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Review

# Two ligands for a GPCR, proton *vs* lysolipid[1]

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## Key words

lysolipid; proton; G-protein-coupled receptor; sphingosylphosphorylcholine; lysophosphatidylcholine; psychosine

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## Abstract

Recently, two different chemicals have been matched as ligands with the same G-protein-coupled receptor (GPCR). Double-pairing of OGR1 family GPCRs with proton and lysolipid raises several questions. First, whether both are the real ligands for the GPCRs. Second, whether modulation of a GPCR by two chemicals could be possible. Third, one of the chemicals is proton. Proton-sensing not only is a new action mode of GPCR activation, but also it could be generalized in other GPCRs. In this review, I'd like to summarize the issue and discuss questions with pharmacological criteria.

## Introduction

G-protein-coupled receptor (GPCR) is the largest gene family of human genome. GPCR is glaringly obvious by the fact that more than 50% of drugs on the market are either agonists or antagonists on GPCRs[1]. Positive or negative modulation of GPCRs with drugs has been successful tools to treat many diseases such as allergy, gastric ulcer, and hypertension. The common structural feature of all GPCRs is a seven-helical transmembrane region. GPCR activations are evoked by stimuli as diverse as light, $Ca^{2+}$, odorants, amino acids, nucleotides, proteins, polypeptides, steroids, and fatty acid derivatives.

The completion of the human genome project has identified about 865 GPCR genes[2]. Except sensory receptors, 367 GPCRs have been considered as receptors for endogenous ligands in the human genome[3]. However, identification of novel members of GPCRs by genome sequencing faces orphan receptor problem, that is, ligands are not yet found[1]. About 150 orphan GPCRs are waiting for discovery of their ligands[4]. Pairing orphan GPCRs with their own ligands (endogenous or surrogate) would advance scientific knowledge and induces discovery of new drugs[5–9]. However, recent double-pairing of OGR1 subfamily GPCRs with two different chemicals, proton and lysolipid, raises several questions.

First, whether both chemicals are the real ligands for OGR1 subfamily. Second, whether modulation of a GPCR by two chemicals could be possible, because classic pharmacological concept is one ligand for one GPCR. Third, one of the chemicals is proton. Although it has been well established for ion channel receptors such as transient receptor potential/vanilloid receptor subtype-1 (TRPV1)[10] and acid-sensing ion channels (ASICs)[11], in GPCR area, not only proton-sensing is a new action mode of GPCR activation, but also it could be generalized in other GPCRs.

## OGR1 subfamily and lysolipids

OGR1 subfamily is composed of four members (OGR1, GPR4, G2A, and TDAG8) and has previously been identified as receptors for lysolipids; sphingosylphosphorylcholine (SPC), lysophosphatidylcholine (LPC) and psychosine (galactosylsphingosine)[12–14]. In 2000, Xu *et al* reported Ovarian cancer G-protein-coupled Receptor 1(OGR1, GPR68) to be a high-affinity receptor for SPC ($K_d$ = 33 nmol/L) and

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SPC was shown to inhibit cell growth of OGR1-transfected HEK293 as well as various ovarian cancer cell lines[12,15]. In 2001, Zhu *et al* reported GPR4 to be the second high affinity receptor for SPC and GPR4 was shown to be activated by structurally-related LPC[13]. However, in contrast to OGR1, GPR4 activation stimulated cell growth and cell migration of GPR4-transfected Swiss3T3 cells[13]. TDAG8 (T-cell death-associated gene 8, GPR65) was reported to be activated by psychosine and its activation was shown to result in multinuclear cell formation[14]. G2A (G2 accumulation protein, GPR132), the last member of the subfamily, was characterized to cause cell cycle arrest in the $G_2$/M phase[16]. LPC was initially reported as a ligand of G2A and T cell chemotaxis to LPC was shown to be mediated through G2A[17].

Yan *et al* reported macrophage chemotaxis to LPC is dependent on G2A function and mutation of the conserved DRY motif of G2A results in loss of function[18]. Wang *et al* demonstrated that murine G2A was spontaneously internalized and accumulated in endosomal compartments, whereas its surface expression was enhanced and stabilized by LPC treatment[19]. Han *et al* reported G2A-mediated up-regulation of CXCR4 in human helper T cells[20]. Murakami *et al* reported G2A-dependent actin stress fiber formation and its inhibition by LPC in G2A-NIH3T3 cells[21]. Also LPC enhances dose-dependently intracellular cAMP accumulation and G2A-induced apoptosis in Hela cells[22]. Ikeno *et al* reported that secretary phospholipase $A_2$ induce neurite outgrowth in PC12 cells through LPC generation and activation of G2A receptor[23]. Lin and Ye reported that G2A displays a significant level of intrinsic signaling via $G\alpha_q$, $G\alpha_s$, and $G\alpha_{13}$ pathways[22].

Lum *et al* showed that inflammatory stress increases GPR4 expression and LPC binding in human microvascular endothelial cells[24]. Recently, Kim *et al* reported that GPR4 plays a critial role in SPC-induced angiogenesis and SPC transactivates VEGF receptor 2 in endothelial cells[25].

Maghazachi *et al* reported that psychosine and glucosylsphingosine induce multinuclear cell formation and apoptosis in TDAG8-expressing natural killer cells[26]. Malone *et al* reported that activation of TDAG8 by psychosine enhanced dexamethasone-induced apoptosis in a TDAG8-dependent manner in lymphomas[27]. Tosa *et al* reported critical function of TDAG8 in glucocorticoid-induced thymocyte apoptosis[28].

These ligand chemicals have similar lysolipid structures (Figure 1) and their significance in pathological conditions and pharmacological application has been discovered. Especially, G2A deficient mouse developed an autoimmune syndrome similar to systemic lupus erythematosus (SLE)[29],

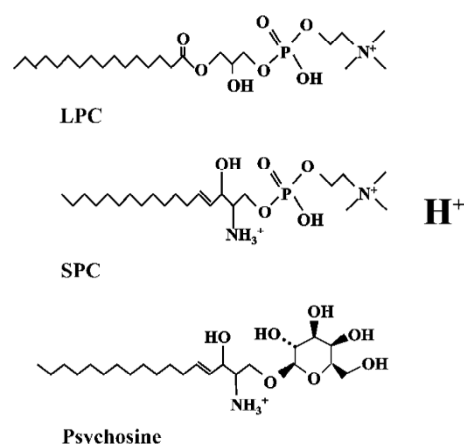


**Figure 1.** Structures of LPC and other lysolipids.

and therapeutic application of LPC for sepsis was proposed in relation with G2A receptor[30].

Overexpression of G2A, GPR4, and TDAG8 in human cancers has been found to play a role in driving or maintaining tumor formation, however, transformation was achieved without addition of lysolipids[31,32]. Bektas *et al* reported ligand-independent signaling of GPR4 and its inability to respond to SPC and LPC in several assay systems, that is, GTPγS binding, receptor internalization, and arrestin translocation[33]. Additionally, the original paper that reported G2A-LPC pairing was recently retracted by authors, because they could not confirm the LPC-binding experiments[34]. Constitutive activation of GPCR and lipid-independent responses raises a possibility, that is, another activator of GPCR is present in the culture medium or secreted from GPCR-transfected cells. Such a possibility has been suggested and supported with proton by five independent groups[21,35–38].

## OGR1 subfamily as proton-sensing GPCRs

Ludwig *et al* (2003) reported OGR1 and GPR4 to be proton-sensing receptors. At pH 7.8, OGR1 was inactive, but activated fully inositol phosphate (IP) formation at pH 6.8. Ludwig *et al* predicted several hydrogen-bond interactions occurring between unprotonated histidines by using a computational 3D model of OGR1. Under alkaline conditions these interactions could stabilize the receptor in an inactive state. Exposure to an acidic pH would destabilize the hydrogen bonds, switching the receptor to its active conformation. Indeed, mutation of several histidines (H17, H20, H84, H169, and H269) to phenylalanines reduced proton-sensing ability of OGR1 (Figure 2)[35]. In the same paper, authors observed that a very similar activation of GPR4 by pH change, but GPR4 activates the Gs-adenylyl cyclase-cAMP pathway[35].

**Figure 2.** Sequence alignment of OGR1 subfamily GPCRs. Histidine residues that have been reported to be involved in proton-sensing are bolded and underlined in OGR1 (H17, H20, H84, H169, and H269), TDAG8 (H10 and H14), and G2A (H174). Residues in GPR4 that are assumed to be critical for proton-sensing based on alignment with OGR1 are also marked. Suggested basic amino acids in G2A instead of histidines in OGR1 are underlined. A histidine in TMVI conserved in all four members of GPCRs is starred. Potential transmembrane regions are overscored; gaps are indicated by dashes.

However, they were not able to observe any effect of SPC and LPC, previously reported ligands, on OGR1 and GPR4. In 2004, Murakami *et al* reported that G2A functions as a proton-sensing GPCR[21]. Transient transfection of G2A caused significant activation of the *zif* 268 promoter and IP accumulation at pH 7.6 and lowering extracellular pH augmented the activation only in G2A-expressing PC12h cells[21]. Site-directed mutation of His-174, which is predicted to be located at the extracellular part of the transmembrane helix IV (Figure 2), reduced partially G2A-dependent signaling at lower pH. They found that LPC and SPC did not cause IP formation at pH 7.6, but LPC inhibited IP formation at pH 6.8

in a dose-dependent manner, suggesting that LPC acts as an antagonist not an agonist. Wang *et al* reported that TDAG8 is also a proton-sensing GPCR stimulating cAMP accumulation[36]. They found that psychosine and SPC are antagonistic on pH-dependent responses in the cells transfected with TDAG8. Psychosine-sensitive and pH-dependent cAMP accumulation was also observed in mouse thymocytes, where TDAG8 is endogenously expressed[36]. Radu *et al* conducted experiments with all 4 members of OGR1 subfamily to test proton-sensibility and confirmed previous reports on OGR1, GPR4, and TDAG8[38]. However, G2A was insensitive or less sensitive to extracellular pH change in their experimental conditions[38]. They suggested that lack of many histidine residues, defined to be involved in pH-sensing of OGR1, could be a cause for insensitiveness of G2A to acidic pH (Figure 2)[38]. Also they suggested that the constitutive activation of G2A in neutral pH might be resulted from maintaining active conformation of G2A via positively charged amino acids in human and mouse G2A instead of conserved histidines (Figure 2)[38]. Ishii *et al* reported that TDAG8 is a proton-sensing GPCR, however, they were not able to observe any inhibitory effect of psychosine on pH-dependent TDAG8 activation[37]. Therefore, a series of publications propose that extracellular proton could be an activator of the OGR1 subfamily of GPCRs. More than two independent groups reported proton-sensing properties of OGR1, GPR4, and TDAG8 (Table 1). In the case of G2A, constitutive activation at pH 7.4 has been observed in many transfected cells by many research groups, however, pH-dependent activation was supported only by one group[21] and was not fully reproduced by another group[38]. Dependence of pH sensing on the histidine residues on the extracellular domains of GPCRs has been tested on OGR1, TDAG8, and G2A[21,35–37]. However, site-directed mutagenesis study of the histidines on GPR4 has not been experimentally reported.

In summary, there are four opinions in the published reports, that is agonism of lysolipid, antagonism of lysolipid, agonism of proton, and no confirmation of lysolipid action on OGR1 subfamily GPCRs. Table 1 shows list of publications supporting each opinion except negative observation or constitutive activation.

## Two ligands for a GPCR, proton *vs* lysolipid

As for lysolipids as ligands, Ludwig *et al* could not confirm such activation of GPCRs with lysolipids[35], and three research groups observed antagonistic effects of LPC, SPC and psychosine on the GPCRs in acidic conditions rather than agonism (Table 1)[21,36–38]. In summary, lysolipids have been suggested as ligands for the OGR1 subfamily GPCRs, however, all four members of the GPCRs have also been proposed as proton-sensing GPCRs. OGR1 subfamily has been considered as a contentious GPCR subgroup, because pairing it with lysolipids has been controversial in the scientific society[4,21,33,35,36]. Additionally, the original G2A paper was retracted[34] and GPR4 paper would be retracted (Y XU, FASEB conference, 2005). However, there are growing numbers of reports supporting actions of lysolipids on OGR1 subfamily GPCRs (Table 1). As proposed by Kim *et al* it can be dependent on cell types. Kim *et al* recently confirmed pH-dependent cAMP production in GPR4-transfected HEK293 cells but not in GPR4-transfected HUVEC or HMEC-1 cells[25]. Cell-type specific functions remind us RAMPS (receptor activity-modulating proteins) which are essential proteins for expression and function of GPCRs such as CGRP (calcitonin gene-related peptide) and adrenomedullin[39]. If

**Table 1.** Summary of publications.

| Receptor | | Agonist | | Antagonist |
|---|---|---|---|---|
| Name | Expression | Lysolipid | Proton | Lysolipid |
| OGR1 (GPR68) | Spleen, testis, small intestine, PBL, brain, heart, lung, placenta, kidney[53] | (SPC)[12] | $(H^+)$[35,36,38] | (Psy and SPC)[36] |
| GPR4 | Ubiquitous (ovary, liver, lung, kidney, lymph node, subthalamic nucleus)[13] | (LPC and SPC)[13]<br>(LPC)[24], (SPC)[25] | $(H^+)$[25,35,36,38] | (Psy and SPC)[36] |
| TDAG8 (GPR65) | PBL, spleen, thymus, lymph nodes[14] | (Psy)[14,26,27] | $(H^+)$[36–38] | (Psy and SPC)[36] |
| G2A (GPR132) | Spleen, thymus, T-lymphocytes, B-lymphocytes, monocytes, macrophages[16] | (LPC)[17,18,21,30] | $(H^+)$[21,38] | (LPC)[21,38] |

there are RAMP-like proteins specific for OGR1 subfamily, the complicated results may be solved. Wang *et al* recently observed spontaneous internalization of murine G2A and reported that LPC induces surface redistribution and stabilization of murine G2A[19]. Such an action of LPC may support spontaneous activation of G2A and explain agonistic and antagonistic effects of LPC. If spontaneous activity of G2A was presumed as control level in neutral pH, LPC-induced action might be interpreted as agonism. However, if spontaneous activation or proton-activated effect was thought as agonism, LPC-induced action might be considered as antagonism. Further investigation on cell-type specificity and receptor distribution in the cells may clarify action mode of OGR1 subfamily GPCRs by both chemicals, proton and lysolipids.

Therefore, it is not easy to say which chemical is the real ligand, although both chemicals could be called as modulators of the GPCRs. Finding of antagonistic effects of lysolipids on the receptors at acidic conditions may advance our understanding and might reconcile the controversy in the future. If both chemicals could activate the same GPCRs in certain conditions, another issue might be two ligands for a GPCR. Dual actions may be a rare example in the GPCR area. However, considering that TRPV1 could be activated by capsaicin, proton, heat and lipids, two chemicals for a GPCR could not be a surprising action mode in biological sciences[10]. Investigation of physiological roles and pathological implications of the GPCRs in the future may lighten importance of discovery of proton-sensing GPCRs, because acidosis is related with many diseases such as cancer[40], asthma[41], atherosclerosis[42], arthritis[43], and osteopenia[35,44].

## Proton as an agonist

Another issue remains; could proton be a ligand? To be an agonist, it should bind to GPCR specifically and reversibly[45]. Furthermore, it should dose-dependently activate GPCR. In five publications, proton has been shown to fulfill all the above criteria, except specificity[21,35–38]. Basal activity of many other GPCRs has been observed in neutral pH without apparent presence of endogenous ligands as like δ-opioid receptor[46,47]. Such basal activity of GPCR has further been confirmed by inverse agonists, which reduce the basal activity in a dose-dependent manner[46,47]. Many GPCRs have histidine residues in extracellular loops and outer segments of GPCR helixes. Thus, if change of extracellular pH could change basal activities of many GPCRs, although the magnitude of change varies, this means lack of specificity. If many GPCRs are activated or inactivated by change of pH without presence of any agonists, proton can not be an agonist, because it lacks specificity, even though OGR1 subfamily activation with proton was the greatest response among GPCRs activated with proton. Indeed, modulation of GPCRs by extracellular pH has been reported in $P2Y_4$ ATP receptor and calcium-sensing receptor[48,49].

Four research groups have used the term "proton-sensing GPCR" for OGR1 subfamily, however, the term for proton, the counterpart, is omitted or the term "ligand" has been used for proton. However, it is inadequate, because of the above-mentioned reason, lack of specificity. GPCR modula-

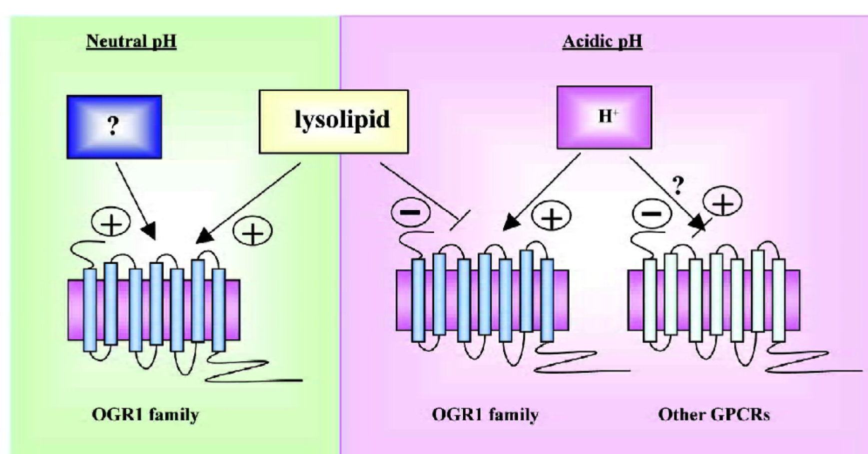


**Figure 3.** Illustration of suggested modulators of OGR1 subfamily. Lysolipids have been suggested as agonists of OGR1 subfamily in neutral pH and as an agonist in acidic pH. Proton has been suggested as agonists of OGR1 subfamily. Two possibilities are also included. One is another chemical activating OGR1 subfamily in neutral pH. The other possibility is proton action on other GPCRs positively and/or negatively. The symbol (+) means activation of GPCR and (-) inactivation.

tor may be the good term for proton, because it changes activity of GPCRs, but it can not be an agonist. So far, the smallest particle ever reported to be a GPCR activator is photon. In this case, photon energy in light activates rhodopsin GPCR by isomerisation of 11-*cis*-retinal to *trans* conformation within the rhodopsin helixes[50,51]. Now, proton might become the second small molecule activating GPCRs. Although lysolipids as the ligand of OGR1 subfamily still remain controversial, proton as a ligand for the GPCRs also need to be considered with caution, because an endogenous ligand might be waiting to be discovered, suggesting possible presence of another chemical to activate OGR1 subfamily in neutral conditions (Figure 3). It may not be a surprise if there is another chemical activating OGR1 subfamily in neutral pH such as prostaglandins and capsaicin modulating TRPV1[52].

In summary, proton activates OGR1 subfamily GPCRs and lysolipids modulate activity of OGR1 GPCRs positively or negatively. However, we need to consider other two possibilities. One is that modulation by proton could be generalized in other GPCRs even though the magnitude varies (Figure 3). Second, there could be another chemical activating OGR1 subfamily in neutral pH (Figure 3).

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