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JB Commentary

What is the natural ligand of GPR55?

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GPR55 is a seven transmembrane G protein-coupled receptor and was originally identified as a putative third cannabinoid receptor. Recently, lysophosphatidylinositol (LPI) was reported to be a GPR55 ligand. Stimulation of GPR55 by LPI activates $G_{12/13}$ and $G_{q/11}$ proteins, induces phosphorylation of the extracellular signal-regulated kinase and increases intracellular calcium concentration. Lysophospholipids are molecularly quite diverse across species and tissues. A recent report showed that the predominant fatty acyl moiety of LPI in rat brain is stearic acid followed by arachidonic acid. The biological activity of arachidonic acid-containing LPI species towards GPR55 was shown to be markedly higher than that of LPI species containing other fatty acyl groups, suggesting that 2-arachidonolyl LPI is the most likely natural ligand of GPR55.

Keywords: GPR55/LPI/natural ligand/lipid/mass spectrometry.

Abbreviations: GPCR, G protein-coupled receptor; LPI, lysophosphatidylinositol; 2-AG, 2-arachidonoyl glycerol; PEA, *N*-palmitoylethanolamine; abn-CBD, abnormal-cannabidiol; Δ^9-THC, Δ^9-tetrahydrocannabinol.

The diversity of physiological effects caused by marijuana and cannabinoids suggests that several different receptors may be responsible for their biological activities. Yet to date, only two receptor subtypes, CB1 and CB2, have convincingly been confirmed as cannabinoid targets. The CB1 receptor, abundantly expressed in the nervous system, is involved in attenuation of synaptic transmission. The CB2 receptor, mainly expressed in the immune system, is implicated in the regulation of inflammatory reactions and immune responses (*1*). Anandamide (*N*-arachidonoylethanolamine) was initially identified as an endogenous ligand for CB receptors, and is now considered a partial agonist (Figure 1). After an intensive search for endogenous ligands for CB receptors, 2-arachidonoylglycerol (2-AG) was identified (*2–8*).

GPR55 was initially identified as a putative cannabinoid receptor, but the validity of this assignment has been the subject of some debate. Oka *et al*. (*9*) reported that while cannabinoids did not appear to activate GPR55, lysophosphatidylinositol (LPI) derivatives resulted in robust stimulation of the receptor. LPI induced phosphorylation of ERK1/2 in hGPR55-expressing HEK293 cells. However, numerous synthetic cannabinoid agonists [2-AG, anandamide, *N*-palmitoylethanolamine (PEA), *N*-oleoylethanolamide, virhodamine, CP55940, HU-210, WIN55212-2, Δ^9-tetrahydrocannabinol (Δ^9-THC), abnormal-cannabidiol (abn-CBD) and SR141716A] failed to show the same effect. The authors also reported that LPI rapidly induced p38 MAP kinase and the phosphorylation of activating transcription factor-2 (ATF-2) (*10*).

Most studies on the pharmacological properties of GPR55 have used HEK293 cells transfected with GPR55. However, despite wide tissue distribution of GPR55 its physiological function *in vivo* remains to be established. The prominent expression of GPR55 within brain, dorsal root ganglion (DRG) neurons and immune cells suggests that GPR55 functions in these tissues, especially in the regulation of inflammatory and neuropathic pain (*8*). Staton *et al*. (*11*) reported that $GPR55^{-/-}$ mice were protected in models of inflammatory and neuropathic pain (*11*). Whyte *et al*. (*12*) found that GPR55 was expressed in osteoclastes and osteoblastes, and played roles in the maintenance of bone mass by affecting the differentiation of these cells.

To identify ligands for orphan GPCRs, the ligand content of the tissue of interest must be quantified. For example, we identified 12(*S*)-hydroxyheptadecatrienoic acid (12-HHT) as an intrinsic ligand for BLT2, which was originally identified as a low-affinity leukotriene B_4 receptor (*13*). We purified BLT2-active lipids from rat small intestine by HPLC, and determined the structure of the natural BLT2 ligand by mass spectrometry. The estimated concentration (~1 μM) of 12-HHT in small intestine is high enough to activate BLT2 according to *in vitro* experiments using BLT2-expressing cells. We concluded that 12-HHT is a natural ligand for BLT2 (*14*).

Lysophosphatidic acid (LPA) is composed of various lengths of acyl chains containing saturated, monounsaturated or polyunsaturated fatty acid at either *sn*-1 or *sn*-2 position. This natural variety in the LPA class is thought to generate ligand specificity and diversity in biological activity. Among the various LPA receptors isolated so far, LPA3, p2y5 and GPR35 have been reported to bind preferentially 2-acyl LPAs over 1-acyl LPAs (*15*). LPI is also composed of various chains at either the *sn*-1 or *sn*-2 position. To identify the molecular species of LPI in rat brain, Oka *et al*. (*16*) extracted total lipids using the Bligh and Dyer

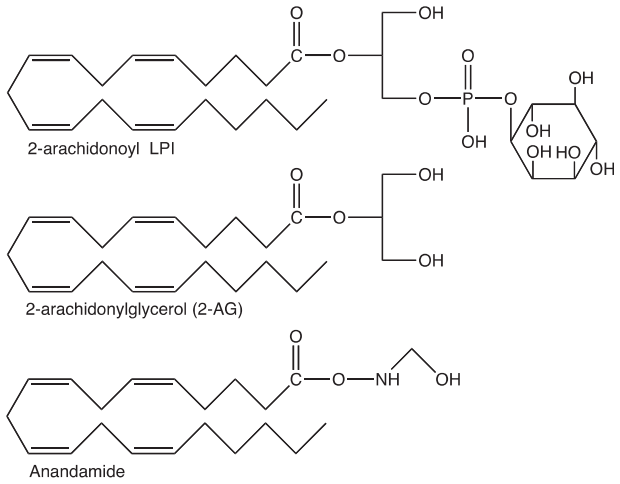

Fig. 1 Structural similarity of agonists for GPR55, CB1 and CB2 receptors 2-arachidonoyl LPI is a ligand for GPR55. 2-AG and anandamide are ligands for CB1 and CB2.

methods and purified LPIs by 2D-TLC (*16*). LPI was extracted from the silica gel and analysed by gas chromatography or LC-MS. The study showed that rat brain contained 37.5 nmol/g tissue of LPI. The most predominant fatty acyl moiety was stearic acid (50.5%) followed by arachidonic acid (22.1%). In addition, the biological activities of individual molecular species of LPI and related molecules were analysed. They found that the biological activity of 2-arachidonyl LPI was much greater than that of other LPI molecules.

Yamashita *et al.* (*17*) reported that intracellular phospholipase A_1 [DDHD1 or DDHD domain containing 1, which contains four conserved amino acids (Asp, Asp, His and Asp)], previously identified as phosphatidic acid (PA)-preferring PLA_1 (PA-PLA_1), is involved in the formation of 2-arachidonoyl-LPI. $iPLA_1$/DDHD1/PA-PLA_1 might be an LPI-producing enzyme. However, recent studies showed that other intracellular PLA_1s ($iPLA_1$s) cleave the fatty acid of PI at the *sn*-1 position (*18*). As $iPLA_1$s are intracellular enzymes that produce LPI, the mechanism of LPI release for GPR55 activation should be clarified in future studies.

The molecular species of LPI in rat brain were determined using whole tissue extracts, including plasma membranes and intracellular components. GPR55 is a membrane protein and the molecular species of lipids in tissue fluids may be different from those in the structural components of tissues. GPR55 is highly expressed in lymphoid organs such as spleen and thymus in addition to brain (*10*). The identification of the molecular species present in these tissues may help the understanding of the physiological function of LPI in the tissues other than brain.

Conflict of interest
None declared.

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