Molecular and Cellular Endocrinology xxx (2017) 1–9

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Lysophosphatidylcholine and its phosphorothioate analogues potentiate insulin secretion via GPR40 (FFAR1), GPR55 and GPR119 receptors in a different manner

Anna Drzazga [a], Hjalti Kristinsson [b], Maciej Sałaga [c], Hubert Zatorski [c], Maria Koziołkiewicz [a], Edyta Gendaszewska-Darmach [a, *], Peter Bergsten [b]

[a] Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, B. Stefanowskiego 4/10, 90-924 Lodz, Poland
[b] Department of Medical Cell Biology, Uppsala University, Husargatan 3, 75123 Uppsala, Sweden
[c] Department of Biochemistry, Medical University of Lodz, Mazowiecka 6/8, 92-215 Lodz, Poland

ARTICLE INFO

Article history:
Received 30 July 2017
Received in revised form
8 November 2017
Accepted 7 December 2017
Available online xxx

Keywords:
Lysophosphatidylcholine (LPC)
GPR40 (FFAR1)
GPR55
GPR119
Insulin secretion

ABSTRACT

Lysophosphatidylcholine (LPC) is an endogenous ligand for GPR119 receptor, mediating glucose-stimulated insulin secretion (GSIS). We demonstrate that LPC facilitates GSIS in MIN6 pancreatic β-cell line and murine islets of Langerhans by recognizing not only GPR119 but also GPR40 (free fatty acid receptor 1) and GPR55 activated by lysophosphatidylinositol. Natural LPCs are unstable when administered *in vivo* limiting their therapeutic value and therefore, we present phosphorothioate LPC analogues with increased stability. All the modified LPCs under study (12:0, 14:0, 16:0, 18:0, and 18:1) significantly enhanced GSIS. The 16:0 sulfur analogue was the most potent, evoking 2-fold accentuated GSIS compared to the native counterpart. Interestingly, LPC analogues evoked GPR40-, GPR55-and GPR119-dependent $[Ca^{2+}]_i$ signaling, but did not stimulate cAMP accumulation as in the case of unmodified molecules. Thus, introduction of a phosphorothioate function not only increases LPC stability but also modulates affinity towards receptor targets and evokes different signaling pathways.

1. Introduction

2-Lysophosphatidylcholines (1-acyl-glycero-3-phosphocholines, 2-LPCs, LPCs), which maintain the acyl chain in *sn-1* position, are the most abundant lysophospholipid in nature (D'Arrigo and Servi, 2010). Lipidomic analysis has revealed a correlation of lower plasma concentrations of LPCs with impaired glucose tolerance and obesity (Zhao et al., 2010; Barber et al., 2012). LPC 16:0 is the most abundant species in human plasma (146 ± 37 μM) followed by LPC 18:0 (56.5 ± 14.9 μM) and LPC 18:1 (28.4 ± 12.5 μM) (Heimerl et al., 2014). Although the presence of LPCs in plasma was observed at the beginning of the twentieth century (Kihara et al., 2015), the original observation from Metz's laboratory on dose-dependent lysophospholipid-induced insulin secretion was shown already in 1986 (Metz, 1986) while the involvement of G protein coupled receptor for the effect of LPC on insulin secretion was identified as GPR119 in 2005 (Soga et al., 2005). GPR119 is preferentially expressed on β-cells of the islets of Langerhans but its expression has also been demonstrated in intestinal L- and K-cells, where its activation was associated with secretion of glucagon-like peptide 1 and glucose-dependent insulinotropic peptide (Overton et al., 2006; Sakamoto et al., 2006; Ahlkvist et al., 2013). GPR119 has been shown to bind a variety of lipid-derived ligands, as well as a range of small synthetic molecules. Recent literature data indicate that lysophospholipids also have the ability to interact with other pancreatic receptors regulating carbohydrate metabolism. GPR55 activated by lysophosphatidylinositols may be another attractive target in type 2 diabetes mellitus (T2DM) (Liu et al., 2016). Treatment of diabetic rats with lysophoshatidylinositol has been found to counteract the symptoms of diabetes such as high blood glucose, lower body weight, increase amplitude of slow wave in stomach smooth muscle, and to improve gastric emptying (Lin et al., 2014). Both GPR119 and GPR55 receptors

Abbreviations: $[Ca^{2+}]_i$, intracellular calcium ions concentration; cAMP, cyclic AMP; GSIS, glucose-stimulated insulin secretion; GPCR, G protein coupled receptor; IBMX, 3-isobutyl-1-methylxanthine; LPC, lysophosphatidylcholine; OEA, oleoylethanolamide; PI, propidium iodide; T2DM, type 2 diabetes mellitus.

* Corresponding author.
E-mail addresses: edarmach@wp.pl, edyta.gendaszewska-darmach@p.lodz.pl (E. Gendaszewska-Darmach).

https://doi.org/10.1016/j.mce.2017.12.002
0303-7207/

are stimulated by endocannabinoids such as palmitoylethanolamide (PEA), oleoylethanolamide (OEA), arachidonoylethanolamide (anandamide, AEA), and 2-arachidonoylglycerol (2-AG) (Godlewski et al., 2009). We have recently proved that ligand specificity of GPR55 is much wider and our studies evidence that GPR55 is activated also by LPC (Drzazga et al., 2017). However, GPR40 (also known as the free fatty acid receptor 1 or FFAR1) is the best-studied of the cell-surface receptors on β-cells. GPR40 is the most potently activated by endogenous free fatty acids (FFAs) with medium and long (C12-C22) aliphatic chains, resulting in amplification of insulin secretion only in the presence of elevated glucose levels (Itoh et al., 2003; Itoh and Hinuma, 2005; Briscoe et al., 2003). The glucose dependency of insulin secretion makes this receptor an excellent target for developing efficacious therapies with a desired safety profile for use in the treatment of T2DM.

LPCs have been previously described as potential anti-diabetic factors due to stimulation of secretory activity from the isolated rodent pancreas, L-type cells, ands insulin-producing cell lines, with LPC 18:1 as the main structure of interest (Soga et al., 2005; Sakamoto et al., 2006; Overton et al., 2008; Lan et al., 2009; Lauffer et al., 2009). Although these studies have shed light on LPC as GPR119 agonist, it should be kept in mind that other pancreatic islet receptors might be involved in LPC-induced anti-diabetic activity. We have previously shown that both native LPCs and their phosphorothioate analogues (Fig. 1) have the ability to stimulate the mobilization of intracellular Ca^{2+} in a murine β-cell line and the amount of Ca^{2+} flux depends on the length of the saturated acyl residue. Additionally, we have noticed that simultaneous phosphorothioate and 2-*OMe* substitution at the *sn*-3 and *sn*-2 position, respectively, within LPC moieties greatly reduce off-target interactions with the cell membrane and make LPC analogues less toxic compared to their natural counterparts (Rytczak et al., 2013; Drzazga et al., 2015). Here, we explored the complexity of GPR119/GPR55/GPR40 signaling in insulin-secreting MIN6 β-cells affected by unique synthetic phosphorothioate analogues of LPC with strictly defined fatty acid residues. The MIN6 cell line was established from insulinoma by targeted expression of the simian virus 40 T antigen gene in transgenic mice (Miyazaki et al., 1990). The cell line produces insulin, has morphological characteristics of primary pancreatic β-cells, and exhibits glucose-inducible insulin secretion albeit less than in normal primary mouse islet cells. Expression of GPR40, GPR55 and GPR119 has previously been confirmed in MIN6 cells (Liu et al., 2016; Itoh et al., 2003; Zhang et al., 2014). Taking into account the favorable properties of phosphorothioate analogues of LPCs over their natural counterparts we examined a set of LPC analogues bearing medium (C12, C14) to long (C16, C18) fatty acid residues as potential modulators of glucose-stimulated insulin secretion (GSIS). The strongest stimulators of GSIS were subjected to further studies and compared to their native LPC counterparts. We investigated basic intracellular signaling in MIN6 cells, namely cyclic AMP (cAMP) and intracellular calcium ($[Ca^{2+}]_i$) levels since these second messengers play a major role in augmenting GSIS (Soga et al., 2005; Itoh et al., 2003; Moran et al., 2014; McKillop et al., 2013; Shibasaki et al., 2004; Yaekura et al., 1996; Hou et al., 2009; Ning et al., 2008; Landa et al., 2005). Due to ligand promiscuity between GPR119 and GPR55 documented in the case of their natural lipid agonists (Godlewski et al., 2009), we studied possible complex activity in the case of our test compounds. GPR40 was included as a potential target as well since lipids of more complex structure than FFAs were not investigated so far as potential ligands of this receptor and because LPCs contain a long-chain fatty acid moiety that theoretically could bind to the receptor.

The goal of the study was to determine the influence of novel phosphorothioate analogues of LPC on insulin secretion and related cell signaling with reference to the most commonly studied GPCR targets engaged in this process. We aimed to delineate structure-dependent efficiency of the test compounds as well as their potential target preferences.

2. Materials and methods

2.1. Chemicals

Unmodified LPC moieties (16:0 and 18:1) were purchased from Avanti Polar Lipids (Alabaster, USA). OEA was purchased from Sigma-Aldrich (Saint Louis, USA). LPC phosphorothioate analogues were synthesized as described in the previous report (Rytczak et al., 2013). Prior to analysis of biological activity, all the investigated compounds were diluted in methanol (MeOH, POCH) at stock concentration of 5 mM. Further dilutions, when needed, were performed in phosphate-buffered saline (PBS, pH 7.4; Life Technologies, Carlsbad, USA). DC 260126 (Hu et al., 2009) (depicted as DC), CID 16020046 (Kargl et al., 2013) (depicted as CID) and ML 193 (Heynen-Genel et al., 2010; Kotsikorou et al., 2013) (depicted as ML) were purchased from Tocris Bioscience (Ellisville, USA). GW 1100 (Briscoe et al., 2006) (depicted as GW) was purchased from Cayman Chemical Company (Michigan, USA). GPR119 antagonist (compound 8, depicted as C8) was kindly provided by Pfizer (Connecticut, USA) (McClure et al., 2011). All synthetic antagonists were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) at stock concentration of 10 mM.

2.2. Culture of MIN6 cell line

The murine adherent insulinoma MIN6 cells were kindly provided by Dr. Jun-ichi Miyazaki from the Division of Stem Cell Regulation Research, Osaka University, Japan (Miyazaki et al., 1990). The applied culture medium consisted of 82.5% Dulbecco's modified Eagle's medium (Life Technologies) containing 4.5 g/L glucose, 10% fetal bovine serum (FBS, Life Technologies), 2 mM L-glutamine, 50 μM β-mercaptoethanol (Sigma-Aldrich) and 1% penicillin/streptomycin (Gibco). Experiments on MIN6 cells were performed between passages 24–29.

2.3. Isolation and culture of murine islets of Langerhans

Murine pancreata were kindly provided from the Department of Biochemistry, Medical University of Lodz, Poland and isolated from naive male C57B1/6 mice (8 weeks old). Isolation of murine islets of Langerhans followed the protocol described previously (Li et al., 2009). Hand-packed murine islets were cultured in RPMI 1640

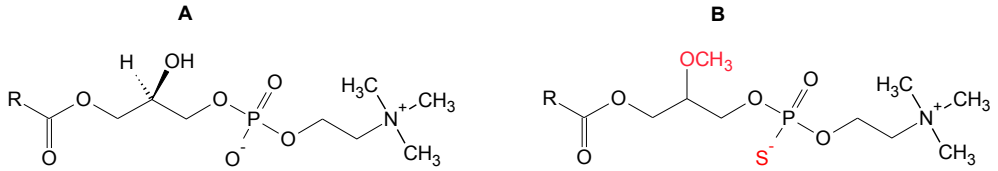

Fig. 1. Structures of native LPCs (**A**) and their corresponding synthetic 2-*OMe*-phosphorothioate analogues (**B**). Set of investigated LPCs involves natural LPC 12:0 (L), LPC 14:0 (M), LPC 16:0 (P), LPC 18:0 (S) and LPC 18:1 (O) and corresponding LPC analogues (LT, MT, PT, ST, OT respectively).

Please cite this article in press as: Drzazga, A., et al., Lysophosphatidylcholine and its phosphorothioate analogues potentiate insulin secretion via GPR40 (FFAR1), GPR55 and GPR119 receptors in a different manner, Molecular and Cellular Endocrinology (2017), https://doi.org/10.1016/j.mce.2017.12.002