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Characterization of metabolic phenotypes of mice lacking GPR61, an orphan G-protein coupled receptor

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ABSTRACT

Aims: GPR61 is an orphan G protein-coupled receptor whose function remains unknown. The purpose of the present study is to elucidate the importance of GPR61 in metabolism by characterization of GPR61-deficient mice.
Main methods: Male GPR61-deficient mice were characterized regarding various metabolic parameters, including food intake, body weight, oxygen consumption, body temperature, locomotor activity, and in a pair feeding study. Hypothalamic gene expression was analyzed using real-time quantitative RT-PCR.
Key findings: GPR61-deficient mice exhibited marked hyperphagia and heavier body weight than wild-type mice. Hyperphagia of GPR61-deficient mice was observed before the differences in body weight became apparent between the genotypes. When body weight difference did become apparent between genotypes, increases in visceral fat pad weight, liver weight, liver triglyceride (TG) content, plasma leptin, and plasma insulin were observed in GPR61-deficient mice, suggesting that GPR61 deficiency caused obesity associated with hyperphagia. Oxygen consumption, body temperature, and locomotor activity were not significantly different between GPR61-deficient and wild-type mice. Pair-fed GPR61-deficient mice had a greater fat mass than wild-type mice despite comparable body weight in both genotypes. The mRNA levels of proopiomelanocortin (POMC) and brain-derived neurotropic factor (BDNF) in the hypothalamus of GPR61-deficient mice were significantly lower than those of wild-type mice.
Significance: GPR61-deficient mice exhibited obesity associated with hyperphagia. These findings suggest that GPR61 is involved in the regulation of food intake and body weight, and may be of importance when considering GPR61 as a therapeutic target for obesity or eating disorders.

Introduction

G-protein coupled receptors (GPCRs) constitute a large and functionally diverse superfamily of cell surface receptors that detect various extracellular stimuli including odor, photons, nutrients, hormones, and neurotransmitters. GPCRs play various physiological roles and convey their effects in a highly specific manner (Fredriksson et al., 2003). GPCRs are thus well-established therapeutic drug targets.

To date, approximately 100 orphan GPCRs (oGPCRs) without any known endogenous ligand have been identified by sequence homology cloning and by analysis of the sequence of the human genome (Fredriksson et al., 2003; Bjarnadóttir et al., 2006; Vassilatis et al., 2003). Given that nearly 50% of drugs on the market are targeted to GPCRs (Drews, 2000), uncharacterized oGPCRs have the potential to provide novel therapeutic opportunities.

GPR61 is an oGPCR that was cloned from the human brain in 2001 (Cikos et al., 2001; Lee et al., 2001). GPR61 is also known as BALGR, *b*iogenic *a*mine receptor-*l*ike *GPCR*, because GPR61 exhibits the highest sequence similarity to the biogenic amine receptors, i.e., serotonin, histamine, adrenaline, and dopamine receptors (approximately 30% identity for each receptor at the amino acid level). GPR61 has a phenylalanine residue in the sixth transmembrane domain, which is conserved in many biogenic amine receptors. This phenylalanine residue is considered to interact with the aromatic ring of biogenic amine ligands (Strader et al., 1995). GPR61 has been classified within the biogenic amine receptor subgroup, rather than the chemokine, peptide, or nucleotide and lipid receptor subgroups based on phylogenetic analysis (Joost and Methner, 2002). However, GPR61 lacks the conserved aspartic acid residue that is present in the third transmembrane domain of biogenic amine receptors. This aspartic acid residue is considered to be important for biogenic amine agonist and antagonist binding (Gantz et al., 1992; Ho et al., 1992). In line

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with these findings, the cognate ligand for GPR61 has not yet been identified. This is one of the reasons why the physiological function of GPR61 remains elusive.

GPR61 has been reported to be predominantly expressed in the brain. Detailed analysis of GPR61 expression in the rat brain revealed that GPR61 is broadly expressed in the brain including in the cortex, hippocampus, thalamus, hypothalamus, and midbrain (Lee et al., 2001). Given that the GPR61 gene has been conserved throughout mammalian evolution and that GPR61 shows high sequence homology between species (Cikos et al., 2001), GPR61 could play important roles in the brain. These combined findings suggest that the ligand for GPR61 could possibly be a key neurotransmitter with biogenic amine-like features. Because biogenic amines are known to be involved in various brain functions, including food intake and energy metabolism, we hypothesized that GPR61 may play a critical role in energy homeostasis.

In the present study, we characterized mice whose GPR61 gene was disrupted by insertion of a LacZ gene into the coding sequence of GPR61 to elucidate the importance of GPR61 in metabolism. To our knowledge, this is the first study of GPR61-deficient mice reported in the literature.

Materials and methods

Animals

Heterozygous GPR61-deficient mice were obtained from Deltagen (San Mateo, USA). The GPR61 gene was targeted by homologous recombination in ES cells derived from the 129/OlaHsd mouse substrain and was injected into blastocysts. Mice carrying a disrupted GPR61 gene were generated by breeding the chimeric mice with C57BL/6J females. Backcrossing of the F1 generation to C57BL/6J females was conducted to produce the N1 generation offspring followed by further backcrossing to C57BL/6J mice. Heterozygote N4 or N5 intercrossing produced GPR61-deficient mice and wild-type littermates that were studied. Mice were housed individually in a controlled animal room (room temperature, 23 ± 2 °C; humidity, $55 \pm 15\%$) using a 12 h light–dark cycle (lights on: 07:00–19:00). Mice were fed either a regular diet (CE-2, CLEA Japan Inc., Tokyo, Japan) or a moderately high-fat (MHF) diet (Oriental Bio-Service Kanto Inc., Ibaraki, Japan) ad libitum except for the pair-feeding study. The regular diet provides 50% energy as carbohydrate, 25.1% as protein and 4.8% as fat (3.4 kcal/g), whereas the MHF-diet provides 52.4% energy as carbohydrate, 15.0% as protein and 32.6% as fat (4.4 kcal/g). All animal experiments were approved by the Banyu Institutional Animal Care and Use Committee.

TaqMan analysis

Gene expression level was determined by real-time quantitative RT-PCR using an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA). For analysis of the tissue distribution of GPR61 in mouse, total RNA from each tissue was purchased from Clontech (Mountain View, CA), BioChain Institute, Inc. (Hayward, CA), or Zyagen Laboratories (San Diego, CA). Total RNA (5 μg) was used as a template for the synthesis of cDNA using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems). The expression of 18s rRNA was measured as an internal control. Mixtures of primers and probes for GPR61 were purchased from Applied Biosystems. The relative expression level of GPR61 in each tissue was calculated by applying the "Comparative Ct Method" as previously described (Livak and Schmittgen, 2001). Under the experimental conditions used, the PCR output displayed relatively large variations when the cycle number of the PCR exceeded 35, presumably due to the progressive loss of polymerase activity. Therefore, the expression level of GPR61 in each tissue was determined when the Ct value of GPR61 was less than 35 ($Ct_{GPR61\ in\ tissue} < 35$). The expression level of GPR61 in the brain was used for calibration. The expression level of GPR61 in tissues relative to that in the brain was calculated using the following formula:

$$\text{Relative expression} = 2^{-\Delta\Delta Ct},$$

where $\Delta\Delta Ct = (Ct_{GPR61\ in\ tissue} - Ct_{rRNA}) - (Ct_{GPR61\ in\ brain} - Ct_{rRNA})$.

To analyze the expression of other genes in the hypothalamus and liver, total RNA was isolated from these tissues using the RNeasy Lipid Tissue or RNeasy kits (QIAGEN-Japan, Tokyo, Japan). The first strand cDNA was synthesized from 5 μg of total RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). The TaqMan primers and probes are described in Supplementary Table 1. The expression of β-actin was measured as an internal control. The PCR and analyses were performed according to the manufacturers' protocols.

X-gal histochemistry

X-gal staining was performed as described (Houtani et al., 2000). Briefly, a heterozygous male GPR61-deficient mouse at 36 weeks of age was perfused with saline (PBS) followed by paraformaldehyde under pentobarbital anesthesia. The brain was removed, soaked in 30% sucrose and cut into sections (30 μm thick) using a freezing microtome. The free-floating tissue sections were rinsed in 0.1 M phosphate buffer (pH 7.3) containing 2 mM $MgCl_2$, 0.01% sodium deoxycholate and 0.02% Nonidet P-40 (X-gal buffer) and were stained by immersion in X-gal buffer containing 1 mg/mL 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), 5 mM $K_3Fe(CN)_6$, and 5 mM $K_4Fe(CN)_6$ for 16–24 h. Stained sections were rinsed with PBS, mounted on glass slides, counterstained with nuclear fast red and covered with a coverslip.

Body weight, food intake, and body composition analysis

The body weight of mice was measured on a weekly basis. Daily food intake was calculated as the average food consumption of each animal per day based on actual food consumption over 3 to 7 consecutive days. Body fat mass was determined using a Bruker Minispec NMR analyzer mq 10 (Bruker Optics, Billerica, MA). Body fat percentages were calculated as the ratio of the body fat mass to the body weight measured just prior to NMR measurement.

Indirect calorimetry

Oxygen consumption (VO_2, ml/kg^{75}/h) and carbon dioxide production (VCO_2, ml/kg^{75}/h) were simultaneously determined using the Oxymax System (Columbus Instruments, Columbus, OH). Mice were placed in individual calorimeter chambers with free access to an MHF-diet and water. After a 17 h acclimation, VO_2 and VCO_2 were measured over 48 h. The VO_2 values during diurnal (07:00–19:00) or nocturnal (19:00–07:00) periods were averaged for analysis. The respiratory quotient (RQ) was calculated as the molar ratio of VO_2:VCO_2.

Body temperature

Rectal temperature was measured in the morning (09:00–11:00) with a digital thermometer (BAT-12, Physitemp Instruments, Clifton, NJ) equipped with a rectal probe. To measure rectal temperature, the probe was gently inserted to a depth of 2 cm from the anus.