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2-Arachidonoyl-*sn*-glycero-3-phosphoinositol: A Possible Natural Ligand for GPR55

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GPR55 is a G protein-coupled receptor. Recently, we obtained evidence that lysophosphatidylinositol (LPI) is a possible endogenous ligand for GPR55. However, no information is currently available concerning the biological activities of the individual molecular species of LPI. Furthermore, little is known concerning the levels as well as the molecular species of LPI in mammalian tissues. In this study, we first examined whether LPI is present in rat brain. We found that rat brain contains 37.5 nmol/g tissue of LPI; the most predominant fatty acyl moiety is stearic acid (50.5%) followed by arachidonic acid (22.1%). We next compared the biological activities of various molecular species of LPI and related molecules using HEK293 cells expressing GPR55. We found that the level of biological activity of the 2-arachidonoyl species is markedly higher than those of others. These results strongly suggest that the 2-arachidonoyl species of LPI is the true natural ligand for GPR55.

Key words: 2-arachidonoyl-*sn*-glycero-3-phosphoinositol, cannabinoid, G protein-coupled receptor, GPR55, lysophosphatidylinositol.

Abbreviations: $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; ERK, extracellular signal-regulated kinase; GC, gas chromatography; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPG, lysophosphatidylglycerol; LPI, lysophosphatidylinositol; PI, phosphatidylinositol; S1P, sphingosine 1-phosphate.

GPR55 is a seven-transmembrane G protein-coupled receptor identified in 1999 (*1*). Recently, several groups reported that GPR55 is a possible novel type of cannabinoid receptor (*2–4*), yet the details remain obscure (*5–7*). Very recently, we explored a possible endogenous ligand for GPR55 using HEK293 cells expressing GPR55 and found that lysophosphatidylinositol (LPI) induces rapid phosphorylation of the extracellular signal-regulated kinase (ERK) and a rapid transient increase in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in these cells (*8*). Importantly, LPI did not induce similar cellular responses in vector-transfected cells. These results strongly suggest that LPI is an endogenous ligand for GPR55.

In the above study, we employed LPI prepared from soybean phosphatidylinositol (PI) by phospholipase A_2 treatment. The predominant fatty acyl moiety of the LPI employed in our previous study was palmitic acid (74%) followed by stearic acid (12%), oleic acid (5%) and linoleic acid (9%). It is well-known that the fatty acyl moieties of PI derived from plants and from mammalian tissues are markedly different from each other. For example, PI derived from mammalian tissues mainly consists of stearic acid esterified at the *sn*-1 position and arachidonic acid esterified at the *sn*-2 position. In contrast, PI derived from plants does not contain arachidonic acid. It is apparent, therefore, that the molecular species of the LPI present in mammalian tissues also markedly differs from that of the LPI present in plants. In relation to this, it should be noted that monoacylglycerols not containing arachidonic acid, such as monopalmitoylglycerol, monostearoylglycerol and monooleoylglycerol, do not bind to cannabinoid receptors (*9*). Thus, it is essential to determine the molecular species of LPI present in mammalian tissues and to evaluate in detail the biological activities of the individual molecular species of LPI.

In the present study, we examined the level as well as the molecular species of LPI present in rat brain. We found that rat brain contains 37.5 nmol/g tissue of LPI. Notably, rat brain contains a substantial amount of the arachidonic acid-containing species (22.1%) as well as the stearic acid-containing species (50.5%). We then compared the biological activities of the individual species of LPI. We found that the level of biological activity of the arachidonic acid-containing species is markedly higher than those of others. These results strongly suggest that the endogenous natural ligand for GPR55 is the arachidonic acid-containing species of LPI, i.e. 2-arachidonoyl-*sn*-glycero-3-phosphoinositol (2-AGPI).

MATERIALS AND METHODS

Chemicals—LPI sodium salt (derived from soybean), lysophosphatidylcholine (LPC) (1-palmitoyl), lysophosphatidylglycerol (LPG) (1-palmitoyl), lysophosphatidic acid (LPA) (1-oleoyl), sphingosine 1-phosphate (S1P) and snake venom phospholipase A_2 (*Naja naja atra*) were purchased from Sigma (St Louis, MO, USA). 1,2-Dipalmitoyl PI and 1,2-dioleoyl PI were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Lipase (*Rhizopus delemar*) was acquired from Seikagaku

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Kogyo Co. Ltd (Tokyo, Japan). The Sep-Pak™ cartridge was obtained from Waters Corp. (Milford, MA, USA). Various species of LPI were chemically or enzymatically synthesized. For example, 2-arachidonoyl LPI was prepared as follows. LPI, obtained from soybean PI following treatment with *Naja naja atra* phosphilipase A_2, was then treated with arachidonic anhydride dissolved in chloroform (ethanol-free) using 4-dimethylaminopyridine as a catalyst at 24°C for 10 min. The resultant PI was separated from the substrate LPI and by-products by TLC using chloroform:methanol:28% ammonia (55:35:5, v/v) as the solvent. The purified PI was then hydrolysed by *Rhizopus delemar* lipase (50 mg) in 2 ml of 50 mM acetate buffer (pH 5.6) containing 10 mM $CaCl_2$ and 100 mM NaCl with vigorous stirring at 24°C for 1 h. The enzyme reaction was terminated by adding 0.4 ml of 200 mM EDTA and 15 ml of chloroform:methanol (1:2, v/v). After the addition of 5 ml of chloroform and 5.6 ml of water, the resultant 2-arachidonoyl LPI was recovered from the water–methanol layer of the Bligh and Dyer extraction mixture and purified using a Sep-Pak™ cartridge. The 1-Arachidonoyl LPI was obtained by the incubation of 2-arachidonoyl LPI in 100 mM Tris–HCl buffer (pH 8.8) at 24°C for 30 min. The purity of the individual LPI was assessed as above 95% by gas chromatography (GC) and liquid chromatography-mass spectrometry (LC-MS). The lipid phosphorus was determined as previously described (*10*).

Analysis of LPI in Rat Brain—Wistar male rats (body weight 250 g) were anaesthesized with diethyl ether. The brain (about 2 g) was quickly removed and homogenized in 120 ml of chloroform:methanol:water (1:2:0.8, v/v) using a Waring blender. Following the addition of 2-heptadecanoyl LPI (50 nmol) as an internal standard, the supernatant was aspirated. We confirmed that LPI was recovered from the water–methanol layer of the Bligh and Dyer extraction mixture (>95%) at neutral or mildly alkaline pH, and it was recovered from the chloroform layer (>95%) following several washes of the upper layer with chloroform when 2 M HCl was employed instead of water (data not shown). The LPI was purified by a two-phase partition in combination with TLC using chloroform:methanol:28% ammonia (50:40:6, v/v) as the solvent system and 2D TLC using the solvent systems, chloroform:methanol:water (65:35:6, v/v) for the first dimension, and chloroform:acetone:methanol:acetic acid:water (4:2:1:1.5:0.5, v/v) for the second dimension. The spot corresponding to the authentic LPI was scrapped off and chloroform:methanol:water (1:2:0.8, v/v) was added to the silica gel. The LPI was extracted from the silica gel by the modified method of Bligh and Dyer (*11*) in which 2 M HCl was employed instead of water. The purified LPI was then treated with 0.5 M methanolic sodium methoxide at 24°C for 30 min. The resultant fatty acid methyl esters were extracted with hexane and analysed in a gas chromatograph (GC-8A, Shimadzu, Kyoto, Japan) equipped with a fused silica column (SP2330, Supelco, Bellefonte, PA, USA).

In some experiments, the purified LPI was analysed by LC-MS using an Acquity UPLC system (Waters) connected to a ZQ4000 mass spectrometer (Waters) operating in the electrospray ionization (ESI) mode. The LPI was detected in the negative ion mode after separation on a reversed-phase column [Acquity UPLC BEH octadecylsilane (C18) column, 1.7 μm, 2.1 × 50 mm]. To determine the positional distribution of the fatty acyl moiety, the brain was homogenized in chloroform:methanol:water (1:2:0.8, v/v). The LPI was recovered from the water–methanol layer of the Bligh and Dyer extraction mixture, separated using a Sep-Pak™ cartridge, and analysed by LC-MS.

Cloning and Cell Culture—A DNA fragment containing the entire open reading frame of human GPR55 (GenBank™ accession number NM_005683) was amplified from human spleen cDNA by PCR as described earlier (*8*). HEK293 cells were grown at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin. The cells were transfected with GPR55-pcDNA4/TO or an empty vector using Lipofectamine™ 2000 reagent. The stably transfected clones were selected in the presence of zeocin (100 ng/ml, Invitrogen). The human GPR35 gene was prepared as follows. A DNA fragment containing the entire open reading frame of the human GPR35 (GenBank™ accession number NM_005301) was amplified from human small intestine cDNA by PCR using Pyrobest® DNA Polymerase (Takara Bio Inc., Shiga, Japan) and oligonucleotides (forward primer containing a KpnI site, a Kozak sequence and a FLAG sequence, 5′-AAAAAAGGTACCGCCACCATGGACTACAAGGACGACGATGACAAGAATGGCACCTACAACACCTGTGGC-3′; reverse primer containing a XbaI site, 5′-AAAAAATCTAGATTAGGCGAGGGTCACGCACAGAGA-3′). The resultant DNA fragment was digested with KpnI and XbaI and subsequently cloned into the mammalian expression vector pcDNA4/TO between the KpnI and XbaI sites. The integrity of the plasmid was verified by DNA sequencing.

RNA Interference Analysis—GPR55-expressing HEK293 cells were transfected with duplex small interfering RNA (siRNA) for GPR55 using the Lipofectamine RNAiMAX reagent as previously described (*8*). Following transfection, the cells were incubated at 37°C for 48 h. A scrambled stealth RNAi (Invitrogen, Carlsbad, CA USA) was used as a negative control.

Western Blot Analysis—Western blot analysis was carried out according to a previously described method (*12*). The intensity of the bands was quantified using NIH *Image*, and the ratio of phospho-ERK to total ERK was calculated. The data were expressed as fold-stimulation (compared to vehicle alone).

Measurement of the $[Ca^{2+}]_i$—The $[Ca^{2+}]_i$ was estimated using a CAF-100 Ca^{2+} analyser (JASCO, Tokyo, Japan) as previously described (*8*).

Statistical Analysis—The data were analysed by analysis of variance (ANOVA) followed by Tukey's test (Figs 2, 3 and 5). Considered significant difference was $P < 0.05$.

RESULTS

Analysis of LPI Present in Rat Brain—We first analysed the level as well as the molecular species of LPI present in rat brain using 2-heptadecanoyl LPI as an internal standard. In this experiment, the fatty acyl moiety of the purified LPI was converted to fatty acid methyl esters and analysed by GC (Table 1). We found that rat brain contains 37.5 nmol/g tissue of LPI.

Table 1. **Fatty acid composition of LPI present in rat brain.**

Fatty acid	16:0	16:1	18:0	18:1	18:2	20:4	22:6	Total
Tissue (nmol/g) (%)	4.8 ± 4.1 (12.8)	0.5 ± 0.7 (1.3)	18.9 ± 8.9 (50.5)	5.0 ± 2.2 (13.3)	trace	8.3 ± 6.0 (22.1)	trace	37.5 ± 18.1 (100)

Fatty acid composition of LPI present in rat brain was analysed as described in MATERIALS AND METHODS section. The ratios 16:0 indicate palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 20:4, arachidonic acid; and 22:6, docosahexaenoic acid (fatty acids are designated as number of carbon atoms:number of double bonds, e.g. 16:0 for palmitic acid). The values are the means ± SD of seven determinations.

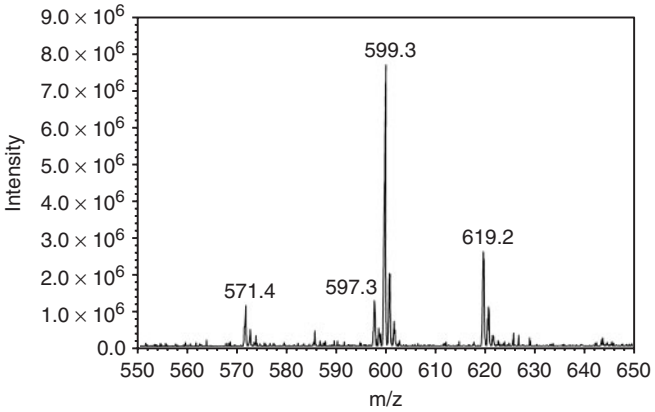

Fig. 1. **Analysis of rat brain LPI by LC-MS.** LC-MS analysis was performed as described in MATERIALS AND METHODS section. The result is representative of three separate experiments.

The most predominant species is that containing stearic acid (18.9 nmol/g tissue; 50.5%). Notably, rat brain contains a significant amount of arachidonic acid-containing species (8.3 nmol/g tissue; 22.1%) in addition to palmitic acid-containing species (4.8 nmol/g tissue; 12.8%), oleic acid-containing species (5.0 nmol/g tissue; 13.3%) and palmitoleic acid-containing species (0.5 nmol/g tissue; 1.3%). On the other hand, the amounts of linoleic acid-containing species and docosahexaenoic acid-containing species were negligible.

The occurrence of various species of LPI in rat brain was also confirmed by LC-MS: *m/z* 599.3 for the stearic acid-containing species, *m/z* 619.2 for the arachidonic acid-containing species, *m/z* 571.4 for the palmitic acid-containing species and *m/z* 597.3 for the oleic acid-containing species (Fig. 1). We confirmed that stearic acid is exclusively esterified at the *sn*-1 position (99 ± 1%, the mean ± SD of three determinations) and arachidonic acid is predominantly esterified at the *sn*-2 position (91 ± 6%, the mean ± SD of three determinations).

Effects of Various Species of LPI and Related Molecules on ERK—We then compared the effects of various molecular species of LPI and structurally related molecules on ERK in vector-transfected or GPR55-expressing HEK293 cells. Figure 2 shows the results of such experiments. Various species of LPI were found to induce the phosphorylation of ERK in a dose-dependent manner (Fig. 2A–F). Interestingly, the potencies of various molecular species of LPI markedly differed. The highest level of activity was observed with 2-arachidonoyl LPI (Fig. 2F): the EC_{50} was 30 nM. Its activity was approximately 8 times greater than that of 2-linoleoyl LPI (Fig. 2E), and 15 times that of 2-oleoyl LPI (Fig. 2D), 1-oleoyl LPI (Fig. 2C) and 1-stearoyl LPI (Fig. 2B). Notably, 1-palmitoyl LPI acted as a weak partial agonist (Fig. 2A). Marginal activity was also observed with LPG (1-palmitoyl) (Fig. 2G), while LPC (1-palmitoyl) did not exhibit any activity (Fig. 2H). Various types of cannabinoid receptor ligands such as 2-AG (Fig. 2I), anandamide (Fig. 2J), CP55940 (Fig. 2K) and O-1602 (Fig. 2L) did not induce the phosphorylation of ERK. Free arachidonic acid was also shown to be inactive (Fig. 2M). The cellular responses induced by various species of LPI are not due to non-specific physicochemical effects; synthetic detergents such as sucrose monolaurate (SM-1200) and sodium dodecylbenzenesulfonate (SDBS) did not affect the phosphorylation of ERK (Fig. 2N and O).

Effects of Various Species of LPI and Related Molecules on $[Ca^{2+}]_i$—We next compared the effects of various molecular species of LPI and structurally related molecules on $[Ca^{2+}]_i$ in vector-transfected or GPR55-expressing HEK293 cells. As depicted in Fig. 3A–F, various species of LPI-induced transient increases in $[Ca^{2+}]_i$ in GPR55-expressing HEK293 cells. The highest level of activity was observed with 2-arachidonoyl LPI (Fig. 3F) and the lowest level with 1-palmitoyl LPI (Fig. 3A), the same as for the effects of various species of LPI on ERK as shown in Fig. 2. LPG acted as a weak partial agonist (Fig. 3G) as in the case of the phosphorylation of ERK. On the other hand, LPC, several types of cannabinoid receptor ligands, free arachidonic acid and detergents did not markedly affect $[Ca^{2+}]_i$ in either the vector-transfected cells or GPR55-transfected cells even at high concentrations (Fig. 3H–O).

Relatively high concentrations (such as 3 μM and 10 μM) of LPI induced small increases in $[Ca^{2+}]_i$ in the vector-transfected cells (Fig. 3A–F). The reason for this is not clear. One possible explanation is that LPI weakly interacted with the LPA receptor(s) to elicit a small response, because pre-treatment of the cells with 10 μM LPA abolished the response to LPI in the vector-transfected cells (data not shown).

In contrast to the vector-transfected cells, pre-treatment with LPA did not markedly affect the LPI-induced change in $[Ca^{2+}]_i$ in the GPR55-expressing cells. As shown in Fig. 4, pre-treatment of the cells with LPA (10 μM) or S1P (10 μM) did not markedly affect the response to 2-arachidonoyl LPI (1 μM). On the other hand, pre-treatment with 2-arachidonoyl LPI (10 μM) markedly reduced the response to 2-arachidonoyl LPI (1 μM). These results suggest that 2-arachidonoyl LPI mainly interacted with a specific receptor, i.e. GPR55, thereby eliciting the increase in $[Ca^{2+}]_i$ in the GPR55-expressing cells.

This was confirmed by an experiment in which siRNA against GPR55 was employed. As shown in Fig. 5,

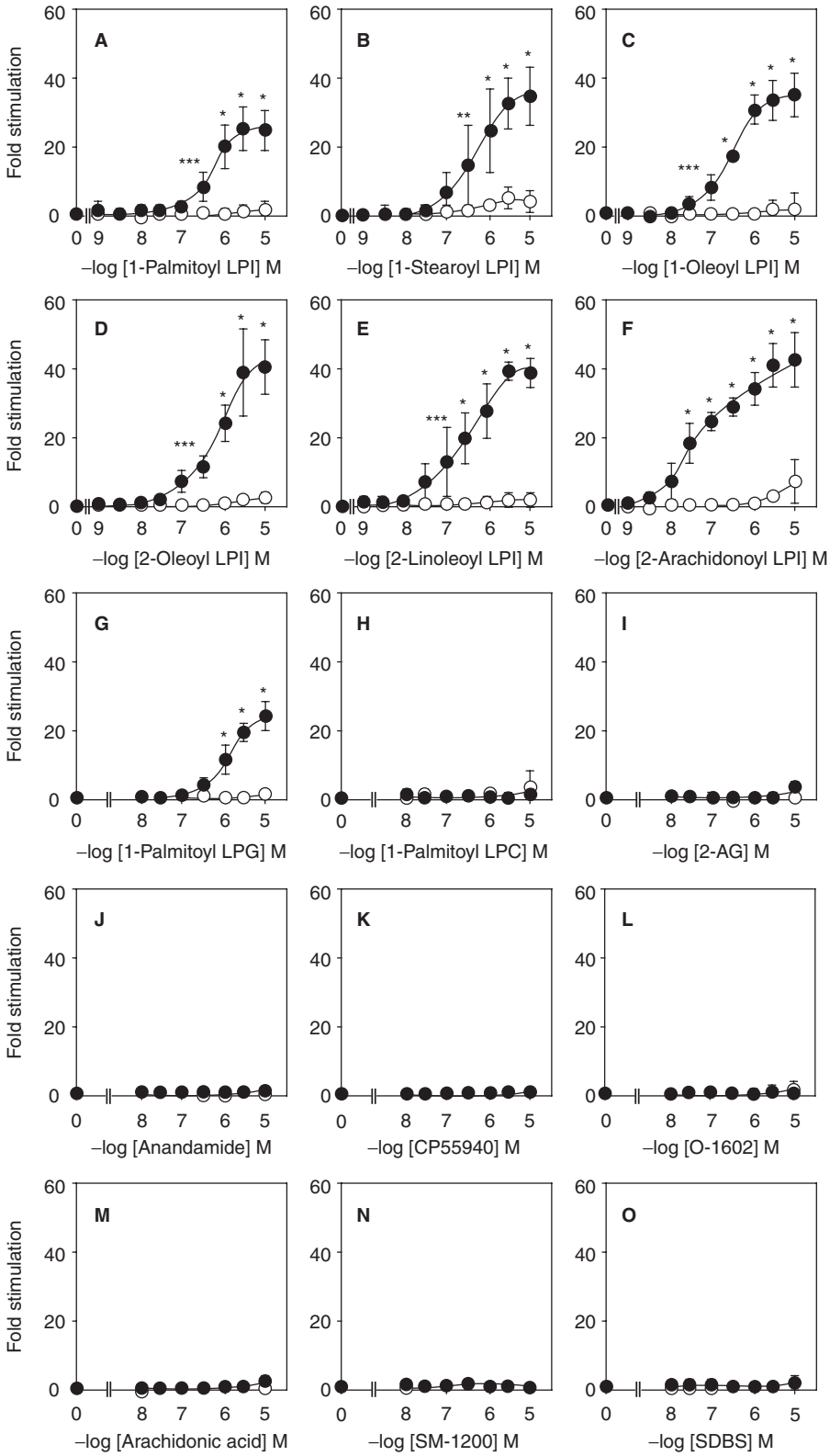

Fig. 2. **Effects of various species of LPI and related molecules on ERK in HEK293 cells stably expressing GPR55.** Vector-transfected cells (open circle) or GPR55-expressing cells (closed circle) were incubated in the presence of various concentrations of LPI and related molecules at 37°C for 5 min. (A) 1-palmitoyl LPI; (B) 1-stearoyl LPI; (C) 1-oleoyl LPI; (D) 2-oleoyl LPI; (E) 2-linoleoyl LPI; (F) 2-arachidonoyl LPI; (G) LPG (1-palmitoyl); (H) LPC (1-palmitoyl); (I) 2-AG; (J) anandamide; (K) CP55940; (L) O-1602; (M) arachidonic acid; (N) sucrose monolaurate (SM-1200); and (O) SDBS. The values are the means ± SD of four determinations. $^{*}P < 0.001$; $^{**}P < 0.01$; $^{***}P < 0.05$.

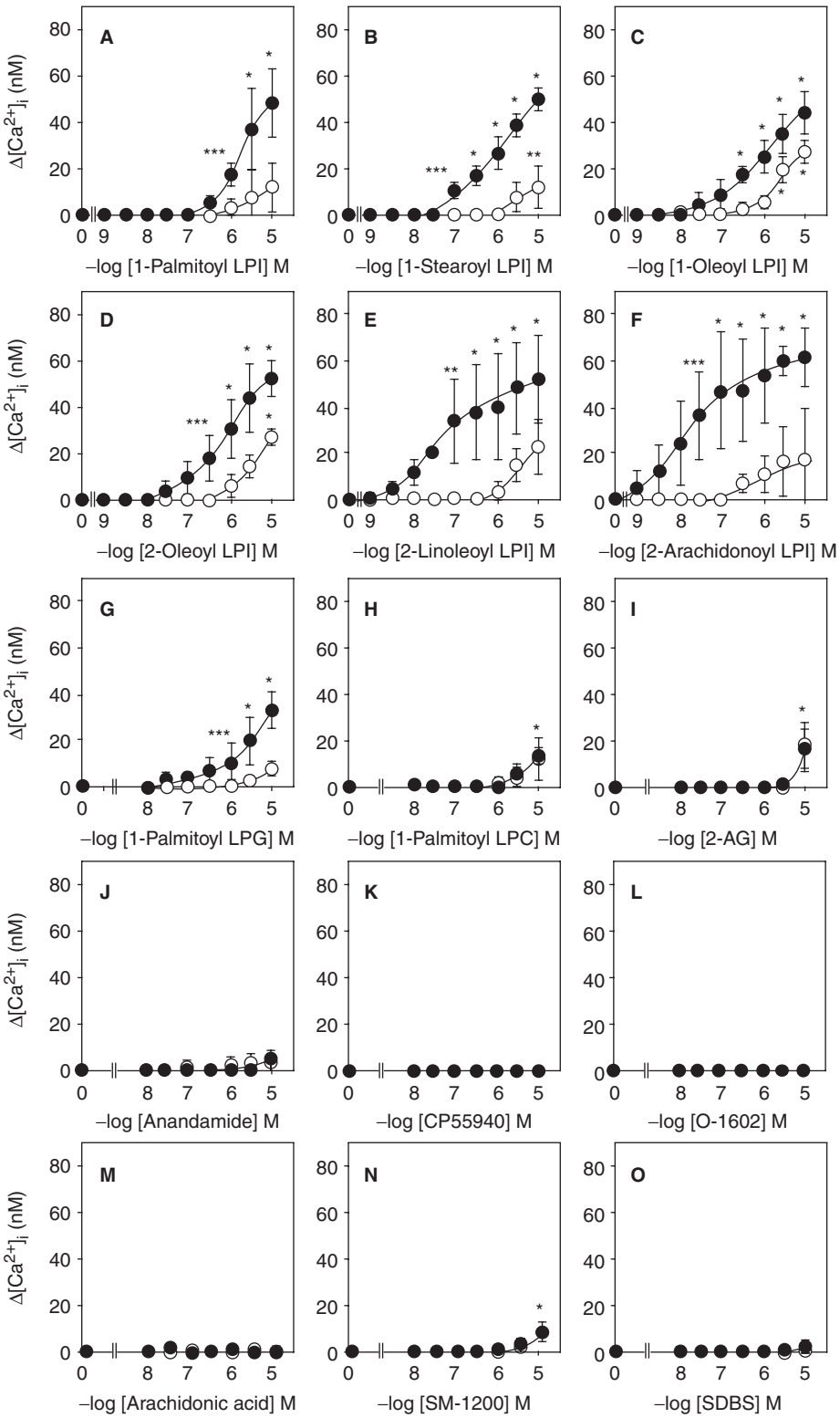

Fig. 3. **Effects of various species of LPI and related molecules on $[Ca^{2+}]_i$ in HEK293 cells stably expressing GPR55.** Various concentrations of LPI and related molecules were added to the vector-transfected cells (open circle) or GPR55-expressing cells (closed circle) loaded with Fura-2/AM and the changes in $[Ca^{2+}]_i$ were analysed. (A) 1-palmitoyl LPI; (B) 1-stearoyl LPI; (C) 1-oleoyl LPI; (D) 2-oleoyl LPI; (E) 2-linoleoyl LPI; (F) 2-arachidonoyl LPI; (G) LPG (1-palmitoyl); (H) LPC (1-palmitoyl); (I) 2-AG; (J) anandamide; (K) CP55940; (L) O-1602; (M) arachidonic acid; (N) sucrose monolaurate (SM-1200); and (O) SDBS. The values ($\Delta[Ca^{2+}]_i$) are the means ± SD of four determinations. $^{*}P < 0.001$; $^{**}P < 0.01$; $^{***}P < 0.05$.

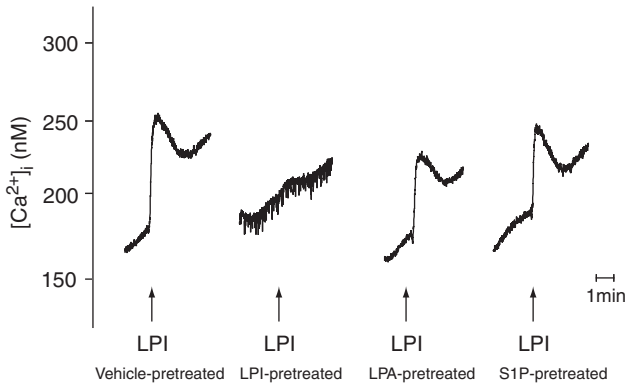

Fig. 4. **Effect of pre-treatment with 2-arachidonoyl LPI, LPA or S1P on the 2-arachidonoyl LPI-induced Ca^{2+} transient in HEK293 cells stably expressing GPR55.** The cells loaded with Fura-2/AM were treated with 2-arachidonoyl LPI, LPA or S1P (final concentration, 10 μM) or the vehicle at 37°C for 1 min, sedimented, and resuspended in HEPES-buffered Tyrode's solution (–Ca^{2+}) containing 0.1% BSA. After adding $CaCl_2$ (final concentration, 1 mM), 2-arachidonoyl LPI (final concentration, 1 μM) was added to the cuvette, and the changes in $[Ca^{2+}]_i$ were analysed. The result is representative of three separate experiments.

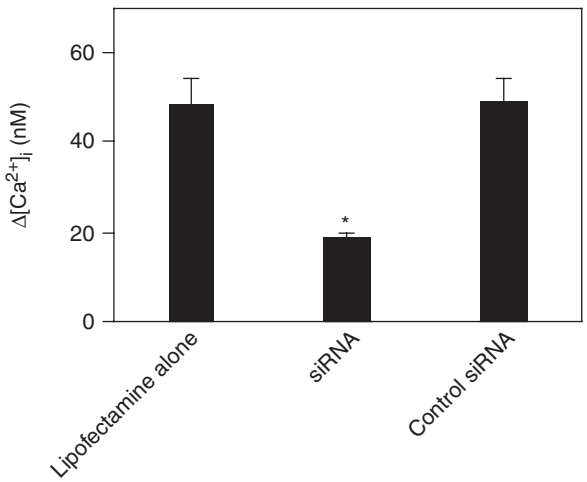

Fig. 5. **Effect of siRNA against GPR55 on the 2-arachidonoyl LPI-induced Ca^{2+} transient in HEK293 cells expressing GPR55.** The GPR55-expressing cells were treated with Lipofectamine alone or siRNA against GPR55 or control siRNA for 48 h. The cells were then loaded with Fura-2/AM. 2-Arachidonoyl LPI (final concentration, 1 μM) was added to the cuvette, and the changes in $[Ca^{2+}]_i$ were analysed. The values are the means ± SD of four determinations. $^*P < 0.001$ (compared to Lipofectamine alone).

treatment of the cells with the siRNA markedly reduced the response induced by the 2-arachidonoyl LPI. In contrast, treatment with a control siRNA did not exert any effect on the 2-arachidonoyl LPI-induced response.

To further confirm that the effects of 2-arachidonoyl LPI on the GPR55-expressing cells are not attributable to non-specific effects on the gene-transfected cells, we examined the effects of 2-arachidonoyl LPI on HEK293 cells expressing a G protein-coupled receptor with

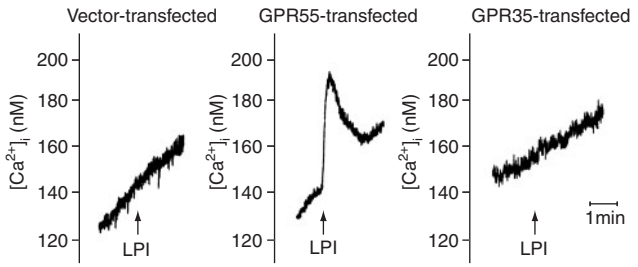

Fig. 6. **Comparison of the effects of 2-arachidonoyl LPI on $[Ca^{2+}]_i$ in HEK293 cells stably expressing GPR55 or GPR35.** 2-Arachidonoyl LPI (1 μM) was added to the cuvette and the changes in $[Ca^{2+}]_i$ were analysed. The result is representative of three separate experiments.

homology to GPR55. Here, we chose GPR35 which has 30% homology to GPR55 (*13*). As demonstrated in Fig. 6, 2-arachidonoyl LPI induced a Ca^{2+} transient in the GPR55-expressing cells. On the other hand, 2-arachidonoyl LPI did not affect $[Ca^{2+}]_i$ in the GPR35-expressing cells as in the case of the vector-transfected cells.

These results strongly suggest that GPR55 is a specific and functional receptor for LPI, especially for 2-arachidonoyl LPI, and that the cellular response induced by 2-arachidonoyl LPI is actually mediated by GPR55.

DISCUSSION

GPR55 is expressed in a variety of mammalian tissues such as the frontal cortex, striatum, hypothalamus, caudate, putamen, adrenal gland, testis, spleen, tonsils and ileum (*1–4*). Evidence is gradually accumulating, which suggests that GPR55 plays essential regulatory roles in living animals, although the details are yet to be determined (*5–7*).

The endogenous ligand for GPR55 remained to be identified until recently. In an earlier study, we found that soybean-derived LPI induces rapid phosphorylation of ERK and a Ca^{2+} transient in GPR55-expressing HEK293 cells (*8*). Notably, these responses were not observed in vector-transfected cells (*8*). The results, together with other experimental data, indicated that LPI is a possible endogenous ligand for GPR55. To the best of our knowledge, this is the first report showing clear evidence for the occurrence of the LPI receptor.

In the present study, we first investigated the level and the molecular species of LPI present in rat brain, inasmuch as the molecular species of plant-derived LPI are postulated to be markedly different from those of mammalian tissue-derived LPI. We found that rat brain contains a substantial amount of 2-arachidonoyl LPI besides other species (Table 1 and Fig. 1). We then compared in detail the biological activities of the individual molecular species of LPI and related molecules and found that the biological activity of 2-arachidonoyl LPI is much greater (8–15 times) than those of others (Figs 2 and 3). We also confirmed that the biological activity of 2-arachidonoyl LPI (the phosphorylation of ERK or the elevation of $[Ca^{2+}]_i$) is 3 times greater than that of 1-arachidonoyl LPI (Oka *et al.*, unpublished results).

These results strongly suggest that 2-arachidonoyl LPI, i.e. 2-arachidonoyl-*sn*-glycero-3-phosphoinositol (2-AGPI), is the true natural ligand for GPR55.

Previously, several groups reported that GPR55 is a possible novel type of cannabinoid receptor (*2–4*). Nevertheless, it is not presently clear whether GPR55 actually acts a cannabinoid receptor, like CB1 and CB2. For example, CP55940, a synthetic cannabinoid, failed to induce the phosphorylation of ERK and a Ca^{2+} transient (Figs 2 and 3). In addition, 2-AG, an endogenous ligand for the cannabinoid receptors (CB1 and CB2), did not exert any apparent effect on cells expressing GPR55 [Figs 2 and 3 and Refs (*4*, *8*)]. In contrast, several investigators demonstrated that 2-AG stimulated the GPR55-expressing cells (*3*). Further detailed studies are needed to answer the questions whether GPR55 is a typical type of cannabinoid receptor and whether LPI is an endocannabinoid.

The physiological significance of GPR55 remains to be determined. Several investigators reported that GPR55 may play some essential role in the vascular system (*14*). On the other hand, Johns *et al*. (*2*) described that the vasodilator effects of several cannabinoids are not mediated by GPR55. The elucidation of the physiological functions of GPR55 and its endogenous ligand LPI awaits future investigation.

The finding that 2-arachidonoyl LPI is the natural ligand for GPR55 is particularly noteworthy, because the 2-arachidonoyl species of monoacylglycerol, i.e. 2-AG, is the true natural ligand for the cannabinoid receptors (CB1 and CB2) (*9*). There is close structural similarity between these two molecules, i.e. the presence of the 2-arachidonoyl moiety attached to the glycerol backbone. 2-Arachidonoyl LPI and 2-AG may constitute a novel bioactive lipid family. In relation to this, it should be noted that rat brain contains a significant amount of 2-arachidonoyl LPA (*10*, *15*) and that the 2-unsaturated fatty acyl LPA appears to be the natural ligand for LPA_3 (*16*). Thus, 2-arachidonoyl LPA may be another member of this novel bioactive lipid family.

LPI can be formed from PI by the action of phospholipase A_2 or phospholipase A_1; the latter enzyme is assumed to be involved in the generation of 2-arachidonoyl LPI from PI. Ueda *et al*. (*17*) previously demonstrated that PI is hydrolysed by phospholipase A_1 to release 2-acyl LPI in rat brain. Alternatively, 2-arachidonoyl LPI may be formed from PI by the reverse reaction of acyl-CoA:LPI acyltransferase (*18*, *19*). Several investigators have demonstrated the generation of LPI in a variety of mammalian tissues and cells upon stimulation (*20*). Various species of LPI including 2-arachidonoyl LPI have been shown to exist in several biological fluids (*21*, *22*). Yet, the molecular mechanisms involved in the formation of LPI in these tissues and cells remain to be determined. Further detailed studies are required to clarify the mechanisms underlying the generation of LPI in various stimulated tissues and cells.

Not much attention has thus far been directed towards LPI as a bioactive lipid. Several investigators have demonstrated that LPI stimulates several cellular functions, such as cell growth (*21*, *23*). Nevertheless, the available information concerning LPI as a bioactive lipid is sparse compared to that for LPA. In particular, little information is available concerning the biological activity of 2-arachidonoyl LPI. The thorough elucidation of the physiological and pathophysiological significance of LPI, especially 2-arachidonoyl LPI, and its receptor GPR55 awaits future investigation.

In summary, we found that rat brain contains a significant amount of arachidonic acid-containing LPI in addition to other molecular species of LPI. We also found the level of biological activity of 2-arachidonoyl LPI to be the highest using the GPR55-expressing HEK293 cells. These results strongly suggest that the intrinsic natural ligand for GPR55 is 2-arachidonoyl LPI.

CONFLICT OF INTEREST

None declared.

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