BIOSYNTHESIS OF PLASMALOGENS FROM ALKYL- AND ALKYL-ACYL-GLYCEROPHOSPHORYL ETHANOLAMINE IN THE RAT BRAIN

F. PALTAUF

Institut für physiologische Chemie, Universität Graz, Austria

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1. Introduction

Results obtained from experiments *in vivo* [1–10] and *in vitro* [11] indicate that *O*-alk-1-enyl glycerol lipids (plasmalogens) are derived from *O*-alkyl glycerol lipids. However, the question has not been answered about the nature of the immediate precursor of plasmalogens. Desaturation of the alkyl glycerol bond to an alk-1-enyl glycerol bond could possibly occur at any stage after the alkyl glycerol linkage has been formed from dihydroxy acetone phosphate and a long chain alcohol [12].

The conversion *in vivo* of 1-*O*-(9,10-$^3H_2$ octadecyl) *sn*-glycerol 3-phosphoryl (2-^{14}C) ethanolamine and its 2-octadecenoyl derivative respectively into doubly labelled plasmalogens without change of the $^3H/^{14}C$ ratio allows us to conclude that alkyl acyl GPE is the direct plasmalogen precursor in the brain. It is further suggested that this conversion represents the main route of degradation of brain alkyl glycerol lipids.

2. Materials and methods

The chemical synthesis of 1-*O*-(9,10-$^3H_2$ octadecyl) 2-octadecenoyl *sn*-glycerol 3-phosphoryl (2-^{14}CO ethanolamine (alkyl acyl GPE) and the 2-lyso derivative (alkyl GPE) will be described elsewhere. The specific radioactivity of the substrate was 1.21×10^6 cpm/μmole ^3H and 0.43×10^6 cpm/μmole ^{14}C. Purity of the substances was checked by thinlayer chromatography employing four different solvent systems. The alkyl acyl GPE had the correct ether:ester:phosphorus: ethanolamine ratio; this was estimated from the specific radioactivities of the alkyl and ethanolamine residues, P analysis and determination of the acyl ester linkages.

1 μmole of the respective substrate was dissolved in 100 μl of 14% Triton WR 1339. 20 μl of this solution were injected into the brains of 14–16 day old Wistar rats each. The animals were killed by decapitation at the times indicated in table 1. Lipids were extracted from the pooled brains and fractionated into individual classes as described earlier [10]. Calculation of the $^3H/^{14}C$ ratio of brain alk-1-enyl-acyl GPE is based on the ^3H radioactivity of aldehydes and ^{14}C radioactivity of lyso phosphatidylethanolamine which are formed by acid catalyzed hydrolysis of the isolated phosphatidylethanolamine (PE) fraction. In order to liberate aldehydes from alk-1-enyl acyl GPE an aliquot of the pure PE fraction was spotted on a 20 × 20 cm TLC plate (coated with 0.5 mm silica gel H) 3 cm from the edges. The plate was then exposed to HCl fumes for 10 min [13]; after HCl had been removed by a stream of air the plate was developed in the first direction with petroleum ether (b.p. 40–60°)-ether (95/5, v/v) and in the second direction with chloroform-methanol-ammonia (100/50/12, v/v/v). Prior to the second chromatography a narrow (1.5 cm) strip of silica gel had been removed at the left edge of the plate (15 cm long, from the top) to prevent aldehydes from migrating with the second developing solvent. Spots corresponding to aldehydes, 1-lyso PE and unchanged PE were visualized with iodine vapor and scraped into counting vials for determination of radioactivity. Recovery of radioactivity was determined by the use of a mixture of synthetic ^3H labelled aldehydes and $^3H/^{14}C$ labelled 1-alkyl GPE which were subjected to the reaction and separation procedure outlined above. Part of the brain PE was subjected to hydrolysis with 90% acetic acid [14] followed by mild alkal-

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ine hydrolysis [15]. The acid and alkali resistant 1-alkyl GPE was isolated by preparative TLC and the $^3H/^{14}C$ ratio was determined.

3. Results and discussion

After intracerebral injection of 1-*O*-(9,10-$^3H_2$-octadecyl) 2-octadecenoyl *sn*-glycerol 3-phosphoryl (2-^{14}C) ethanolamine (alkyl acyl GPE) as well as of the 2-lyso derivative (alkyl GPE) the aldehydrogenic chains and the ethanolamine part of brain alk-1-enyl acyl GPE became labelled with ^3H and ^{14}C respectively (table 1), the $^3H/^{14}C$ ratio being equal to that of alkyl acyl GPE isolated from brain lipids.

The more rapid incorporation of radioactivity into plasmalogens in experiments 3 and 4, when alkyl GPE was the substrate, as compared to experiments 1 and 2, when alkyl acyl GPE was administered, is most likely due to a facilitated transport of the lyso derivative through cell membranes. It has been shown previously that 1,2-dialkyl GPE, which is resistant to phospholipases A and therefore cannot be converted to a lyso-derivative, penetrates the epithelial cell of the intestinal mucosa to a very limited degree [16], whereas the 1-alkyl GPE is easily absorbed (own results, unpublished).

If the same pattern applies to brain cell membranes, then the alkyl GPE would reach the interior of the cell, where acylation and plasmalogen formation occur, more rapidly than does the alkyl acyl derivative.

No radioactivity was associated with acid labile groups of the lyso PE fraction in experiments 3 and 4; no incorporation of radioactivity into lyso PE was observed in experiments 1 and 2. Taking all this into account is seems justified to assume that the intact alkyl acyl GPE is the immediate plasmalogen precursor in the brain.

The $^3H/^{14}C$ ratio of plasmalogens in experiments 2 and 4 was essentially equal to that of the administered substrates, whereas in experiment 3 it was increased. Whatever the reason for this as yet unexplained phenomenon might be, it does not speak against the proposed concept of a direct conversion of alkyl acyl- to an alk-1-enyl acyl GPE, since the same increase of the $^3H/^{14}C$ ratio was observed in alkyl acyl GPE isolated from brain lipids in this experiment.

In neither experiments was ^{14}C incorporated into lecithin indicating the absence of an enzyme system that would methylate alkyl acyl or alk-1-enyl acyl GPE. Only traces of ^3H were recovered from phospholipid fatty acids after mild alkaline hydrolysis; however, approximately 2.5% in experiment 3 and 15% in experiment 4 of the total ^3H radioactivity of brain lipids were recovered from cerebrosides. This is in agreement with the observation of Segal and Wysocki [17] that fatty acids produced by degradation of brain plasmalogens are predominantly incorporated into cerebroside fatty acids.

Pfleger et al. [18] found that in brain tissue homo-

Table 1

Biosynthesis of plasmalogens from 1-*O*-(9,10-$^3H_2$ octadecyl) 2-octadecenoyl *sn*-glycerol 3-phosphoryl (2-^{14}C) ethanolamine and 1-*O*-(9,10-$^3H_2$ octadecyl) *sn*-glycerol 3-phosphoryl (2-^{14}C) ethanolamine in the rat brain.

Exp.	Substrate administered[a]	time (hours)	% of administered[b] ^3H radioactivity recovered in brain lipids	% of total ^3H radioactivity recovered with phosphatidyl-ethanolamine (PE)[c]	% of ^3H radioactivity in PE associated with alk-1-enyl acyl GPE[d]	$^3H/^{14}C$ ratio of alkyl acyl GPE isolated from PE
1	alkyl acyl GPE	3	16 [3.0][e]	97 [3.0]	0	–
2	alkyl acyl GPE	24	10 [3.2]	90 [3.1]	13 [3.1]	[3.1]
3	alkyl GPE	3	21 [3.7]	37 [4.1]	18 [4.1]	[4.1]
4	alkyl GPE	15	30 [4.3]	50 [3.1]	43 [3.0]	[3.1]

[a] $^3H/^{14}C$ ratio of administered substrates was [2.9].
[b] With some animals losses occurred during injection.
[c] The phosphatidylethanolamine fraction comprises 1,2-diacyl-, 1-alk-1-enyl 2-acyl- and 1-alkyl 2-acyl GPE.
[d] $^3H/^{14}C$ ratio was calculated as described in Materials and methods.
[e] $^3H/^{14}C$ ratios are in brackets.

genates the activity of the alkyl glycerol cleaving enzyme was low. This raises the question as to the mechanism of alkyl glycerol lipid degradation in the brain. The identity of labelled fatty acid distribution in the experiments reported here and in experiments where labelled plasmalogens were injected into rat brains [17] as well as the lag of production of fatty acids from alkyl glycerol lipids (experiments 3 and 4) behind the rate of plasmalogen formation might indicate an alternative pathway of alkyl glycerol phospholipid degradation. This would imply desaturation of the alkyl glycerol bond rather than its oxidative cleavage [19], giving rise to an alk-1-enyl glycerol lipid which is subsequently degraded by a plasmalogenase [20]

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