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Recent Progress: Solving the Native Structure of BMP and Probing the Ligand Binding Pocket of GPR 55

The glycerophospholipid bis(monoacylglycerol)phosphate (BMP) is a minor constituent in mammalian cells, but is specifically enriched in internal membranes of digestive organelles, such as late endosomes (LE) and lysosomes (Lyso). The digestive organelles are the waste treatment and recycling center in the cells, separating the hydrolytic enzymes needed to digest endocytosed particles or cellular waste, including aged lipids, proteins and even whole organelles, from the remainder of the cell. In this acidic environment, BMP plays a vital role by providing the suitable environment for these hydrolytic enzymes to work and is involved in cholesterol egress. Perturbation of BMP metabolism strongly affects glycosphingolipid degradation, cholesterol homeostasis and may also be a relevant factor in atherosclerosis. Probing the native acylation pattern of lysophospholipids is difficult due to the inherent ability of ester groups to migrate between adjacent hydroxyl functions. The regioisomer composition of native BMP has been the subject of some scientific discussion and Luquain et al [1] demonstrated that TLC-purified BMP is primarily esterified on the primary hydroxyl function. To overcome possible ester migration during isolation, a simple LC-MS/MS based assay system capable to separate BMP based on its fatty acid composition as well as its regioisomer was developed and the native BMP regioisomer identified.

Recently, we have demonstrated [2] that phosphatidyl-β-D-glucoside derived lysophosphatidyl-β-D-glucoside (lyso-PtdGlc) is the first lipid derived regulator of central projections in nociceptive (pain) sensory afferents during embryonic development. This lyso-PtdGlc associated patterning is mediated by G-protein coupled receptor 55 (GPR55). GPR55 has originally been deorphanized as a cannabinoid receptor, but has later been described as lysophospholipid-responsive receptor. GPR55 upregulation has been reported in a variety of cancers, while GPR55 knock out mice show abnormal responses to inflammation and mechanical stimuli, suggesting a role of GPR55 in neuropathic pain and inflammatory processes. Furthermore, GPR55 has been linked to a variety of physiological and pathological processes, such as synaptic transmission, obesity, bone development and gastrointestinal functions. To characterize lysolipid-GPR55 interaction and more specifically the ligand binding pocket, we established synthetic access to lyso-PtdGlc and prepared a variety of synthetic lyso-PtdGlc analogues. The biological activity of our synthetic analogues was evaluated using our previously

developed functional assay, based on primary cultured DRG sensory neurons endogenously expressing GPR55. To overcome the lack of structural information of GPR55, we established and validated a GPR55 homology model [3] and performed molecular dynamics (MD) simulations of GPR55 in the presence of natural and synthetic lysolipid ligands. The results of our MD simulation were in good agreement with our biological assay results, providing novel insight into the ligand binding pocket specifics.

[1] C Luquain, R Dolmazon, JM Enderlin, C Laugier, M Lagarde, JF Pageaux; Biochem. J. 2000, 351, 795.
[2] A.T. Guy, Y. Nagatsuka, N. Ooashi, M. Inoue, A. Nakata, P. Greimel, A. Inoue, T. Nabetani, A. Murayama, K. Ohta, Y. Ito, J. Aoki, Y. Hirabayashi, H. Kamiguchi, Science 2015, 349(6251), 974-977.
[3] A.T. Guy, K. Kano, J. Ohyama, H. Kamiguchi, Y. Hirabayashi, Y. Ito, I. Matsuo, P. Greimel ACS Chem. Neurosci. 2019, 10, 716-727.