HIGH CONTENT OF DOCOSAHEXAENOATE AND OF TOTAL DIACYLGLYCEROL IN RETINA (*)

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Received June 15,1972

SUMMARY. The toad retina diacylglycerol level is eight times that of the brain; this molecule in the former tissue is also more unsaturated. The difference is mainly due to a very high content of docosahexaenoate (42%): arachidonate represents only about 7%. On the contrary the diacylglycerides in brain contain 3 and 25% respectively of those fatty acids. It is suggested that highly unsaturated diacylglycerides play a unique and very active precursor role of phosphoglycerides in the retina.

INTRODUCTION. Excitable biomembranes contain polar lipids with large amounts of long-chain highly unsaturated acyl components (1-6). The phospholipids of the rod outer segments of the retina are also very richly endowed with polyenoic fatty acids (2-5).

Only few studies have been published on the content and structure of acyl glycerols in the nervous tissue (7-9). Attention has not been paid to the level, composition and metabolic significance of diacylglycerols of the retina. In the present communication we report a high amount of docosahexaenoic acid and of total diglycerides in the toad retina.

METHODS. Brains and retinas were obtained with normal artificial laboratory light after decapitation from the toad Bufo arenarum, Hensel.

The lipids were extracted (10), filtered through a sintered glass filter under a nitrogen stream and the diacylglycerols isolated by a one step development on gradient-thickness thin layer chromatography (11-12). The development of the chromatogram was done with hexane-diethyl ether-acetic acid 40-60-2,5 (V/V/V) at 4°C.

(*) Reprint request to N.G. Bazán.
(**) In partial fulfilment of the requeriments for Doctorate degree in Biochemistry.

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Methanolysis was carried out during 45 minutes at 100°C (8, 13).

For the separation and quantification of the individual fatty acid methyl esters a Varian Aerograph Model 1700 Gas Chromatograph with a hydrogen flame ionization detector was used (injector port 225°C; oven 200°C; detector 210°C and carrier gas (N_2) 25 ml/ min). This was equiped with a coiled stainless steel column (inside diameter 2,3 mm; length 2.2 meters) packed with 6% diethylene glycol succinate on Diatoport S, 80-100 mesh (Hewlett-Packard). The runs were carried out with nonadecanoate methyl ester as internal standard after having determined the endogenous content of that fatty acid in the samples. The identification of the individual fatty acids was performed as described elsewhere (8).

RESULTS AND DICUSSION. The diacylglycerols studied here are a mixture of the 1,2 and 1,3 isomers. They have not been subfractionated because we have obtained evidence showing that at least most of the native pool of diacylglycerols in the neural tissue is not a mixture of both isomers (14).

In table I a striking difference can be seen in the level of total diacylglycerides of retina and brain. The former tissue has a pool about eight times larger than that of the brain and contains about (42%) of its total fatty acids as docosahexaenoate while in the brain this molecule amounts only to 3%. Docosapentaenoic acid also appear to be more concentrated in the retina (data not shown). Arachidonate on the contrary represents 27% of the total acyl groups from the brain diglycerides and only 7% in the retina. The ratio between total saturated to unsaturated components indicates a predominance of the later in both parts of the central nervous system surveyed and is lower in retina than in brain.

There is evidence that the diacylglycerols are active metabolites in the central nervous system (15); however the data reported here represent, to the best of our knowledge, the first study on the fatty acid composition of the diacylglycerols of the retina. The content of arachidonate and docosahexaenoate in brain diglycerides is similar to mouse brain diglycerides content in that the former fatty acid is much greater than the later (7).

Table I

Level, unsaturation and major unsaturated fatty acids in diacylglycerides of brain and retina.

Total diacylglycerols, 22:6 ω 3 and 20:4 ω 6 content represent amounts of methyl esters as determined by gas-liquid chromatography using nonadecanoate methyl ester as an internal standard. Figures for these molecules indicate mean μg/100 mg of tissue dry weight ± S.E.M. Dry weight was determined in the residues after the lipid extraction. The number between parentheses indicates the number of samples. Each retina sample contained ten toad retinas and each brain sample contained from five to fifteen toad brains. From each sample at least two analysis were performed.

Parameter	Retina	Brain
Total diacylglycerols	404 ± 51.00 (9)	56,00 ± 13,30 (13)
22:6 ω 3	185 ± 35.00 (6)	2,26 ± 0.74 (4)
20:4 ω 6	25 ± 3.82 (6)	15.93 ± 5.97 (4)
Ratio total saturated to total unsaturated acyl components	0.69 ± 0.05 (9)	0.82 ± 0.14 (5).

The high level in docosahexaenoate in toad retinal diglycerides is one of the highest reported values for a particular lipid class in any tissue. Only in etanolamine phosphatides from the electric organ of the Torpedo marmorata a slightly higher content (43%) of 22:6 was found (3). Ethanolamine, serine and choline phosphatides from the bovine rod outer segments contain 29.3, 16.8 and 16.1% respectively of the docosahexaenoate (16), while in the total lipids from the same portion of the bovine retina there is 34,3% (4). Very recently the presence of triglycerides very rich in docosahexaenoate was reported in sand trout retina (17).

The diacylglycerols are precursors of phosphatides and triacylglycerols (18). A requeriment of exogenously added diglyce-

rides have been demostrated for the biosynthesis of brain (18-20) and retina phosphatides (21). Further it was shown that phosphatidylcholine formation was increased when certain D - α, β-diglycerides were added, diolein being most actively incorporated (19-20).

The retina phospholipids fatty acid composition resembles retina diglycerides, with some difference: the later are richer in docosahexaenoate, while the analogy is not so obvious when a comparison is made between the composition of toad brain phosphatides with diglycerides (14). It is also interesting to note that a tendency to compensation of unsaturation is seen in both neural pool of diacylglycerides.

The marked differences in these diglyceride pools likely indicates that in the retina they play a unique and very active precursor role of phospholipids.

ACKNOWLEDGEMENTS. This work was supported in part by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina) and by a Research Fellowship to M.I.A. from the same institution.

REFERENCES

1. Kishimoto, Y., Agranoff, B.W., Radin, N.S. and Burton, R.M., J. Neurochem. 16 (1969) 397.
2. Nielsen, M.C., Fleischer, S. and Mc Connell, D.G., Biochim. Biophys. Acta 211 (1970) 10.
3. Breckeridge, W.C. and Vincendon, G., Compt. Rend. 273 (1971) 1337.
4. Borggreven, J.M.P.M., Dalman, F.J.L. and Bonting, S.L., Biochim. Biophys. Acta 202 (1970) 374-381.
5. Anderson, R.E., Feldman, L.S., Feldman, G.L. Biochim. Biophis. Acta. 202 (1970) 367-373.
6. Rouser, G. and Yamamoto, A. in Handbook of Neurochemistry. I Plenum. Press, New York, p 121 (1969).
7. Sun, G.Y., J. Neurochem. 17 (1970) 445.
8. Bazán, N.G., Biochim. Biophys. Acta 218 (1970) 1.
9. Rowe, C.E., J. Neurochem. 16 (1969) 205.
10. Folch Pi J., Lees, M. and Sloane-Stanley, G.H.J. Biol. Chem. 226 (1957) 497.
11. Bazán, N.G., and Joel, C.D., J. Lipid Res. 11 (1970) 42.
12. Bazán, N.G., and Cellik, S., Anal. Biochim 45 (1972) 309.
13. Morrison, W.R., and Smith, L.M., J. Lipid. Res. 5 (1964)600.

14. Bazán, N.G., and Aveldaño, M.I., Unpublished.

15. Sun, G.Y., and Horrocks, L.A., J. Neurochem. 16 (1969) 181.

16. Poincelot, R.P., and Abrahamson, E.W., Biochim. Biophys. Acta. 202 (1970) 383.

17. Nicol, J.A.C., Arnott, H.J., Mizuno, G.R., Ellison, E.D., and Chipanlt J.R., Lipids 7 (1972) 171.

18. Rossiter, R.J. In Metabolic Pathways. II Ed Greenberg, D.M. Academic Press, London p. 69 (1968).

19. Strickland, K.P., Subrahmayan, D., Pritchard, E.T., Thompson, W. and Rossiter, R.J. Biochim. J. 87 (1963) 128.

20. Mc Caman, R.E. and Cook, K., J. Biol. Chem. 241 (1966) 3390.

21. Swartz, J.G. and Mitchell, J.E., J. Lipid Res. 11 (1970) 544.