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様式１０２

学位論文の内容の要旨

専　攻	医学	部　門 (平成27年度以前入学者のみ記入)	
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論文題目	Involvement of the γ Isoform of $cPLA_2$ in the Biosynthesis of Bioactive *N*-Acylethanolamines		

Introduction:

Long-chain fatty acyl ethanolamides (*N*-acylethanolamines, NAEs) are lipid molecules widely present in animal and plant tissues, and exhibit various biological activities. For example, arachidonylethanolamide (anandamide) acts as an endogenous ligand of cannabinoid receptors, while other NAEs, such as palmitylethanolamide and oleylethanolamide, show analgesic, anti-inflammatory, and appetite-suppressing effects through other receptors. In mammalian tissues, NAEs are biosynthesized from membrane phospholipids in two enzyme reactions. In the first reaction, *N*-acyl-phosphatidylethanolamines (NAPEs), a class of phospholipids with three fatty acyl chains, are produced by the catalysis by *N*-acyltransferase. In the second reaction, NAEs are directly produced from NAPEs by the phospholipase D-type enzyme NAPE-PLD or by a combination of two or more hydrolases via *N*-acyl-lysophosphatidylethanolamines (lysoNAPEs).

The cytosolic phospholipase A_2 ($cPLA_2$) family is a group of phospholipid-metabolizing enzymes, consisting of six isoforms (α, β, γ, δ, ε, and ζ). Among them, the ε isoform ($cPLA_2ε$, also referred to as PLA2G4E) is known to function as a Ca^{2+}-dependent *N*-acyltransferase to produce NAPEs. However, it remained unclear whether other five isoforms are also involved in the biosynthesis of NAEs.

Aim:

In this study, we investigated whether the isoforms (α, β, γ, δ, and ζ) other than ε in the $cPLA_2$ family could be involved in the biosynthesis of NAEs using cells overexpressing recombinant enzymes as well as purified enzymes.

Methods and Results:

I constructed vectors expressing each of the six isoforms of mouse $cPLA_2$ and transiently overexpressed recombinant isoform proteins alone or in combination in HEK293 cells. These cells were metabolically labeled with [^{14}C]ethanolamine, followed by the stimulation with Ca^{2+} ionophore. Radiolabeled lipids were then extracted, separated by thin-layer chromatography, and quantified.

Firstly, when the cells overexpressing one of the isoforms were analyzed, the increase in the production of [^{14}C]NAPE was observed only with the ε isoform-expressing cells. Secondly, when the cells co-expressing ε and one of the other isoforms were analyzed, the increase in [^{14}C]lysoNAPE and [^{14}C]NAE was seen with the combination of ε and γ isoforms. Furthermore, the purified preparation of recombinant γ isoform hydrolyzed not only NAPE to lysoNAPE (by phospholipase A_1/A_2 activity), but also lysoNAPE to glycerophospho-*N*-acylethanolamine (by lysophospholipase activity), which could be further hydrolyzed to NAE by another enzyme, glycerophosphodiesterase 1.

Conclusion:

These results suggested that five isoforms (α, β, γ, δ, and ζ) other than ε do not have *N*-acyltransferase activity but the γ isoform is involved in the biosynthesis of NAE by its phospholipase A_1/A_2 and lysophospholipase activities.

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