Fatty Acid Profiles of Various Lipids in the Cerebrospinal Fluid[1] (35478)

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Recent studies have demonstrated that both free fatty acids (FFA) and β-hydroxybutyrate were oxidized when introduced into the subarachnoid space (1, 2), thus reviving interest in the potential role of CSF[3] to supply metabolites locally for certain parts of the central nervous system. In view of these findings, it was the aim of the present studies to investigate the fatty acid concentration and profiles of the various CSF lipids in dogs under control conditions, with special emphasis on the free fatty acid content of CSF.

TABLE I. Fatty Acid Concentration of Four Major Lipids in CSF and Plasma (μmole/liter).[a]

	CSF	Plasma
FFA	25.4 ± 1.8	458 ± 64
TG	7.9 ± 0.7	609 ± 80
CE	10.8 ± 1.8	1578 ± 131
PL	16.5 ± 1.0	3044 ± 206

[a] Mean of 13 dogs ± standard error of the mean.

TABLE II. Concentration of FFA in CSF and Plasma (μmole/liter).

Dog no.	CSF	Plasma
1	27.1	233
2	27.0	513
3	5.7	327
4	24.7	611
5	29.0	499
6	29.1	324
7	25.6	703
8	24.5	173
9	25.3	402
10	28.6	175
11	23.2	725
12	27.9	813
13	32.5	—

Materials and Methods. Experimental procedure. The experiments were performed on 13 adult male mongrel dogs after they had been deprived of food for 16 hr. They were anesthetized with sodium pentobarbital given

TABLE III. Fatty Acid Composition of the Four

		C14=0	C14=1	C16=0	C16=1	C18=0	C18=1
FFA,	CSF	3.47 ± 0.46	2.70 ± 0.25	51.10 ± 1.89	3.20 ± 0.55	14.33 ± 0.80	13.31 ± 2.13
	plasma	1.68 ± 0.25	—	32.98 ± 1.92	3.89 ± 0.29	13.05 ± 0.92	37.89 ± 2.37
TG,	CSF	4.89 ± 0.93	0.47 ± 0.32	27.78 ± 0.83	8.96 ± 1.19	11.80 ± 0.89	34.05 ± 1.57
	plasma	1.11 ± 0.22	—	22.50 ± 0.59	4.41 ± 0.52	8.91 ± 0.87	41.52 ± 1.58
CE,	CSF	7.46 ± 0.97	16.21 ± 1.28	22.46 ± 1.46	8.26 ± 1.51	9.31 ± 1.51	23.43 ± 1.60
	plasma	0.66 ± 0.14	—	10.26 ± 0.40	2.47 ± 0.25	1.98 ± 0.30	25.17 ± 1.25
PL,	CSF	1.40 ± 0.29	—	45.40 ± 1.91	1.56 ± 0.55	15.29 ± 0.94	29.97 ± 0.96
	plasma	0.18 ± 0.03	—	23.94 ± 0.77	0.73 ± 0.13	33.67 ± 1.50	13.85 ± 0.91

[a] Mean of 13 dogs ± standard error.

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[3] The following abbreviations are used: FFA, free fatty acids; CSF, cerebrospinal fluid; TG, triglyceride; CE, cholesterol ester; PL, phospholipid.

intravenously (30 mg/kg). Arterial blood samples were obtained by introducing a catheter into the femoral artery through a side branch. Simultaneous CSF samples were obtained from the cisterna magna. All specimens were handled at 4°. No anticoagulant was given to the animals.

Analytical methods. Arterial blood samples were centrifuged in a refrigerated centrifuge. One ml of plasma or 5 ml of CSF were added to 20 or 100 ml of chloroform–methanol mixture, 2:1 parts by volume (3). After the lipid extraction, the major lipid classes were separated by thin-layer chromatography using glass plates coated with silica Gel G (Merck) of 0.25 mm thickness impregnated with 0.02% 2′-7′-dichlorofluorescein. The plates were developed in hexane–diethyl ether–glacial acetic acid, 156:40:4 parts by volume (4). The PL, FFA, TG, and CE spots were scraped into 80-ml tubes and methylation was carried out by the addition of 5 ml of methanol, 0.1 ml of 2,2-dimethoxypropane and 0.2 ml of concentrated sulfuric acid, followed by incubation overnight in a 40° water bath.

Five-μl samples of the methylated fatty acids were chromatographed using a Model No. 100 Barber-Coleman gas–liquid chromatograph equipped with a glass column (0.25 in. by 6 ft) packed with diethylene glycol succinate on 100–200 mesh chromsorb $\overline{\text{W}}$ (Applied Science Lab., Pa.).

The operating conditions were as follows: column temperature, 180°; temperature of strontium ionization detector, 250°; injection port temperature, 250°, rate of flow of the carrier argon, 80 ml/min. The samples were quantitated by the use of heptadecanoic acid as an internal standard and the area under the peaks was expressed as a product of width at half-height by peak height. The peaks were identified by comparing their retention time with those of a standard mixture of fatty acids obtained from Applied Science Lab.

To avoid any contamination by lipid soluble substances, all the glassware was acid-washed before use. This precaution appeared to be necessary in dealing with such low concentration of lipids as are present in the CSF.

Results. Fatty acid concentration of the four major lipids are shown in Table I. It is apparent that the concentration of FFA is higher in the CSF than that of the other lipids. On the average, the CSF contained approximately 5.5% of plasma FFA, 1.3% of TG, 0.6% of CE, and 0.5% of PL. Table II shows the individual values of CSF and plasma FFA for each of the 13 dogs. As shown, (with the exception on one animal) the variation of FFA concentration in the CSF among the individual dogs was quite small.

Table III shows a comparison of the fatty acid composition of 4 major lipid classes of CSF and plasma from normal dogs. The fatty acid composition of FFA in the CSF differs markedly from that in the plasma. The percentage of palmitic acid is markedly higher, that of oleic and linoleic acids is significantly lower in the CSF. FFA in the CSF also contains some longer chain fatty acids that

Major Lipid Classes of Normal Dog CSF and Plasma (%).[a]

C18=2	C20=0	C18=3	C20=2	C22=0	C20=4	C22=1	Total sat.	Total unsat.
1.55 ± 0.59	2.52 ± 0.61	—	—	6.79 ± 2.08	0.75 ± 0.51	0.28 ± 0.28	78.21	21.79
10.08 ± 0.80	—	—	—	—	0.42 ± 0.35	—	47.71	52.28
12.06 ± 2.29	—	—	—	—	—	—	44.47	55.54
16.60 ± 1.34	—	—	—	—	4.91 ± 0.74	—	32.52	67.44
8.72 ± 1.08	—	1.67 ± 0.99	2.07 ± 1.24	—	0.42 ± 0.42	—	60.78	39.23
44.11 ± 1.56	—	—	—	—	15.24 ± 1.22	—	12.90	86.99
3.46 ± 1.03	—	0.32 ± 0.32	—	—	2.61 ± 1.53	—	62.09	37.92
12.43 ± 1.05	—	—	—	—	15.04 ± 1.83	—	57.79	42.05

are not present in plasma FFA. While 78% of the total FA is saturated in CSF, only 48% is in the plasma.

Although statistically significant differences also exist between CSF and plasma for all the other classes of lipids, involving a number of fatty acids, the biological significance of some of these differences may not be great, due to the very low concentration of some of these lipids. However, it is of interest to note that in all the other lipid classes, (TG, CE, PL) the percentage values for myristic, palmitic, and palmitoleic acids are higher, and that for linoleic acid is lower in the CSF than in plasma. It is also noteworthy that the plasma fatty acids are generally more unsaturated than are the CSF fatty acids. This difference is especially marked in the case of CE, where 87% is unsaturated in the plasma, but only 39% in the CSF.

Discussion. The present investigation represents a quantitative and qualitative study of the fatty acids of the CSF and plasma lipids. Although several studies dealt with the total lipids of CSF (5–7) only a few described the FA composition of individual lipid classes (*i.e.*, TG, PL, and CE) in the human CSF (8–10). No such information is available concerning FFA that is present in CSF.

The concentration of FFA in the CSF is higher than that of any other lipid. The fatty acid composition of CSF lipids differs from that of the plasma. Generally, the differences are characterized by higher fractions of myristic and palmitic acids, and a lower fraction of linoleic acid in the CSF. The percentage of unsaturated acids are generally lower in the CSF. These differences agree with those described by Tichy *et al.* (10) for TG, PL, and CE and by Blomstrand (11) for CE.

The origin of the CSF lipids is—at the present time—only a subject of speculation. Tichy *et al.* (10) have recently speculated that in addition to the plasma and brain lipids, "the cells of CSF and the cells lining the CSF space might also be contributory."

The relatively high concentration of FFA in the CSF is of interest. In view of recent findings that FFA was readily oxidized when introduced into the parietal subarachnoid space (1), or into the ventricles (Wolf, E. H. and J. J. Spitzer, unpublished observation), it is tempting to speculate that the FFA present in the CSF may serve a nutritional role, supplying energy-yielding metabolites locally to certain portions of the brain. Further studies are required to establish the validity and physiological importance of this postulated function of FFA in the CSF.

Summary. Fatty acid concentration and composition of various lipids were studied in the CSF and in the plasma of control dogs. The average concentration of FFA, TGFA, PLFA, and CEFA in the CSF were 5.5%, 1.3%, 0.5%, and 0.6% of the respective fatty acid fractions of the plasma. The concentration of FFA in the CSF was higher than that of other lipids. CSF lipids contained a higher percentage of saturated fatty acids than did plasma lipids, mostly due to a higher fraction of palmitic and a lower fraction of oleic and linoleic acids.

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