

Report

Expression and Distribution of GPR55, a Receptor for Lysophosphatidylinositol, in Mouse Tissues and Cells

Takashi Tanikawa,^{*a,b} Saori Oka,^a Keisuke Nakajima,^a Yasuhiro Hayashi,^a Yoko Nemoto-Sasaki,^a Yoichiro Arata,^a Takayuki Sugiura,^a and Atsushi Yamashita^{*a}

^aFaculty of Pharma-Science, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan; ^bFaculty of Pharmacy and Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan

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GPR55 is a G protein-coupled receptor that is proposed as a novel type of cannabinoid receptor. Lysophosphatidylinositol is an endogenous ligand for GPR55. The physiological roles of GPR55 have not yet been elucidated in detail. In the present study, the expression of *Gpr55* mRNA was evaluated in various mouse tissues and organs using real-time RT-PCR. *Gpr55* mRNA expression was highest in testis, the male reproductive system, among mouse tissues. *Gpr55* mRNA expression was high in immune organs such as the spleen, lymph nodes, and thymus. *Gpr55* mRNA was also detected in the small and large intestines. The expression of *Gpr55* mRNA was relatively low in a mouse brain. The distribution of *Gpr55* in mice is very similar to that in humans, however, the rank order was somewhat different. The sub-fractionation revealed that *Gpr55* mRNA was expressed in both germinal cells and somatic cells in the testis. In the small intestine, *Gpr55* was expressed in the duodenum, jejunum, and ileum. *Gpr55* was highly expressed in B and T lymphocytes and dendritic cells in the mouse spleen.

Key words GPR55, bioactive lipid messenger, lysophosphatidylinositol, expression in mouse tissues

INTRODUCTION

GPR55 is a seven-transmembrane G protein-coupled receptor (GPCR).¹⁾ The human GPR55 gene is mapped to chromosome 2q37 and encodes a 319 amino acid protein.¹⁾ The mouse *Gpr55* gene is mapped to chromosome 1 C5 and encodes 327 amino acids.^{2,3)} GPR55 was initially reported as a novel cannabinoid receptor.²⁻⁶⁾ However, it has low sequence identity with cannabinoid receptors, such as CB1 (13.5%) and CB2 (14.4%).^{2,4-6)} Since an endogenous agonist for the receptor was unknown, we searched for an endogenous ligand for GPR55 using human GPR55-expressing HEK293 cells. We found that lysophosphatidylinositol (LPI) induced the rapid phosphorylation of extracellular signal-regulated kinase (ERK) as well as a transient increase in intracellular Ca²⁺ concentrations in a GPR55-dependent manner.⁷⁾ The structure-activity relationship of LPI species revealed that GPR55 responds to the arachidonic acid-containing species of LPI, *i.e.* 2-arachidonoyl-*sn*-glycero-3-phosphoinositol (2-arachidonoyl LPI) were the most potent agonist among other species of LPI.⁸⁾ We also found that PA-PLA1/DDHD1, which is highly expressed in the brain, was involved in the stimulus-dependent formation of 2-arachidonoyl LPI.⁹⁾

We previously examined the distribution of GPR55 in various human tissues and found out that it was highly expressed in several inflammatory and immune tissues besides the nervous system.¹⁰⁾ Similar findings were reported by other groups.^{1,3,11-13)} We herein investigated its expression and localization in mouse tissues and compared to the distribution in humans.

MATERIALS AND METHODS

Mice and Reagents Specific pathogen-free female and male ICR mice were obtained from Sankyo Labo Service Corporation Inc. (Tokyo, Japan) and used at 6–10 weeks of age. Animal care and experiments were undertaken in compliance with the ethical guidelines for the animal facilities of Teikyo University. Lympholyte-M was purchased from Cedarlane (Ontario, Canada). Percoll was purchased from GE Healthcare Bio-Sciences AB (Helsinki, Sweden). Bovine serum albumin (BSA) fraction V, collagenase, and hyaluronidase were purchased from Sigma-Aldrich (St. Louis, MO, USA). F12/DMEM medium was purchased from Wako Pure Chemicals (Osaka, Japan). Isogen II was purchased from NIPPON GENE CO., LTD. (Tokyo, Japan). Fluorescein isothiocyanate (FITC)-conjugated rat anti-human/mouse CD45R (B220) (RA3-6B2), R-Phycoerythrin (PE)-conjugated Armenian hamster anti-mouse CD3ε (145-2C11), allophycocyanin (APC)-conjugated rat anti-mouse CD11b (M1/70), and PE-Cy7-conjugated Armenian hamster anti-mouse CD11c (N418) were purchased from eBioscience Inc. (San Diego, CA, USA). The PrimeScript RT reagent kit and SYBR Premix Ex Taq were purchased from Takara Inc. (Shiga, Japan).

Separation of Spermatogenic and Somatic Cells from the Testis Spermatogenic cells were isolated using a previously described protocol¹⁴⁾ with modifications. Briefly, decapsulated testes were incubated with 0.5 mg/mL collagenase at room temperature for 15 min with gentle agitation and then filtered through nylon meshes. The seminiferous tubules were resuspended in 0.5 mg/mL of collagenase at room temperature

*To whom correspondence should be addressed. e-mail: tanikawa@josai.ac.jp; ayamashi@pharm.teikyo-u.ac.jp

for 20 min. After filtration, the tubules were incubated with 0.5 mg/mL hyaluronidase at room temperature for 15 min with agitation, and cells were washed with F12/DMEM medium (Crude cells). The cells were cultured in F12/DMEM medium supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), and 10% FBS in a humidified atmosphere with 5% CO₂ at 32°C. Spermatogenic cells that did not adhere to culture dishes 24 h after the culture were collected. This step removed somatic cells, including Sertoli cells, that adhered to culture dishes.

Preparation of Various Layers and Cells from the Small Intestine After washing the small intestine to remove digested materials, the mucosal and muscle layers were obtained by scraping. Intestinal epithelial cells (IECs) and intraepithelial lymphocytes (IELs) were enriched by the previously described procedure with minor modifications.¹⁵ The inverted small intestine was minced, and incubated with HBSS supplemented with 5% FBS at 37°C for 45 min with shaking. Cells were suspended in 30% Percoll and centrifuged at 400 × g for 20 min. After centrifugation, cells at the top of 30% Percoll were collected for enriched intestinal epithelial cells (IECs). Cells at the bottom were suspended in 44% Percoll and layered over 70% Percoll. After centrifugation, intraepithelial lymphocytes (IELs) were isolated from the interface.

Preparation of Lymphocytes from the Mouse Spleen Spleen cells were released by smashing the spleen in RPMI 1640 medium and passing cells through a nylon mesh. Red blood cells were removed by lysis with a hypotonic buffer. Lymphocytes from the spleen were purified by density gradient centrifugation on Lympholyte-M. To isolate T and B lymphocytes, lymphocytes were labeled with FITC- rat anti-human/mouse CD45R and PE-Armenian hamster anti-mouse CD3ε. To isolate dendritic cells and macrophages, spleen cells were labeled with APC-rat anti-mouse CD11b and PE-Cy7 Armenian hamster anti-mouse CD11c. The cells were sorted with FACSaria (BD Biosciences, San Jose, CA, USA).

RNA Isolation and Real-Time RT-PCR Analysis Total RNA was extracted using Isogen II according to the manufacturer's instructions and used for each RT reaction. cDNAs

were synthesized by PrimeScript® RT reagent kit (Perfect Real Time). Real-time RT-PCR was performed using SYBR® Premix Ex Taq™ (Takara Bio, Inc.).

The following primers were used: mouse *Gpr55*: 5'-GAGCAGAGGCACGAACATGA-3' (sense), 5'-ACGGAATC-GAATGAGCAGTTG-3' (antisense); mouse *Gapdh*: 5'-GCA-CAGTCAAGGCCGAGAAT-3' (sense), 5'-GCCTTCTC-CATGGTGGTGAA-3' (antisense); mouse *Dbil5*: 5'-CCCAGGGCGACTGTAACATC-3' (sense), 5'-GCAATGTA-GATCCTCATGGCAT-3' (antisense); mouse *Sycp3*: 5'-AGC-CAGTAACCAGAAAATTGAGC-3' (sense), 5'-CCACT-GCTGCAACACATTCATA-3' (antisense); mouse *Rhox5*: 5'-CACCAGGACCAAAGTGGCC-3' (sense), 5'-GGTATGGAA-GCTGAGGGTT-3' (antisense). Relative expression was calculated according to the Ct value with normalization to *Gapdh*.

Statistical Analysis ANOVA followed by Dunnett's test (Fig. 3) or Tukey's test (Fig. 4) was used for multiple comparisons. $P < 0.05$ was considered to be significant.

RESULTS

Expression of *Gpr55* in Various Tissues To investigate the distribution of GPR55 in mice, we initially investigated whether *Gpr55* is expressed in different tissues using real-time RT-PCR (Fig. 1). The results showed that *Gpr55* mRNA expression was highest in testis, the male reproductive system, among mouse tissues. However, its expression was low in female genital organs, such as the ovaries, uterus, and fallopian tubes. *Gpr55* mRNA was highly expressed in immune organs, including the spleen, lymph nodes, and thymus. *Gpr55* mRNA was also found in the small and large intestines. The expression of *Gpr55* mRNA was relatively low in a mouse brain.

Expression of GPR55 in the Brain Previous studies reported the mRNA expression of *Gpr55* in the human brain.^{1,3,16} The present study revealed that *Gpr55* mRNA expression was low, but detectable in the mouse brain (Fig. 1). Next, we examined *Gpr55* mRNA expression in various parts of the mouse brain. *Gpr55* mRNA expression was the highest in the olfactory bulb. However, detectable levels were also observed

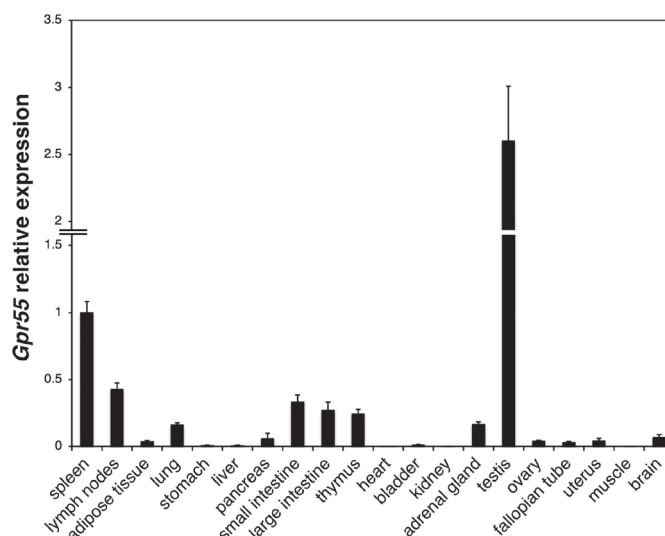


Fig. 1. *Gpr55* mRNA Expression in Various Mouse Tissues and Organs

Total RNA was extracted from mouse tissues and organs. Relative expression of *Gpr55* mRNA in various mouse tissues and organs was assessed by real-time RT-PCR. Results are normalized against *Gapdh* mRNA expression and expressed as means ± standard deviation (SD); n = 3–4. The relative expression of *Gpr55* mRNA was shown as a comparison to the expression in the spleen.

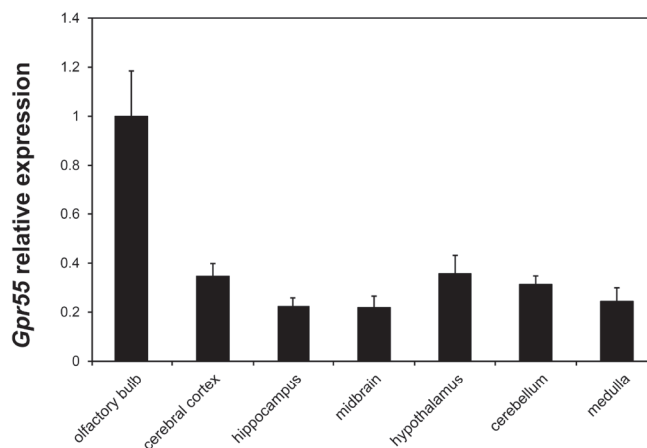


Fig. 2. *Gpr55* mRNA Expression in Various Parts of the Mouse Brain

Relative expression of *Gpr55* mRNA in various parts of the mouse brain was examined by real-time RT-PCR. Results are normalized against *Gapdh* mRNA expression and expressed as means \pm SD; n = 4. The relative expression of *Gpr55* mRNA was shown as a comparison to the expression in the olfactory bulb.

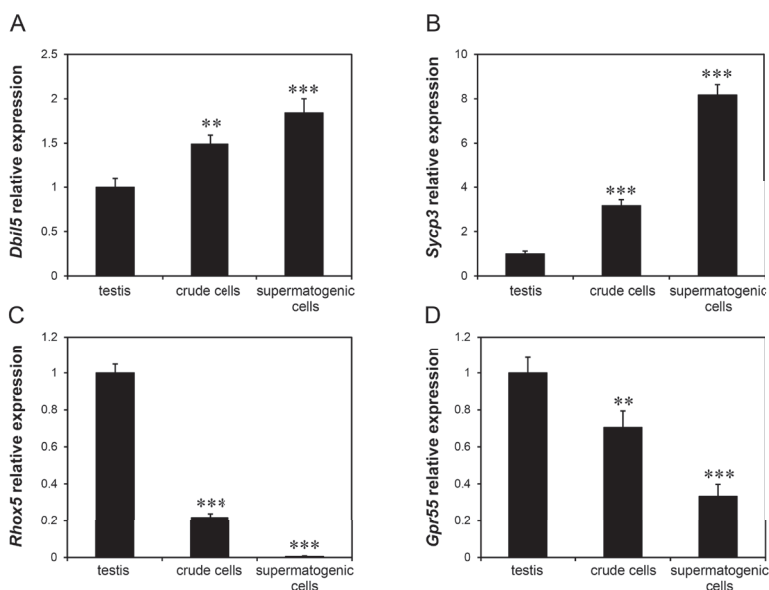


Fig. 3. *Gpr55* mRNA Expression in Mouse Reproductive Tissues

(A–C) Spermatogenic cells were identified by the expression of marker genes. The mRNA expression of marker genes was assessed by real-time RT-PCR: (A) *Dbil5* for elongating spermatids and round spermatids, (B) *Sycp3* for pachytene spermatocytes, and (C) *Rhox5* for Sertoli cells. (D) Relative expression of *Gpr55* mRNA was examined by real-time RT-PCR. Results are normalized against *Gapdh* mRNA expression and expressed as means \pm SD; n = 3–4. The relative expression of *Gpr55* mRNA was shown as a comparison to the expression in testis. ** $P < 0.01$; *** $P < 0.001$.

in other parts, such as the cerebral cortex, hippocampus, mid-brain, hypothalamus, cerebellum, and medulla (Fig. 2).

Expression of GPR55 in Reproductive Organs Since the highest *Gpr55* mRNA expression level was observed in the testis (Fig. 1), its expression in spermatogenic cells was examined. Spermatogenic cells were isolated and enrichment was assessed by detecting the mRNAs of marker genes; *Dbil5* and *Sycp3* are markers for spermatogenic cells whereas *Rhox5* is a marker for Sertoli cells.¹⁷⁾ After the separation, the expression of *Dbil5* and *Sycp3* increased, whereas that of *Rhox5* decreased, suggesting that spermatogenic cells were enriched (Fig. 3 A–C). However, relative expression of *Gpr55* mRNA was decreased, suggesting that *Gpr55* is expressed at a much higher level in somatic cells such as Sertoli cells than in spermatogenic cells (Fig. 3D), although *Gpr55* is highly expressed even in spermatogenic cells.

Expression of GPR55 in Digestive Organs The expression of *Gpr55* mRNA was relatively high in the digestive organs including small and large intestines (Fig. 1). After the sections of the duodenum, jejunum, and ileum were isolated, the expression of *Gpr55* mRNA was examined (Fig. 4A). All three parts expressed *Gpr55* mRNA, with higher levels being observed in the ileum than in the duodenum and jejunum. When the mucosal and muscle layers of the small intestine were separated, *Gpr55* mRNA expression was detected in both layers but was higher in the former (Fig. 4B). We then examined which cell population expressed *Gpr55* mRNA in the mucosal layer. *Gpr55* mRNA expression was higher in the intraepithelial lymphocytes (IELs) than in intestinal epithelial cells (IECs) (Fig. 4C).

Expression of GPR55 in Spleen Cells Since *Gpr55* mRNA expression was high in immune organs, including the spleen

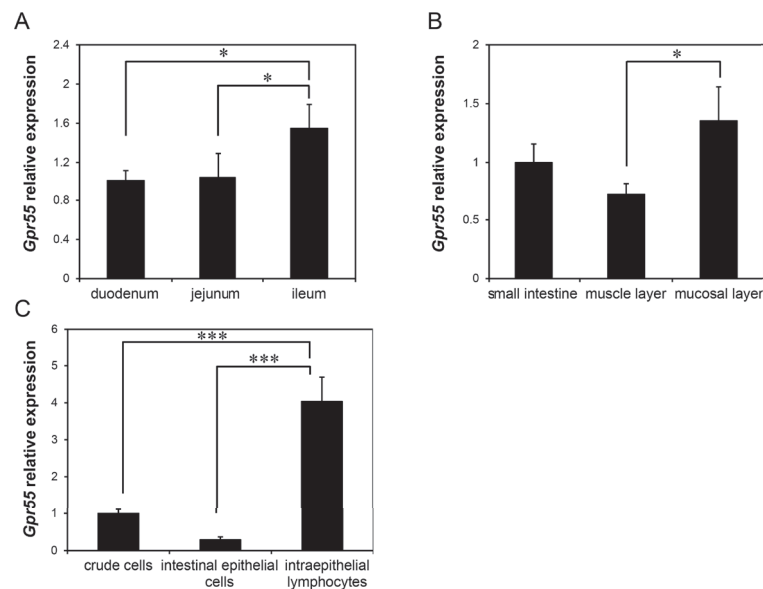


Fig. 4. *Gpr55* mRNA Expression in the Mouse Small Intestine

(A) Relative expression of *Gpr55* mRNA in the mouse duodenum, jejunum, and ileum was examined by real-time RT-PCR. (B) Relative expression of *Gpr55* mRNA in the mouse muscle and mucosal layers of the intestine was assessed by real-time RT-PCR. (C) Relative expression of *Gpr55* mRNA in mouse intestinal epithelial cells (IECs) and intraepithelial lymphocytes (IELs) was evaluated by real-time RT-PCR. Results are normalized against *Gapdh* mRNA expression and expressed as means \pm SD; n = 3–4. The relative expression of *Gpr55* mRNA was shown as a comparison to the expression in duodenum (A), small intestine (B), and crude cells (C). * $P < 0.05$; *** $P < 0.001$.

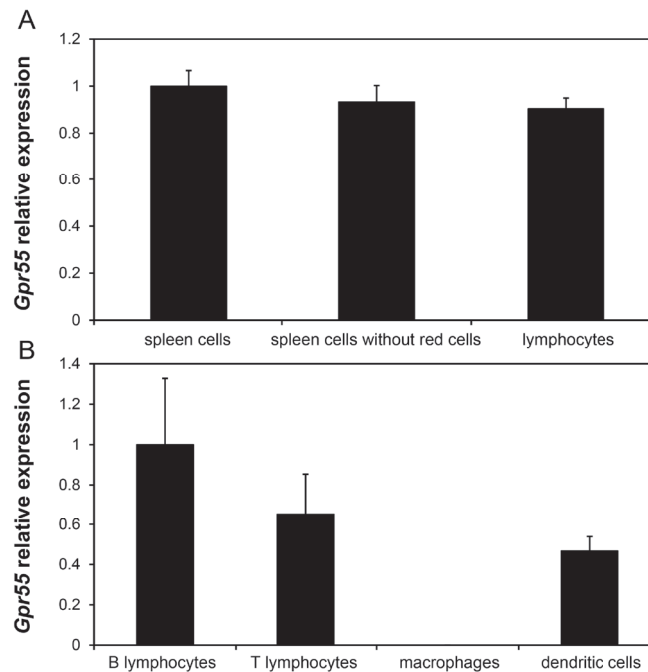


Fig. 5. *Gpr55* mRNA Expression in the Mouse Spleen

(A) Relative expression of *Gpr55* mRNA in mouse spleen cells, spleen cells without red cells, and lymphocytes from the spleen was examined by real-time RT-PCR. (B) Total RNA was isolated from sorted B lymphocytes, T lymphocytes, macrophages, and dendritic cells. Relative expression of *Gpr55* mRNA in mouse B lymphocytes, T lymphocytes, macrophages, and dendritic cells was assessed by real-time RT-PCR. Results are normalized against *Gapdh* mRNA expression and expressed as means \pm SD; n = 3–4. The relative expression of *Gpr55* mRNA was shown as a comparison to the expression in spleen cells (A) and B lymphocytes (B).

and lymph nodes, we investigated whether lymphocytes in the spleen express *Gpr55*. Spleen cells expressed *Gpr55* mRNA and its level was not affected by the exclusion of red blood cells (Fig. 5A). We also found that *Gpr55* mRNA expression was not altered by the enrichment of lymphocytes from spleen cells with Lympholyte-M, suggesting expression of *Gpr55* in lymphocytes in the mouse spleen. B and T lymphocytes, mac-

rophages, and dendritic cells were fractionated by a cell sorter with different gatings; B lymphocytes gated on CD45R⁺CD3⁻, T lymphocytes gated on CD45R⁻CD3⁺, macrophages gated on CD11b⁺, and dendritic cells gated on CD11c⁺. *Gpr55* mRNA was highly expressed in B and T lymphocytes and dendritic cells, but not in macrophages (Fig. 5B).

DISCUSSION

GPR55 was originally reported as an orphan GPCR that is abundantly expressed in the human brain.¹⁾ Although the expression of GPR55 in the whole brain was not high in humans¹⁰⁾ and in mice (Fig. 1) compared to those in the spleen and testis, significant expression of *Gpr55* mRNA was detected in certain parts of the brain (Fig. 2). In particular, the relative expression of *Gpr55* in the brain was lower in mice than in humans.¹⁰⁾ Accumulating evidence supports the presence of GPR55 in the central nervous system and various functions have been proposed.^{1,3,16)} However, the precise function of GPR55 in the different regions of the mouse brain remains to be elucidated.

We previously demonstrated that GPR55 was expressed in the human testis.¹⁰⁾ In the present study, we confirmed the highest level of expression in the mouse testis (Fig. 1). The testis is composed of different types of cells, including spermatogenic cells, Sertoli cells, and Leydig cells. During the enrichment of spermatogenic cells, the expression of *Gpr55* was decreased (Fig. 3). These results indicate that the expression of *Gpr55* in spermatogenic cells may be lower than in somatic cells. However, the expression in spermatogenic cells is very high compared with other tissues and cells (Fig. 1 and 3).

High *Gpr55* mRNA expression was observed in small and large intestines in mice (Fig. 1), with a similar distribution in the duodenum, jejunum, and ileum (Fig. 4). These results are consistent with previous findings showing that *Gpr55* mRNA was expressed in the rat duodenum, ileum, and colon¹⁸⁾ and mouse intestine.^{3,19)} In the present study, *Gpr55* mRNA expression was detected in both the mucosal and muscle layers of the mouse small intestine (Fig. 4B), and the sub-fractionation of the mucosal layer revealed higher expression levels in intraepithelial lymphocytes (IELs) than in intestinal epithelial cells (IECs) (Fig. 4C). These results are consistent with previous findings showing *Gpr55* mRNA expression in Peyer's patches and intraepithelial lymphocytes (IELs).²⁰⁾ We demonstrated that *Gpr55* was also expressed in the muscle layer of the mouse small intestine (Fig. 4B), which is in agreement with previous findings showing that a GPR55 agonist reduced electrical field stimulation-evoked contractions in ileal segments.¹⁹⁾

In conclusion, the present results demonstrated that *Gpr55* is highly expressed in the testis, immune organs (the spleen, lymph nodes, and thymus), and digestive organs (the small and large intestines). The next goal should be to clarify the physiological functions of GPR55 in these tissues and cells in near future.

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Conflict of interest The authors declare no conflict of interest.

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