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科学研究費助成事業　　研究成果報告書

令和　５ 年　 ６ 月　 ７ 日現在

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研究課題名（和文）Investigation of the role of radial glia in regulating LysoPtdGlc required for nervous system development

研究課題名（英文）Investigation of the role of radial glia in regulating LysoPtdGlc required for nervous system development

研究代表者

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研究者番号：３０６３４７７９

交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：私は以前、リゾホスファチジルグルコシドという誘導因子が脊髄の痛覚ニューロンの神経回路構築を制御することを解明した。本研究では、ラジアルグリアが分泌するリゾホスファチジルグルコシドの異性体を二つ発見しました：R型(R-LysoPtdGlc)とS型（S-LysoPtdGlc)。R型とS型は同じ受容体GPR55を特異的に活性化しますが細胞内のシグナル伝達は異なる下流パスウェイが活性化されることを発見した。R型はGalpha13/Rho-ROCKパスウェイ、S型はGalphaS/adenylyl cyclaseパスウェイを活性化することで異性体によるGPR55の機能選択肢的活性化だと考えられます。

研究成果の学術的意義や社会的意義
I determined that the axon guidance cue LysoPtdGlc can exist as one of two biologically active isomers: R-LysoPtdGlc and S-LysoPtdGlc. I found that downstream of GPR55, R-LysoPtdGlc activates Galpha13 whilst S-LysoPtdGlc activates GalphaS, strongly suggesting that they are biased ligands of GPR55.

研究成果の概要（英文）：I previously found that lysophosphatidylglucoside (LysoPtdGlc) is a lipid axon guidance cue required for the development of nociceptive axon circuits in spinal cord. In this project, I have discovered that radial glial LysoPtdGlc can exist in two isomeric forms, R-LysoPtdGlc and S-LysoPtdGlc. I found that whilst both R- and S-forms activate the orphan receptor GPR55, they signal via different downstream intracellular cascades: R-LysoPtdGlc induces Galpha13 activation, whilst S-LysoPtdGlc activates GalphaS. In an assay of axon chemotropism, I discovered that the two isomers, although they both activate GPR55, induce cellular chemotropic response of opposite polarity: R-form induces chemorepulsion, S-form induces chemoattraction, strongly suggesting a mechanism of functional selectivity or ligand bias at GPR55. Neither form induces a response in neurons genetically lacking GPR55. I have also obtained preliminary data for R- and S-forms in an in vivo model of mouse neuropathic pain.

研究分野：Developmental neurobiology

キーワード：Lipid biology　Developmental biology　Neuroscience　Molecular biology　Cell biology

１．研究開始当初の背景

(1) To build a functioning nervous system, nascent neurons must extend axons and form the appropriate synaptic connections required for life.　One mechanism by which organisms achieve this is called axon guidance, a form of chemotropism where signaling molecules at midway choice-points or at the innervation target tissue guide extending axons to their correct position. In 2015 I discovered a novel axon guidance cue called lysophosphatidylglucoside (LysoPtdGlc), that is a diffusible lysolipid and the endogenous ligand for the orphan G protein-coupled receptor GPR55. In developing spinal cord, GPR55 is activated by its endogenous ligand LysoPtdGlc, which is a derivative of the membrane lipid phosphatidylglucoside (PtdGlc) produced by radial glia. Since then, I have discovered that in addition to the spinal cord, PtdGlc is expressed in multiple regions of the brain, including olfactory bulb and the cerebellum, where PtdGlc levels are highest. However, the biological significance of high levels of PtdGlc in different brain regions is completely unknown.
(2) Endogenous phospholipids are highly heterogenous in chemical composition, such as differences in fatty acid chain length and composition, and 3D structure (isomerism), with different structural isomers often possessing different chemical properties. Since isomers often have different biological activities in living organisms, I hypothesized that different isomers of LysoPtdGlc may have different functions or interactions with GPR55 receptor.

２．研究の目的

The objective of this study was to gain insight into mechanisms of cell signaling mediated by LysoPtdGlc activation of GPR55, with respect to nervous system development.　GPR55 was cloned in 1999 yet its biology is poorly understood and it has remained enigmatic and controversial, particularly the identity and activity of its endogenous ligands *in vivo*.　In particular, the existence of *R*- and *S*-configuration structural isomers of PtdGlc (the precursor of LysoPtdGlc) was described when PtdGlc was first reported by Y. Nagatsuka and co-workers in 2006, yet the significance of this has never been elucidated in the lab.　This study sought to identify a biological significance of isomeric LysoPtdGlc and shed light on their activities at GPR55.

３．研究の方法

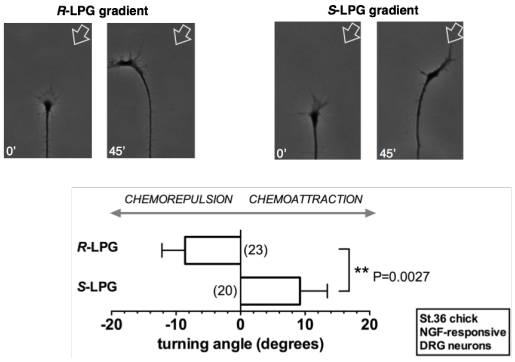
I will use an *in vitro* axon growth cone turning assay to assess the chemotropic signaling activities of different isomers of LysoPtdGlc acting on primary cultured chick embryonic dorsal root ganglion (DRG) sensory neurons. This assay is a well-established technique to analyze chemotropic responses of neurons expressing normal levels of endogenous receptors including GPR55.　Neurons can be treated with pharmacological agents or inhibitory peptides that interfere with Gα protein function,

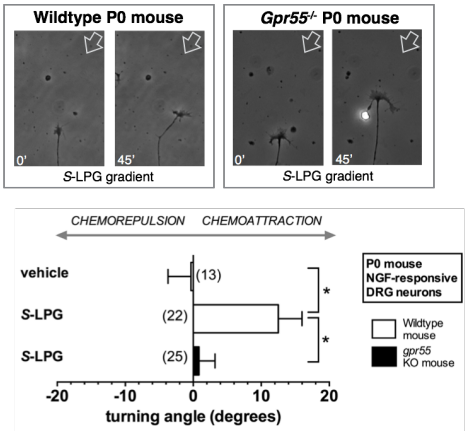
to further probe the intracellular pathways downstream of GPR55 activation by LysoPtdGlc. I plan to establish a timecourse of cerebellum development in the chick correlated to PtdGlc expression as visualized using antibody staining, with the objective of identifying the exact cell type that produces PtdGlc and therefore, LysoPtdGlc. Compared to mammals, the cerebellum of chick develops rapidly during embryonic stages, making it easier to study and a suitable model for my experiments.

４．研究成果

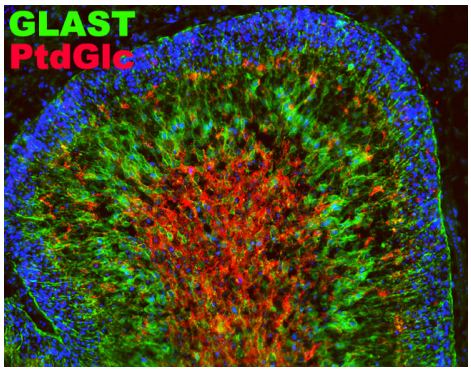
(1) Discovery of a novel signaling mechanism at GPR55 with characteristics of biased agonism.

I discovered that two isomers of LysoPtdGlc called *R*-LysoPtdGlc and *S*-LysoPtdGlc induced differential cellular responses, but both were mediated by activation of GPR55. I therefore concluded that this was evidence of biased agonism (sometimes called ligand selectivity): where different ligands can bind to the same receptor but induce different cellular responses by activating distinct downstream pathways. In my experiments, I observed that *R*-LysoPtdGlc induced chemorepulsion in the growth cone turning assay, a test of chemotropism of axonal growth cones in response to a microscopic concentration gradient of signaling molecules in the culture dish. In contrast, in the same assay *S*-LysoPtdGlc induced chemoattraction in axonal growth cones, indicating positive chemotropism. In neurons in which GPR55 was genetically deleted, *S*-LysoPtdGlc had zero chemotropic effect on the axonal growth cones, indicating that in the wild-type, chemoattraction was mediated by *S*-LysoPtdGlc activating GPR55, the same receptor that *R*-LysoPtdGlc activates. By using pharmacological inhibitors such as Rp-cAMPS or selectively blocking individual Gα subunits with inhibitory peptides, I eventually discovered that whilst *R*-LysoPtdGlc signals via $G\alpha_{13}$, *S*-LysoPtdGlc induces chemoattraction via activation of $G\alpha_S$. Taken together, these data strongly suggest that the two isomers were functioning in a biased agonism mechanism. These data are currently in preparation as a manuscript for submission to a peer-reviewed international journal.





(2) In cerebellum development, PtdGlc is produced by a non-neuronal population separate from Bergmann glia. Using antibody staining in parasagittal sections of developing chick cerebellum, I found that PtdGlc-expressing cells in the developing cerebellum are distributed mostly in the granular layer with weak expression of GLAST, although a few PtdGlc$^+$ cells can be detected in the prospective Purkinje cell layer. Unfortunately, due to the temporary shutdown of my institute in 2020 due to pandemic conditions and reduction of possible laboratory work, and then my subsequent move to Kyoto University in 2021, I did not have enough time to gather sufficient data for the next experiment, which was FACS-isolation of PtdGlc-expressing cells and cell culture for cell identification and fate mapping analyses *in vitro*. I am currently performing this experiment now, after the end of the period funded by this grant-in-aid. I am now also investigating the effect of LysoPtdGlc on the migration of cerebellar granule cells using a transwell migration assay, since the distribution of PtdGlc (in the deep inner granular layer) suggests that is may be involved in the radial migration of granule cells from outer to inner cerebellar layers.



５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　0件）

１．著者名 Guy Adam T、Kamiguchi Hiroyuki	４．巻 66
２．論文標題 Lipids as new players in axon guidance and circuit development	５．発行年 2021年
３．雑誌名 Current Opinion in Neurobiology	６．最初と最後の頁 22～29
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.conb.2020.09.003	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Guy Adam T、Kamiguchi Hiroyuki	４．巻 66
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１．著者名 Guy Adam T.、Ding Feiqing、Abe Junpei、Inoue Mariko、Hirabayashi Yoshio、Ito Yukishige、Kamiguchi Hiroyuki、Greimel Peter	４．巻 11
２．論文標題 Lysolipid Chain Length Switches Agonistic to Antagonistic G Protein-Coupled Receptor Modulation	５．発行年 2020年
３．雑誌名 ACS Chemical Neuroscience	６．最初と最後の頁 3635～3645
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acschemneuro.0c00521	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Abe Junpei、Guy Adam T.、Ding Feiqing、Greimel Peter、Hirabayashi Yoshio、Kamiguchi Hiroyuki、Ito Yukishige	４．巻 18
２．論文標題 Systematic synthesis of novel phosphoglycolipid analogues as potential agonists of GPR55	５．発行年 2020年
３．雑誌名 Organic & Biomolecular Chemistry	６．最初と最後の頁 8467～8473
掲載論文のDOI（デジタルオブジェクト識別子） 10.1039/D0OB01756F	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計4件（うち招待講演　0件／うち国際学会　2件）

1．発表者名
Guy Adam T, Inoue Mariko, Hirabayashi Yoshio, Yamamoto Kei, Murakami Makoto, Kamiguchi Hiroyuki

2．発表標題
Radial glial secreted phospholipase A2 group V modulates LysoPtdGlc/GPR55-mediated axon guidance of nociceptive sensory afferents

3．学会等名
Japan Neuroscience Society Meeting "Neuro2021"

4．発表年
2021年

1．発表者名
Guy Adam T., Inoue Mariko, Yamamoto Kei, Murakami Makoto, Kamiguchi Hiroyuki

2．発表標題
Radial glial sPLA2 V enzyme modulates LysoPtdGlc/GPR55-mediated axon guidance of nociceptive sensory afferents

3．学会等名
Cold Spring Harbor Laboratory meeting (virtual) "Molecular Mechanisms of Neuronal Connectivity"（国際学会）

4．発表年
2020年

1．発表者名
Guy Adam T., Inoue Mariko, Hirabayashi Yoshio, Hanafusa Kei, Yanagida Mitsuaki, Iwabuchi Kazuhisa, Yamamoto Kei, Murakami Makoto, Kamiguchi Hiroyuki

2．発表標題
Radial glial secreted phospholipase A2 group V enzyme modulates LysoPtdGlc/GPR55-mediated nociceptive axon guidance in spinal cord development

3．学会等名
Cold Spring Harbor Laboratory meeting (virtual) "Molecular Mechanisms of Neuronal Connectivity"（国際学会）

4．発表年
2022年

1．発表者名
Guy Adam T., Inoue Mariko, Hirabayashi Yoshio, Hanafusa Kei, Yanagida Mitsuaki, Iwabuchi Kazuhisa, Yamamoto Kei, Murakami Makoto, Kamiguchi Hiroyuki

2．発表標題
Secreted phospholipas A2 group V expressed by radial glia modulated LysoPtdGlc/GPR55-mediated nociceptive axon guidance in spinal cord development

3．学会等名
Japan Neuroscience Society Meeting "Neuro2022"

4．発表年
2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関