.p.). Both hatched bars, tyramine-treated. (*D*) Effects of propionate on heart rate in *Gpr41*^{−/−} mice (i.p.; *n* = 5–7). (*E*) Effects of octanoate on heart rate in *Gpr41*^{−/−} mice. Measurement of heart rate at 20 min after octanoate administration (i.p.; *n* = 4–5). (*F*) After pretreatment with hexamethonium (20 mg/kg) or propranolol (4 mg/kg) for 10 min, at time 0, a bolus of propionate (1 g/kg), carbachol (50 μg/kg), or isoproterenol (3 μg/kg) was administered intraperitoneally (*n* = 4–6). Data of was measured at 20 min (propionate) and at 3 min (carbachol and isoproterenol). Mice were analyzed at 12 wk of age. *P < 0.05; **P < 0.005.

Gallein (Gβγ blocker) for the purpose (20, 21). GPR41-mediated generation of action potential and rise in heart rate was effectively blocked by Gallein and PTX treatment, whereas NF023 had no inhibitory effects (Fig. 3 *D* and *E*). We confirmed that PTX and NF023 successfully inhibited GPR41-mediated cAMP inhibition, whereas Gallein had no such an inhibitory effect (Fig. S6G). These series of pharmacological studies showed that GPR41-mediated excitatory responses in SCG are mediated by Gβγ, but not by Gα(i/o), coupled to cAMP inhibition. We further examined the intracellular signaling for GPR41-induced sympathetic activation by using RNA interference. As it was difficult to transfer small interfering RNA (siRNA) into the primary-cultured sympathetic neurons, we adopted mouse neuroblastoma cell line Neuro2A cells as a surrogate of sympathetic neurons (22, 23).

Neuro2A cells express barely *Gpr41* or *Gpr43* (Fig. S6H). Propionate significantly increased beat rate when cardiomyocytes were cocultured with Neuro2A cells expressing GPR41 (Fig. 3F and Fig. S6I). NGF, known to stimulate noradrenaline release from sympathetic neurons (24), increased beat rate when cocultured with Neuro2A cells transfected either with or without *Gpr41* (Fig. 3F). Treatment of GPR41-expressing Neuro2A cells with siRNA for PLCβ2/3, but not those for β-arrestin1/2 and GRK2 (25, 26), significantly inhibited the propionate-induced rise in beat rate of cardiomyocytes when cocultured (Fig. 3G and Fig. S6 *J* and *K*). Moreover, siRNA for ERK1/2, but not Gαq, showed a significant inhibitory effect (Fig. 3G). These results indicate that GPR41 activation of sympathetic neurons may involve Gβγ, PLCβ, and MAPK, but not Gα(i/o), β-arrestin, and GRKs.
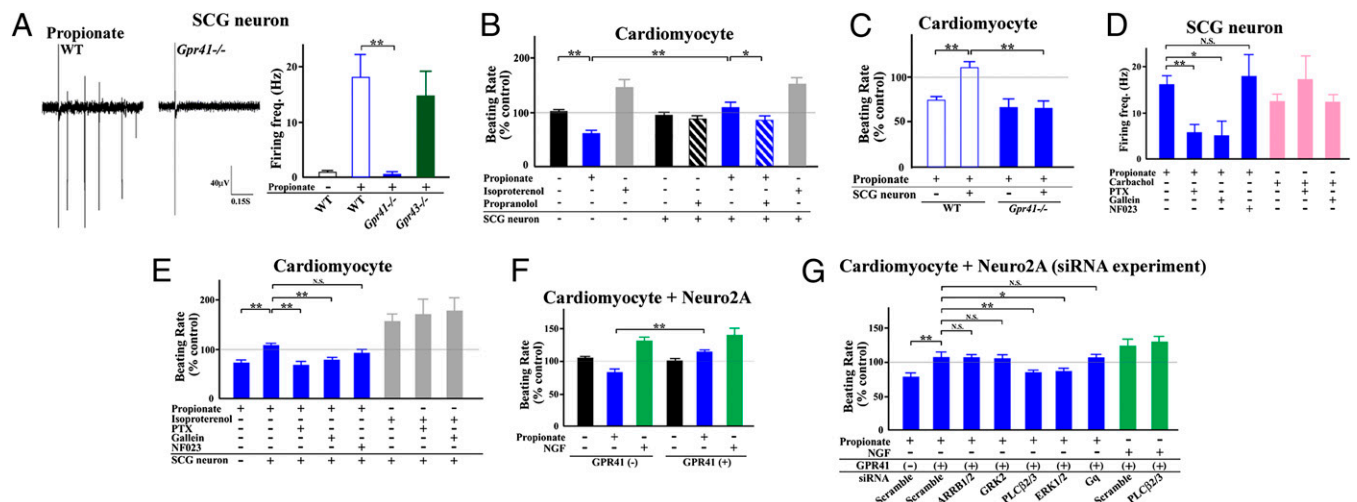

Fig. 3. SCFA-GPR41 signaling in sympathetic neurons. (*A*) Action potentials and firing frequency in *Gpr41*^{−/−} sympathetic neurons following stimulation with propionate (10 mM) (*n* = 3–8). (*B*) Change in myocyte beating rate in sympathetic neurons with and without propionate (1 mM) treatment and effects of propranolol (0.2 μM) (*n* = 5–6). Isoproterenol (10 μM) was used as a positive control. (*C*) Effects of propionate on change in myocyte beating rate in *Gpr41*^{−/−} cardiomyocytes and sympathetic neurons (*n* = 6–8). (*D*) Firing frequency in sympathetic neurons (*n* = 4–9) by propionate (10 mM) stimulation after pretreatment with or without PTX (100 ng/mL), Gallein (10 μM), or NF023 (10 μM) for 2 h. Carbachol (1 μM), a cholinergic agonist, was used as a positive control. (*E*) Effects of propionate on change in myocyte beat rate in cardiomyocytes and sympathetic neurons (*n* = 6–11). Cells were stimulated by propionate (1 mM) after pretreatment with or without PTX (100 ng/mL), Gallein (10 μM), or NF023 (10 μM) for 1 h. Isoproterenol (10 μM) was used as a positive control. (*F*) Effect of propionate (1 mM) and NGF (50 ng/mL) on the beating rate of cardiomyocytes when cocultured with Neuro2A cells either with or without transfection of *Gpr41* (*n* = 6). (*G*) Effects of siRNA on propionate-induced increase in the beating rate of cardiomyocytes when cocultured with Neuro2A cells expressing GPR41 (*n* = 6). *P < 0.05; **P < 0.005.

Ketone Body β-Hydroxybutyrate Inhibited Sympathetic Activity by Antagonizing GPR41. Under ketogenic conditions such as fasting, low-carbohydrate diet feeding, and diabetes, fatty acids and ketone bodies are used as the main energy sources (27). In assessing the effects of SCFAs and ketone bodies in GPR41-expressing HEK293 cells (16), we found that β-hydroxybutyrate has a potent antagonistic effect on GPR41, whereas acetoacetate, another major ketone body, has no significant effect (Fig. S7A). β-Hydroxybutyrate suppressed propionate-induced ERK1/2 activation in a dose-dependent manner (Fig. 4A) and inhibited the propionate-induced reduction in cAMP production in GPR41-expressing HEK293 cells (Fig. 4B). β-Hydroxybutyrate also inhibited both propionate-evoked firing frequency in primary-cultured sympathetic neurons (Fig. 4C), and propionate-induced rise in beat rate of cardiomyocytes cocultured with sympathetic neurons (Fig. 4D). Administration of β-hydroxybutyrate (500 mg/kg, i.p.), but not of another major ketone body, acetone (0.5 g/kg, i.p.), caused a significant decrease in heart rate in wild-type mice, whereas either β-hydroxybutyrate or acetone caused less change in heart rate in $Gpr41^{-/-}$ mice (Fig. 4E and Fig. S7 B–E). Moreover, β-hydroxybutyrate also inhibited the propionate-induced increase in heart rate in wild-type mice but not in $Gpr41^{-/-}$ mice (Fig. S7F). Treatment with hexamethonium did not affect the β-hydroxybutyrate-induced decrease in heart rate, whereas propranolol completely suppressed the response (Fig. S7G). The results show that β-hydroxybutyrate may also inhibit SNS activity at the level of the sympathetic ganglion. Hence, it may be possible for β-hydroxybutyrate to suppress the propionate-induced SNS activation as an antagonist for GPR41.

Reduced Sympathetic Activity Under Ketogenic Conditions Is Partly Due to GPR41 Antagonism by β-Hydroxybutyrate. We further assessed the effect of β-hydroxybutyrate, which can be endogenously produced under ketogenic conditions, on SNS activity. Following 48-h starvation, metabolic parameters (body weight and plasma levels of glucose, triglycerides, and free fatty acids) were comparable between wild-type and $Gpr41^{-/-}$ mice (Fig. S8 A–D). Plasma concentration of β-hydroxybutyrate was significantly higher in $Gpr41^{-/-}$ mice compared with wild-type mice (Fig. S8E). During fasting, heart rate can decline due to sympathetic depression (6, 10). The fasting-associated decline in heart rate was significantly lower in $Gpr41^{-/-}$ compared with wild-type mice (Fig. 5A). At 48-h starvation, an excessive increase in NA stores in the heart was observed in wild-type mice, which may reflect the decreased SNS activity; however, such an excessive increase in NA stores was not observed in $Gpr41^{-/-}$ mice (Fig. 5B). Furthermore, the reduction in heart rate by propranolol was markedly smaller during starvation compared with fed conditions in wild-type mice (Fig. 5C). On the other hand, in $Gpr41^{-/-}$ mice, the reduction in heart rate by pro-

pranolol was not affected by 48-h starvation (Fig. 5C). Taken together, the results showed that starvation-associated sympathetic depression appears to be lacking in $Gpr41^{-/-}$ mice.

Ketone bodies increase dramatically in diabetes (10). In streptozotocin (STZ)-induced diabetes in mice, heart rate declined during the progression of diabetes (28). Reduction of heart rate in the diabetic condition was significantly smaller in $Gpr41^{-/-}$ compared with wild-type mice (Fig. 5D), although the STZ-induced diabetic condition had similar effects on body weight and plasma levels of glucose and β-hydroxybutyrate in both groups (Fig. S8F). Furthermore, propranolol induced a significantly smaller reduction in heart rate in diabetic mice compared with control wild-type mice (Fig. 5E). In $Gpr41^{-/-}$ mice, on the other hand, propranolol did not cause any effect on heart rate, either with or without STZ treatment (Fig. 5E).

Collectively, our results indicate that GPR41 is required to induce sympathetic depression under ketogenic conditions, caused by either starvation or diabetes. However, the heart rate progressively decreased with time elapsed under ketogenic conditions in both wild-type and $Gpr41^{-/-}$ mice. Also, propranolol treatment decreased heart rate by ∼130 in wild-type mice (Fig. 5 C and E), whereas under ketogenic conditions it decreased heart rate by ∼300 (Fig. 5 A and D), respectively, indicating that ketone bodies not only inhibit sympathetic activity, but also may have a direct inhibitory effect on the heart rate.

Effects of GPR41-Mediated Regulation of Sympathetic Activity on Energy Expenditure. We further assessed to what extent the observed effects of SCFAs and ketone bodies on SNS activity affect energy expenditure. Oxygen consumption during feeding was significantly higher in wild-type mice than in $Gpr41^{-/-}$ mice (Fig. 6A). The oxygen consumption was significantly decreased by treatment with tyramine and during starvation in wild-type mice, whereas these effects were not exhibited in $Gpr41^{-/-}$ mice (Fig. 6A). As total activity was comparable between wild-type and $Gpr41^{-/-}$ mice during feeding and starvation (Fig. S9A), the change in oxygen consumption of $Gpr41^{-/-}$ mice may be due to the metabolic effect but not to physical activity. Also, the respiratory exchange ratio was comparable between wild-type and $Gpr41^{-/-}$ mice during feeding and starvation (Fig. S9B). Furthermore, body temperature and $Ucp1$ expression in brown adipose tissue were significantly lower in $Gpr41^{-/-}$ compared with wild-type mice (Fig. 6 B and C). As observed with changes in heart rate, propionate (1 g/kg, i.p.) increased and β-hydroxybutyrate (500 mg/kg, i.p.) decreased the oxygen consumption in wild-type mice, respectively. These responses were abolished by tyramine treatment (Fig. 6 D and E). In contrast, propionate, β-hydroxybutyrate, and tyramine treatment did not cause any change in the oxygen consumption of $Gpr41^{-/-}$ mice. Moreover, β-hydroxybutyrate inhibited the propionate-induced increase in oxygen consumption (Fig. 6F). Our results indicate that
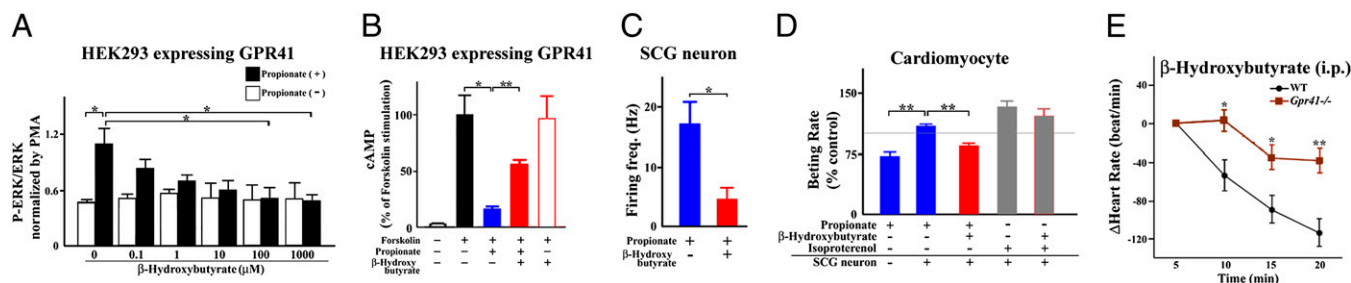

Fig. 4. Inhibitory effects of β-hydroxybutyrate on GPR41 sympathetic activity. (A) Antagonistic effects of β-hydroxybutyrate on ERK1/2 phosphorylation by propionate (1 mM) in GPR41-expressing HEK293 cells (n = 3). (B) Reduction in cAMP levels in response to propionate (0.1 mM) treatment in GPR41-expressing HEK293 cells and inhibitory effects of β-hydroxybutyrate (100 mM) (n = 3). (C) Firing frequency in sympathetic neurons (n = 5) after propionate (10 mM) stimulation with or without β-hydroxybutyrate (10 mM). (D) Inhibitory effects of β-hydroxybutyrate (500 μM) on change in myocyte beat rate in cardiomyocytes and sympathetic neurons with propionate (1 mM) treatment (n = 5–8). (E) Effects of β-hydroxybutyrate on heart rate of $Gpr41^{-/-}$ mice (500 mg/kg i.p.; n = 8). Mice were analyzed at 12 wk of age. *P < 0.05; **P < 0.005.
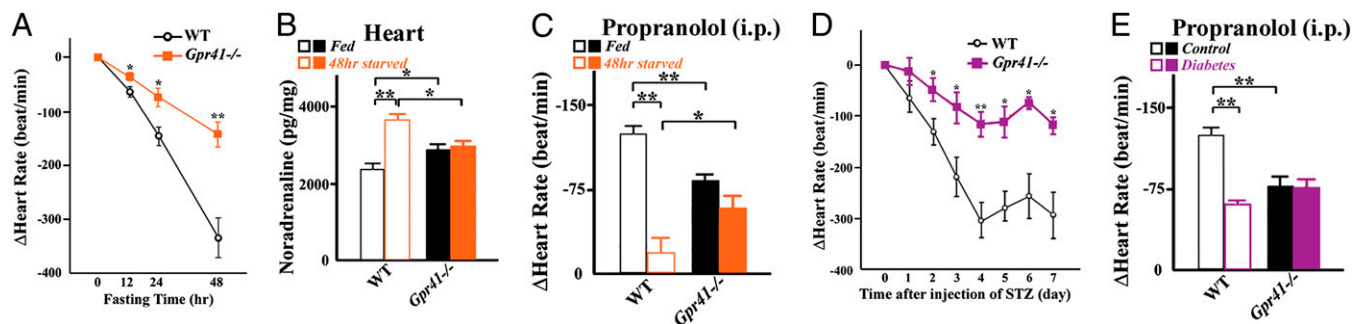
Fig. 5. Inhibitory effects of ketone bodies on GPR41 sympathetic activity during fasting or diabetes. (*A*) Heart rate following fasting in *Gpr41*$^{-/-}$ mice (*n* = 7–8). (*B*) NA content in the heart after 48-h starvation (*n* = 9–10). (*C*) Effects of sympathetic nerve blocking on heart rate of *Gpr41*$^{-/-}$ starved mice (*n* = 9–11). (*D*) Change in heart rate following the induction of diabetes (*n* = 4–10). (*E*) Effects of sympathetic nerve blocking on heart rate in *Gpr41*$^{-/-}$ diabetic mice (*n* = 4–5). Mice were analyzed at 12–14 wk of age. *$P < 0.05$; **$P < 0.005$.

the effects of SCFAs and ketone bodies on oxygen consumption, which reflects energy expenditure, were well correlated with changes in heart rate, indicating that both physiological responses are controlled by GPR41-mediated SNS activation.

Discussion

The high expression level of *Gpr41* in sympathetic ganglia indicates that GPR41 might play an important role in these cells. A series of in vitro and in vivo studies with *Gpr41*$^{-/-}$ mice showed that an SCFA propionate potently activates SNS at sympathetic ganglia. The mechanism of GPR41-mediated activation of sympathetic ganglion neurons is not mediated by cAMP inhibition, but rather involves Gβγ and MAPK signaling. Furthermore, the major ketone body β-hydroxybutyrate antagonizes SCFA-GPR41 signaling and thereby inhibits SNS. As SCFAs and ketone bodies reflect the nutrient conditions, these monocarboxylic metabolites appear to control energy balance by directly regulating GPR41-mediated sympathetic activation. The GPR41-SNS activation pathway may work as one of the important physiological mechanisms for these metabolic fuels to regulate body energy balance.

Gpr41 had been reported to be expressed in adipose tissue in mice (19) and humans (14) and to stimulate leptin secretion (18, 19). Recent studies, however, have reported that *Gpr41* is not

expressed in mouse adipose tissues (29–32). Our quantitative RT-PCR (qRT-PCR) analysis also found that *Gpr41* is not detected in mouse adipose tissues (Fig. S9*C*). In contrast to *Gpr41*, we found that *Gpr43* is highly expressed in mouse adipose tissues by qRT-PCR (Fig. S9), confirming previous reports (28). Because propionate can activate GPR43 (14), it is possible that propionate can affect the SNS by GPR43-mediated effects on adipocytes, and especially by secretion of leptin that can potently activate the SNS (33). However, administration of propionate induced a similar extent of alterations in metabolic parameters and leptin levels in wild-type and *Gpr41*$^{-/-}$ mice (Fig. S5*C*), showing that propionate-activated GPR43 responses in *Gpr41*$^{-/-}$ mice were similar to wild-type mice. The results further confirmed the previous reports that SCFAs can promote leptin secretion from adipose and not via GPR41 (29, 30). Taken together, the results showed that the effects of SCFAs on SNS activity may not be mediated by their effects on adipocytes from which adipocytokines such as leptin that can change sympathetic nerve activity can be secreted.

GPR41 is a Gi/o-coupled G protein-coupled receptor (GPCR) related to the inhibition of cAMP production (17). In general, Gi/o-coupled GPCRs work as an inhibitory system. However, our study shows that activation of GPR41 by SCFA excites the SCG neu-
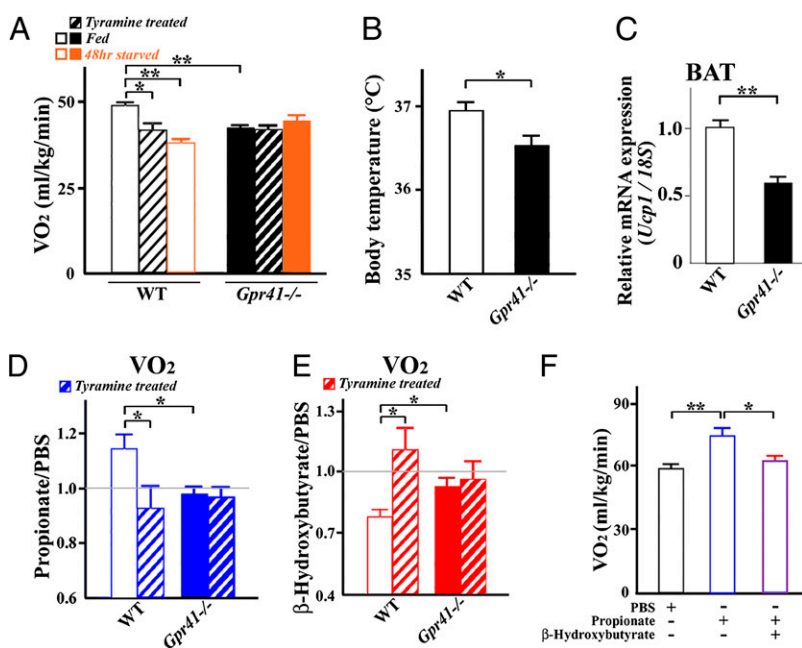

Fig. 6. Effects of GPR41-mediated regulation of sympathetic activity on energy expenditure. (*A*) Effects on oxygen consumption in *Gpr41*$^{-/-}$ mice during feeding and at 48-h starvation. Measurement of oxygen consumption at 24 h after tyramine administration (100 mg/kg i.p.) (*n* = 5–7). (*B*) Body temperature of *Gpr41*$^{-/-}$ mice during feeding (*n* = 6). (*C*) *Ucp1* expression in brown adipose tissue (BAT) in *Gpr41*$^{-/-}$ mice during feeding (*n* = 6). Internal control: *18S* rRNA expression. (*D*) Rate of oxygen consumption in propionate and PBS administration. Oxygen consumption was measured at 40 min after propionate administration (1 g/kg i.p.) (*n* = 4–8). (*E*) Rate of oxygen consumption in β-hydroxybutyrate and PBS administration. Oxygen consumption was measured at 50 min after β-hydroxybutyrate administration (500 mg/kg i.p.) (*n* = 5–7). Propionate and β-hydroxybutyrate were administrated after 24 h treatment of tyramine. Hatched bars, tyramine-treated (*A*, *D*, and *E*). (*F*) Inhibitory effects of β-hydroxybutyrate on oxygen consumption. After pretreatment with β-hydroxybutyrate (500 mg/kg) for 10 min, at time 0, a bolus of propionate (1 g/kg) with or without β-hydroxybutyrate (500 mg/kg) was administered intraperitoneally (*n* = 8). Data were measured at time 40 min. Mice were analyzed at 14–16 wk of age. *$P < 0.05$; **$P < 0.005$.

rons by generating action potential and thereby releases noradrenaline from sympathetic nerve terminals, showing that GPR41 mediates an excitatory signaling. Recently, however, many excitatory responses in a variety of cell systems have been reported to be mediated by Gi/o-coupled GPCRs, and those responses were mediated via Gβγ but not via Gα(i/o) (34). Our study with selective antagonists for Gα(i/o) and Gβγ also showed that GPR41-mediated excitation of SCG neurons is mediated by Gβγ signaling but cAMP inhibition by Gα(i/o) is not. Furthermore, our siRNA experiments showed that GPR41-mediated activation of sympathetic neurons involves PLCβ and MAPK signaling, but not β-arrestin and GRK signaling. Taken together, because the catecholamine release in neurons is regulated by MAPK signaling (35, 36) and because Gβγ signaling activates MAPK signaling via PLCβ in Gi/o-coupled GPCRs (34), our results may indicate that GPR41-induced sympathetic activation involves Gβγ-PLCβ-MAPK signaling.

Under fed conditions, a substantial proportion of total dietary energy intake derives from SCFAs produced via the colonic fermentation of dietary fibers by gut microbiota (5–10%) (37). Levels of SCFAs in the gastrointestinal tract vary significantly, depending on the amount of nondigestible fiber in the diet and also relate to the composition of the gut microbiota. Change in the amount and composition of gut microbiota has been implicated in cardiovascular diseases and in obesity in humans (38, 39). Ketogenic conditions have been suggested as a reason for the increased incidence of cardiac disorders (40, 41). Furthermore, SCFAs have recently been reported to profoundly affect inflammatory responses via the chemoattractant receptor GPR43 (15). Because a plethora of physiological processes are regulated by SNS, SCFA–GPR41 interactions that regulate SNS could represent a central mechanism to account for the effects of diet, prebiotics, and probiotics on body homeostasis and may represent avenues for understanding and potentially manipulating physiology and disease.

Materials and Methods

RNA extraction, qRT-PCR, in situ hybridization, immunohistochemistry, animal and diabetic models, generation of HEK293 cells expressing mouse GPR41, cultures of sympathetic neurons, Neuro2A and cardiomyocytes, transfection and knockdown by siRNA, Western blotting and cAMP determination, cardiographic recording, biochemical analyses, electrophysiological recording, indirect calorimetry, thermometry, and statistical analyses are described in *SI Materials and Methods*. The sequences of primers for qRT-PCR and of siRNAs for knockdown are shown in Tables S1 and S2, respectively.

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