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様式１０２

学位論文の内容の要旨

Summary of the Substance of Dissertation

専攻 Major Field	分子情報制御医学	部門 Department	分子細胞医学
学籍番号 Student No.	13D731	氏名 Name	ｲｯﾌｧｯﾄ ｱﾗ ｿﾆｱ ﾗﾊﾏﾝ
論文題目 Thesis Subject	Calcium-dependent generation of *N*-acylethanolamines and lysophosphatidic acids by glycerophosphodiesterase GDE7		

Introduction:

N-Acylethanolamines (NAEs) are ethanolamides of long-chain fatty acids. They include anandamide (an endocannabinoid), palmitoylethanolamide (an analgesic and anti-inflammatory substance), and oleoylethanolamide (an appetite-suppressor). In animal tissues, they are synthesized from a unique phospholipid, *N*-acyl-phosphatidylethanolamine (NAPE), in one-step reaction by NAPE-specific phospholipase D or through multi-step pathways via *N*-acyl-lysophosphatidylethanolamine (lysoNAPE). LysoNAPE is hydrolyzed by lysophospholipase D (lysoPLD)-type enzyme(s) to generate NAEs. Lysophosphatidic acid (LPA), a well-known lipid mediator, is also generated as another product of this reaction.

The glycerophosphodiesterase (GDE) family is a protein family characterized by highly conserved GDE domain and has seven members (GDE1-7) in mammals. GDE4 was recently shown to have lysoNAPE-hydrolyzing lysoPLD activity to generate NAEs. In the present study, I examined GDE7, together with GDE4, whether it contributes to the NAE and LPA biosynthesis by its lysoPLD activity.

Methods:

Recombinant GDE7 and GDE4 from humans and mice were overexpressed in HEK293 cells, and the membrane fractions or whole cells were subjected to Western blotting, enzyme assay, and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The tissue distributions of mRNAs for GDE7 and GDE4 in humans and mice were examined by RT-PCR. Tissue homogenates were prepared from male C57BL/6 mice and used for enzyme assays.

Results:

Following confirmation of the expression of recombinant GDE7 and GDE4 by Western blotting,

both the proteins were revealed to have lysoPLD activities hydrolyzing various lysoNAPEs with different *N*-acyl species as well as the 1-alkenyl analog, *N*-acyl-lysoplasmalogen, to generate their corresponding NAEs and LPAs. GDE7 also hydrolyzed lysophosphatidylcholine to produce LPA. The activity of GDE7 was stimulated by micromolar concentrations of Ca^{2+}, but not by 2 mM Mg^{2+}. In contrast, GDE4 activity was increased by 2 mM Mg^{2+}, but not by 2 mM Ca^{2+}. LC-MS/MS analyses showed that most of NAE and LPA species were increased in GDE7-overexpressing HEK293 cells. Although GDE7 and GDE4 mRNAs were widely distributed in various tissues of humans and mice, GDE7 mRNA was abundant in kidney and GDE4 was highly expressed in brain and testis. In consistence with high expression levels of GDE7 in mouse kidney, EGTA, a calcium-specific chelator, decreased NAE-forming activity of mouse kidney homogenate.

Discussion:

I showed for the first time that GDE7 has a lysoPLD activity toward lysoNAPE. The activation of GDE7 by submicromolar concentration of Ca^{2+} suggested that GDE7 could produce NAE and LPA in response to physiological stimuli to increase intracellular Ca^{2+} levels. The decrease in the lysoPLD activity of mouse kidney homogenate by EGTA suggested that the lysoPLD activity in this organ may be at least partially attributed to GDE7.

These results suggested that GDE7 is a novel Ca^{2+}-dependent lysoPLD, which is involved in the biosynthesis of NAEs and LPAs.

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（備考）論文要旨は，日本語で１，５００字以内にまとめてください。
(Recital) Sum up the within １５００ letters.