DISPLACEMENT INTO INCUBATION MEDIUM BY ALBUMIN OF HIGHLY UNSATURATED RETINA FREE FATTY ACIDS ARISING FROM MEMBRANE LIPIDS

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1. Introduction

The concentration, composition and metabolic regulation of the free acid (FFA) pool in tissues other than the adipose are not well known. However, it has been reported that many cellular processes are affected by or are dependent upon the components of the FFA pool. FFAs, among other effects are potent uncouplers of the oxidative phosphorylation from the respiratory chain (for references see [1 and 2]; they are necessary as precursors of prostaglandins [3, 4]; they may be involved in the onset of irreversible brain damage caused by ischemia [5, 6]; they inhibit Na-K-dependent ATP-ase in brain [7] and they are inhibitors of key glycolytic enzymes (for references see [1]. Certain aspects of the intrinsic FFAs have been explored but only in a few tissues, e.g., in Ehrlich ascites tumor cells [8, 9], human platelets [10], cultured cells [11] and bran [1, 5, 6, 12, 13].

Bovine retina neutral [14] and polar-lipids [15] are richly in long-chain polyenoic fatty acids. FFAs are a relatively small and labile pool, also composed of a large proportion of polyunsaturated constituents. This letter reports remarkable features of endogenously produced retina FFAs, showing that they arise from membrane lipids, and that a different behavior is apparent in the rate of release of individual FFA from the tissue in vitro. The present data may be of interest with regard to the mechanisms involved in cellular uptake and metabolism of FFA.

2. Materials and methods

Bovine eyes were brought to the laboratory from a slaughterhouse (C.A.P., Cuatreros, General Cerri, Buenos Aires) packed within crushed ice. The retinas were incubated in 6 ml of the medium described by Ames [16], containing glucose (2 mg/ml) and FFA-free bovine serum albumin in the concentrations indicated in each experiment, at 37°C and gassed with O_2–CO_2 (95:5, v/v). At the end of the incubation periods the retinas were homogenized and extracted with $HCCl_3$:CH_3OH (2:1 v/v) as previously described [5]. The incubation media were centrifuged at about 8000 *g* in a refrigerated Sorvall and the residue extracted as above. The supernatants were extracted following the Dole procedure [17]. FFAs and triglycerides were isoalted by preparative gradient-thickness thin-layer chromatography [18]. Then they were treated with 14% B_3F in methanol and the methyl esters were isolated, identified and quantified by gas-liquid chromatography [5, 19].

3. Results and discussion

The FFA concentration in unincubated retinas is 125 ± 14 μg per 100 mg of dry weight and contains a high proportion of polyenoic constitutents [14] that undergo rapid changes during short-term incuba-

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Table 1
Distribution of free fatty acids between the retina and the incubation medium

FFA	5 min		20 min		% of FFA in the medium	
	Tissue	Medium	Tissue	Medium	5 min	20 min
16:0	30.4 ± 6.1	12.6 ± 2.9	56.5 ± 8.5	33.0 ± 2.4	29	37
16:1	3.6 ± 0.5	2.8 ± 0.3	8.3 ± 3.2	3.9 ± 0.7	44	32
18:0	43.6 ± 13.7	9.3 ± 1.5	73.4 ± 12.6	20.8 ± 2.1	18	22
18:1	23.0 ± 5.9	9.1 ± 1.1	60.9 ± 4.0	25.4 ± 3.7	28	29
18:2	3.6 ± 0.5	2.4 ± 0.5	11.1 ± 3.9	5.8 ± 1.9	40	34
20:4	11.3 ± 1.7	4.7 ± 1.2	11.0 ± 2.0	15.8 ± 2.3	29	59
20:5	1.0 ± 0.2	1.2 ± 0.3	1.4 ± 0.2	4.1 ± 0.6	55	75
22:5	1.7 ± 0.3	1.1 ± 0.3	3.0 ± 0.6	4.4 ± 0.8	39	59
22:6	7.7 ± 1.5	4.1 ± 1.4	15.3 ± 2.0	20.1 ± 2.8	35	57

Results are given in μg/mg of lipid-free dry weight. Figures are mean values ± S.E.M. for four samples, each containing two retinas. 0.5% bovine serum albumin was included in the medium. The FFA content in both tissue and medium was determined by gas–liquid chromatography using methyl-nonadecanoate as internal standard.

tions. A 2-fold increase in the retina FFA pool size was found (table 1) within 5 to 20 min in the medium described by Ames [16] in the presence of glucose, oxygen and 0.5% of albumin. In addition, about 30% of total FFAs were recovered in the incubation medium. Examining the concentration of individual FFAs, it was disclosed that a large and rapid efflux of polyunsaturated fatty acids from the retina occurs when incubated under these conditions. Free docosahexaenoic and arachidonic acids are the major polyunsaturated FFAs appearing in the medium, rising in concentration about 5- and 3-fold respectively. Eicosapentaenoic and docosapentaenoic acids, although they are minor constituents, also show similar changes, whereas all other FFAs increase in the incubation medium only about twice the concentration found at 5 min; the earliest incubation time studied. This is mainly the case for the largest FFA of the retina, palmitic, stearic and oleic acids. After 20 min of incubation, most FFAs double their concentration in the tissue, with the exception of oleic acid, which increases about three-times. Surprisingly the tissue concentration of arachidonic and eicosapentaenoic acids remains unchanged.

Since the membrane lipids of the retina are composed by a large proportion of long-chain polyenoic fatty acids, we decided next to explore the probable source of the produced FFAs. The measurement of the acyl groups of polar lipids was not attempted because the changes in the amount of FFA only represent a vanishingly small part of them. The monoacyl-

Table 2
Total free fatty acids and acyl groups of triglycerides in the retina incubated in the presence of albumin

Fatty acid	Free fatty acids		Triglycerides	
	5 min	20 min	5 min	20 min
16:0	43.9 ± 9.2	89.5 ± 9.1	35.9 ± 4.4	40.8 ± 2.2
16:1	6.4 ± 0.7	12.2 ± 2.7	5.9 ± 0.4	9.1 ± 1.4
18:0	55.4 ± 14.7	94.2 ± 12.3	20.5 ± 3.2	18.1 ± 2.3
18:1	32.9 ± 7.1	86.3 ± 5.5	20.6 ± 1.5	28.5 ± 5.1
18:2	6.2 ± 0.6	16.9 ± 2.8	2.7 ± 0.5	2.7 ± 0.3
20:4	15.7 ± 2.9	26.7 ± 4.1	4.6 ± 0.6	4.1 ± 0.7
20:5	2.2 ± 0.5	5.5 ± 0.7	0.7 ± 0.1	0.7 ± 0.2
22:5	2.8 ± 0.5	7.4 ± 1.4	2.6 ± 0.5	2.4 ± 0.2
22:6	12.6 ± 4.2	35.4 ± 4.7	18.6 ± 3.8	15.6 ± 2.6
Total	183.8 ± 10.5	401.2 ± 29.9	124.0 ± 11.0	150.0 ± 15.0

Results are expressed as in table 1. Total FFA content was obtained by the sum of the amount present in the tissue and in the medium.

phosphoglycerides were not studied along with the FFAs: firstly, because their determination implies the isolation of as many monoacylphosphoglycerides as phosphoglycerides are present in the retina, and secondly because a sequential deacylation may take place resulting in the release of both non-polar side chains of phospholipids. Thus, the approach followed consisted in the simultaneous measurement of the individual acyl groups of retina triglycerides to involve or discard them as a source of the produced FFAs. As shown in table 2 it is evident that the retina triglyceride acyl groups are also highly unsaturated. In unin-

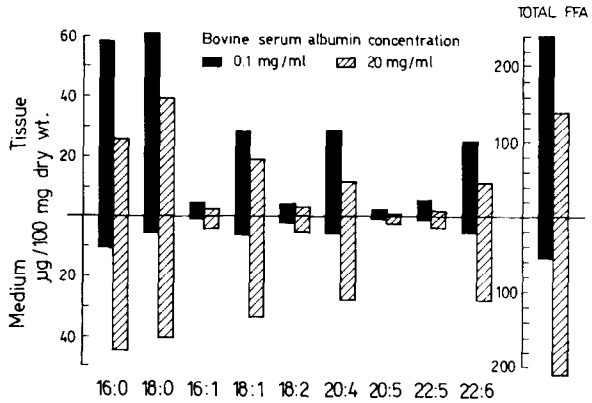

Fig. 1. Quantitative distribution of individual free fatty acids between the retina and the incubation medium at two different albumin concentrations. The samples were incubated during 20 min. Each sample contained one retina.

cubated retinas they are similar in concentration to the FFA. However, while the total retina FFAs significantly rise during incubation, the triglycerides do not decrease, on the contrary they slightly increase. Palmitate, palmitoleate and oleate appear to be responsible for this minor enhancement. Since the acyl groups of the triglyceride fraction were analyzed directly after methanolysis and dimethylacetals were not isolated, the possibility remains that this enlargement may be ascribed to newly formed ether-bonded triglyceride-like compounds [20], as a result of a relative oxygen defficiency due to the low tissue to medium volume ratio used in the present experiments. Alternatively, the increment may represent acylation of diacylglycerols using part of the produced FFAs remaining in the tissue. From the data presented in table 2 we conclude that the FFAs under the present experimental conditions arise mainly from non-polar side chains of membrane lipids. This possibility is further strengthened by the highly unsaturated nature of long-chain fatty acids known to be present in retina phospholipids [15, 21].

The FFA distribution between the retina and the incubation medium is deeply altered as a function of albumin concentration. In fig. 1. the quantitative distribution of individual FFA between the tissue and the medium at 0.01 and 2.0% albumin is presented. About 18% of the total FFAs are released into incubation medium in the lower albumin concentration, in contrast with about 60% that is found in the latter. In addition, it can be seen that a vast proportion of the polyenoic FFAs is displaced to the extracellular compartment. In spite of the large redistribution of FFAs it is apparent that their total content is not significantly altered.

The uneven efflux of FFA can be ascertained by the percentage of each component present in the medium at a given albumin concentration: thus, 1% albumin causes the release of as much as 70% or more of the long-chain polyenoic components, whereas all other FFAs show percentages in the medium between 30 and 60% (data not shown). Larger albumin concentrations increase the total FFA appearance in the medium, but the proportion of polyenoic FFA is not further enlarged. This suggests that the FFA efflux from the retina is not exclusively dependent upon the presence of albumin, moreover there may be a membrane involvement in the regulation of release. This fact, and the selective behavior of individual FFA, could be related with the possibility that at least part of the FFA transport mechanisms across cell membranes may not be a passive process, as has been proposed in adipose tissue [22]. Preliminary experiments with several albumin concentrations indicate that the chain length and unsaturated bonds are not the determinant factor in the efflux or retention of FFAs in the retina in vitro.

The results presented point to the presence of active phospholipase A which hydrolyse membrane lipids in the retina as has been suggested to occur in the brain at the onset of the ischemia [1, 5, 6, 12] and following electroshock (5). The precise source of free arachidonic and docosahexaenoic acids remains to be investigated. The latter is a major constituent of the phosphoglycerides of the photoreceptor membranes [21, 23] and also of neuronal membrane lipids [24, 25], whereas the former is an important acyl group of phosphatidylinositol [15]. Moreover, arachidonate must be unesterified to serve as a prostaglandin precursor [3, 4].

Since increments in the neural tissue-FFA content may be involved in the onset of irreversible brain damage caused by ischemia [1, 5, 6, 12] the results presented open the possibility of looking for therapeutic chemicals that may displace the enlarged tissue FFAs to the extracellular space as albumin does in vitro.

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