REVERSIBILITY OF ETHANOLAMINE AND CHOLINE PHOSPHOTRANSFERASES (EC 2.7.8.1 AND EC 2.7.8.2) IN RAT BRAIN MICROSOMES WITH LABELLED ALKYLACYLGLYCEROLS

Gianfrancesco GORACCI*, Lloyd A. HORROCKS** and Giuseppe PORCELLATI*
Department of Physiological Chemistry, The Ohio State University, Columbus, Ohio 43210, USA

Received 10 June 1977

1. Introduction

While using 1-[^{3}H]alkyl-2-linoleoyl-*sn*-glycerol as the labelled substrate for the synthesis of 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholines (alkylacyl-GPC) by the cholinephosphotransferase in rat brain microsomes, a significant labelling of 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamines (alkylacyl-GPE) was observed without any addition of CDP-ethanolamine. A possible explanation is given in fig.1, namely that the reverse reaction of rat brain ethanolamine-phosphotransferase may degrade endogenous microsomal ethanolamine phosphoglycerides. In the present paper we report the enhancement of the reverse reaction of both phosphotransferases by addition of CMP, a product of phosphotransferase reactions.

2. Experimental

Sprague–Dawley rats (6–8 weeks of age) were decapitated after ether anesthesia. The brains were immediately removed and homogenized in 0.32 M sucrose. The microsomal fraction was obtained as previously described [1] and then resuspended in ice-cold sucrose just before the incubation.

*Permanent address: Istituto di Chimica Biologica, Facoltà di Medicina, Università di Perugia, Policlinico Monteluce, 06100 Perugia, Italy

**Correspondence to: Dr Lloyd A. Horrocks, Dept. of Physiol. Chem., 1645 Neil Avenue, Columbus, Ohio 43210, USA

After saponification of rat fish *(Chimera monstrosa)* liver (Western Chemical Industries Ltd., Vancouver, Canada), 1-*O*-alkyl-*sn*-glycerols were separated by preparative thin-layer chromatography, and then reduced with tritium at New England Nuclear Laboratories, Boston, Mass. The product of the reaction consisted of a mixture of 1-*O*-[^{3}H]octadecyl-*sn*-glycerol (63%) and 1-*O*-[^{3}H]hexadecyl-*sn*-glycerol (28%). The labelled alkylglycerols (1.17 mg) had a specific radioactivity of 11.0 Ci/mmol. They were mixed with unlabelled alkylglycerols that were prepared from the same source and hydrogenated with PtO_2 as catalyst. The [^{3}H]alkylglycerols were acylated with linoleoyl chloride (Sigma Chemical Co., St. Louis, Mo.) in the presence of pyridine as described by Chacko and Hanahan [2]. The 1-*O*-[^{3}H]alkyl-2,3-dilinoleoyl-*sn*-glycerols obtained as a product of this reaction were isolated and purified with column chromatography on Unisil silicic acid (Clarkson Chemical Co., Williamsport, Pa.), according to Carrol and Serdarevich [3]. This product was incubated with pancreatic lipase (Steapsin, Nutritional Biochemical Corp., Cleveland, Ohio) as described by Radominska-Pyrek and Horrocks [4]. The 1-*O*-[^{3}H]alkyl-2-linoleoyl-*sn*-glycerols were then purified by column [5] and thin-layer [4] chromatography. The amount of labelled alkylacylglycerols was measured from the content of the ester bonds by the method of Stern and Shapiro [6] using methyl palmitate as a standard. We obtained 240 μmol of 1-*O*-[^{3}H]alkyl-2-linoleoyl-*sn*-glycerols with a specific radioactivity of 0.517 Ci/mol. Just before use, an 8 mM alkylacylglycerol emulsion was prepared by sonication [4] in a convenient volume of 50 mM

Tris–HCl, pH 7.6, containing 0.5% Tween 20. Incubations were carried out as reported in tables 1 and 2. The reaction was stopped by adding 4.5 ml of chloroform–methanol 2:1 (v/v) and the lipids were extracted [7]. Protein contents were determined according to Lowry et al. [8]. The isolation of lipids was carried out by thin-layer chromatography as described previously [4] except chloroform–methanol–H_2O, 65:25:4 (v/v/v) was used as the solvent for the first dimension. The spots corresponding to acid-stable ethanolamine and choline phosphoglycerides were scraped directly into counting vials. Distilled water (3 ml) and 10 ml Instagel (Packard, Downers Grove, Ill.) were added and the mixture was counted [9].

3. Results

Increasing amounts of [^{3}H]alkylacylglycerols were incorporated into choline phosphoglyceride and ethanolamine phosphoglyceride with increasing time (table 1). Although no CDP-ethanolamine was added, the proportion of radioactivity in the ethanolamine phosphoglyceride was about 3% of the total incorporated. The presence of absorbed CDP-ethanolamine on the microsomes is unlikely because ^{3}H in the ethanolamine phosphoglyceride was not detected at 2 min, but then labelled ethanolamine phosphoglyceride increased with time. If the labelled ethanolamine phosphoglycerides were due to the liberation of CDP-ethanolamine by CMP (fig.1), then the addition of CMP to the incubation medium should increase the proportion of radioactivity in the ethanolamine phosphoglyceride. In the experiment reported in table 2, this proportion was increased from 1.5 to 2.1%. Nearly 9% of the radioactivity was incorporated into choline phosphoglyceride when CDP-ethanolamine was the added nucleotide (table 2). This proportion was increased to 31% by inclusion of CMP in the incubation medium.

Table 1
Incorporation of [^{3}H]alkylacylglycerols into the alkylacyl-GPC and alkylacyl-GPE of rat brain microsomes as a function of time in the presence of CDP-choline

Incubation (min)	Incorporation (nmol/mg protein) into	
	Alkylacyl-GPC	Alkylacyl-GPE
2	2.70	N.D.
4	5.22	0.17
8	12.0	0.31
15	15.9	0.43
30	29.8	0.94

Incubation mixture: Tris–HCl, pH 8.0 (58 mM); $MgCl_2$ (20 mM); dithiothreitol (0.08 mM); CDP-choline (1.066 mM); 1-[^{3}H]alkyl-2-linoleoyl-*sn*-glycerols (1.33 mM); Tween 20 (0.008%); brain microsomal protein (0.9 mg). Final volume: 0.3 ml. Temperature: 39°C.
N.D., not detected.

[^{3}H]alkylacylglycerol + CDP-choline → [^{3}H]alkylacyl-GPC + CMP (1)

CMP + endogenous EPG → CDP-ethanolamine + diglycerides (2)

CDP-ethanolamine + [^{3}H]alkylacylglycerol → [^{3}H]alkylacyl-GPE + CMP (3)

CMP + endogenous CPG → CDP-choline + diglyceride (4)

Fig.1. Enzymic steps for production of [^{3}H]alkylacyl-GPE from [^{3}H]alkylacylglycerol and CDP-choline (reactions 1–3), and for production of [^{3}H]alkylacyl-GPC from [^{3}H]alkylacylglycerol and CDP-ethanolamine (reactions 3,4 and 1). Reactions 1 and 4 are catalyzed by choline phosphotransferase. Abbreviations are: CPG, choline phosphoglyceride and EPG, ethanolamine phosphoglyceride.

4. Discussion

Brain choline and ethanolamine phosphotransferases seem to be reversible as judged by results of CMP addition (table 2). Reversibility of both phosphotransferases has been described in other tissues [10–15]. Although the brain enzymes are quite active [4,7,16–22], they were thought to be irreversible because the specific radioactivities of the phosphoglycerides do not equilibrate with specific radioactivities of the CDP-choline or CDP-ethanolamine in vivo [23,24]. A competitive inhibition by CMP of the choline phosphotransferase from chicken brain has been described [18].

The incorporation of labelled alkyl groups into choline phosphoglyceride in the presence of CDP-ethanolamine and into ethanolamine phosphoglyceride in the presence of CDP-choline can be explained by the reactions in fig.1. Snyder et al. [25] reported similar results after incubation of labelled alkylacylglycerophosphates with microsomes from preputial gland tumors. Similar reverse

Table 2
The effect of CMP addition on the incorporation of [^{3}H]alkylacylglycerol into alkylacyl-GPC and alkylacyl-GPE of rat brain microsomes

Addition	Incorporation (nmol/mg protein/h) into	
	Alkylacyl-GPC	Alkylacyl-GPE
CDP-choline	43.4	0.69
CDP-choline + CMP	43.1	0.95
CDP-ethanolamine	3.48	36.3
CDP-ethanolamine + CMP	8.21	18.5

Incubation mixture: Tris–HCl, pH 8.0 (58.0 mM); $MgCl_2$ (20 mM) or $MnCl_2$ (10 mM); dithiothreitol (0.08 mM); 1-[^{3}H]alkyl-2-linoleoyl-*sn*-glycerol (1.07 mM); Tween 20 (0.008%); brain microsomal protein (0.8 mg). Final volume: 0.3 ml. Temperature: 39°C. Incubation time: 30 min. CDP-choline, CDP-ethanolamine or CMP were added (1 mM) as specified. $MgCl_2$ or $MnCl_2$ were used with CDP-choline and CDP-ethanolamine, respectively.

reactions may explain part of the labelling of plasmalogens in previous experiments carried out with alkylacylglycerols and labelled nucleotides [4,20–22] and may account for the phosphoethanolamine exchange proposed by Wykle and Lockmiller [26].

Diglycerides are released from brain phosphoglycerides during ischemic and electroconvulsive states [27,28] and can be further degraded by a lipase [29]. The release could be mediated by a phospholipase C [30,31] with release of the phosphorylated base or by reversal of a phosphotransferase with release of the choline phosphoglyceride nucleotide. The latter may be responsible for the effects found in our experiments.

Acknowledgements

This research was supported in part by Research Grants NS-08291 and NS-10165 from the US Public Health Service.

References

[1] Gaiti, A., Goracci, G., De Medio, G. E. and Porcellati, G. (1972) FEBS Lett. 27, 116–120.
[2] Chacko, D. G. and Hanahan, J. D. (1968) Biochim. Biophys. Acta. 164, 252–271.
[3] Carrol, K. K. and Serdarevich, B. (1967) Lipid Chromatographic Analysis (Dekker, Inc. New York), Vol. 1, 205–207.
[4] Radominska-Pyrek, A. and Horrocks, L. A. (1972) J. Lipid Res. 13, 580–587.
[5] Kiyasu, Y. and Kennedy, E. P. (1960) J. Biol. Chem. 235, 2590–2594.
[6] Stern, J. and Shapiro, B. (1953) J. Clin. Pathol. 6, 158–165.
[7] Binaglia, L., Goracci, G., Francescangeli, E. and Porcellati, G. (1974) Lipids 9, 738–747.
[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[9] Porcellati, G. and Goracci, G. (1976) Lipids, (Raven Press, New York), Vol. 1, 203–213.
[10] Weiss, S. B., Smith, S. W. and Kennedy, E. P. (1958) J. Biol. Chem. 231, 53–64.
[11] Bjørnstad, P. and Bremer, J. (1966) J. Lipid Res. 7, 38–45.
[12] Kanoh, H. and Ohno, K. (1973) Biochim. Biophys. Acta. 306, 203–217.
[13] Kanoh, H. and Ohno, K. (1973) Biochim. Biophys. Acta. 326, 17–25.
[14] Kanoh, H. and Ohno, K. (1975) Biochim. Biophys. Acta. 380, 199–207.
[15] Sarzala, M. G. and Van Golde, L. M. G. (1976) Biochim. Biophys. Acta. 441, 423–432.
[16] Porcellati, G., Biasion, M. G. and Pirotta, M. (1970) Lipids 5, 734–742.
[17] Ansell, G. B. and Metcalfe, R. F. (1971) J. Neurochem. 18, 647–665.
[18] Freysz, L. and Mandel, P. (1974) FEBS Lett. 40, 110–113.
[19] Binaglia, L., Goracci, G., Porcellati, G., Roberti, R. and Woelk, H. (1973) J. Neurochem. 21, 1067–1082.
[20] Roberti, R., Binaglia, L., Francescangeli, E., Goracci, G. and Porcellati, G. (1975) Lipids 10, 121–127.

[21] Radominska-Pyrek, A., Strosznajder, J., Dabrowiecki, Z., Chojnacki, T. and Horrocks, L. A. (1976) J. Lipid Res. 17, 657–662.
[22] Radominska-Pyrek, A., Strosznajder, J., Dabrowiecki, J., Goracci, G., Chojnacki, T. and Horrocks, L. A. (1977) J. Lipid Res. 18, 53–58.
[23] Ansell, G. B. and Spanner, S. (1968) Biochem. J. 110, 201–206.
[24] Ansell, G. B. and Spanner, S. (1967) J. Neurochem. 14, 873–885.
[25] Snyder, F., Blank, M. L. and Malone, B. (1970) J. Biol. Chem. 245, 4016–4018.
[26] Wykle, R. L. and Lockmiller, J. M. S. (1975) Biochim. Biophys. Acta. 380, 291–298.
[27] Alvedano, M. I. and Bazan, N. G. (1975) J. Neurochem. 25, 919–920.
[28] Bazan, N. G. (1970) Biochim. Biophys. Acta. 218, 1–10.
[29] Cabot, M. C. and Gatt, S. (1976) Biochim. Biophys. Acta. 431, 105–115.
[30] Williams, D. J., Spanner, S. and Ansell, G. B. (1973) Biochem. Soc. Trans. 1, 466–467.
[31] Abra, R. M. and Quinn, P. J. (1975) Biochim. Biophys. Acta. 436–441.