BBA 63494

Lecithin: cholesterol acyl transferase activity in human cerebrospinal fluid

It has been shown that in cases of multiple sclerosis, demyelination is accompanied by loss of phospholipid and esterification of the cholesterol in brain tissue membranes[1,2]. It is of interest, therefore, to know the nature of the enzymes involved in phospholipid and sterol metabolism in cerebrospinal fluid bathing this tissue. The occurrence of phospholipases A_1 and A_2 in cerebrospinal fluid (CSF) has been reported previously[3] and the present study relates to lecithin: cholesterol acyl transferase and cholesterol esterase activities. The association of lecithin: cholesterol acyl transferase with the high-density lipoprotein of serum is well established[4-6]. Lecithin and cholesterol of high-density lipoprotein have also been shown[7-9] to be the preferred co-substrates for this enzyme.

Samples of normal human CSF having a protein content of 25–35 mg % were selected after clinical examination from both child and adult patients. Pooled samples from two groups of the former and one group of the latter were centrifuged at 20000 × *g* for 20 min to remove any cellular material prior to 30-fold concentration by ultrafiltration through a membrane filter (Amicon N.V.) with an upper limit of permeability to molecules of approx. 1000 mol. wt. The protein content of the concentrated CSF was thus raised to 1.0%. For comparison tests, samples of fresh human serum were also diluted with water to a similar protein concentration of approx. 0.8%.

The lecithin: cholesterol acyl transferase activity was determined by incubating samples of concentrated CSF or diluted serum with either [4-^{14}C]cholesterol or biosynthetically prepared[10] [β-1-^{14}C]oleoyl lecithin and determining the resultant radioactivity appearing in cholesterol esters. For assays involving [4-^{14}C]cholesterol, a stock substrate solution was prepared containing 1 μC [4-^{14}C]cholesterol (specific activity 55.8 mC/mM) dispersed with 1 μmole lysolecithin in 2.0 ml of 0.1 M NaCl, using a Whirlimixer (Fisons Scientific Ltd., England). Aliquots of 0.1 ml were then taken for each assay. A similar dispersion of [β-1-^{14}C]oleoyl lecithin ($1.04 \cdot 10^6$ disint./min per μmole per 2 ml) in 0.1 M saline was also prepared, 0.1 ml aliquots again being used as substrate.

Since the level of high-density lipoprotein is relatively low in CSF, two assay procedures were employed with each substrate: In the first, 0.5 ml concentrated CSF was added to 0.1 ml of the radioactive substrate and, after mixing the contents, the tube was stoppered and incubated at 37° for 20 h. In the second procedure, 0.4 ml of fresh human serum, which had previously been heat-treated at 60° for 30 min to destroy its lecithin: cholesterol acyl transferase activity[11-13], was emulsified with 0.1 ml of the substrate solution and then 0.5 ml concentrated CSF added. Mixing and incubation were then carried out as above. Comparison tests with 0.5 ml aliquots of the diluted serum were also assayed under similar conditions as were controls for each procedure containing water in place of CSF.

After incubation, lipids were extracted by the method of BLIGH AND DYER[14]. For the assays performed in the absence of heat-inactivated serum 3 μmoles each of cholesterol stearate and either free cholesterol or lecithin were added as carriers for the isolation of the appropriate labelled compound. The labelled cholesterol esters were then resolved from either lecithin or free cholesterol by thin-layer chromato-

TABLE I

LECITHIN:CHOLESTEROL ACYL TRANSFERASE ACTIVITY IN HUMAN CEREBROSPINAL FLUID

CSF concentrate contained approx. 1% protein. Diluted serum contained approx. 0.8% protein. Protein was estimated in each preparation using the Folin-Ciocalteu reagent.[23].

Substrate	*Radioactivity in cholesterol esters (disint./min per 5 mg enzyme protein)**	
	CSF (concentrated)	*Diluted Serum (1:9)*
[4-^{14}C]Cholesterol	270 ± 40	4300 ± 300
[4-^{14}C]Cholesterol *plus* heat-inactivated serum	2030 ± 360	4900 ± 100
β-[1-^{14}C]Oleoyl lecithin	10	n.d.
β-[1-^{14}C]Oleoyl lecithin *plus* heat-inactivated serum	97 ± 15	178 ± 12

* Net radioactivity after deduction of blank readings for controls.

graphy on 0.25 mm layers of silica gel H (A. G. Merck, Germany). The developing solvent was 7% diisopropyl ether in light petrol (R_F values for cholesterol esters, free cholesterol and lecithin were 0.8, 0.1 and 0.0 respectively). The plates were sprayed with a 10% solution of scintillation fluid (as prepared for radioassay) in alcohol and the bands visualised under ultraviolet illumination and scraped off the plate. Free and esterified cholesterol were eluted from the silica with 18 ml ether. Lecithin was eluted with 18 ml methanol. After evaporation of the solvents 10 ml of scintillation fluid were added to each and radioactivity was assayed using a Beckman LS 200B Liquid Scintillation Counter. Control experiments showed that recovery of radioactivity from the plates exceeded 90%. The results for the net incorporation of radioactivity into cholesterol esters after deduction of control blanks were calculated for a given concentration of enzymic protein. The values are set out in Table I.

Clearly, the concentrated human CSF in the presence of heat-inactivated serum catalysed the formation of ^{14}C-labelled cholesterol esters from added [4-^{14}C]cholesterol or β-[1-^{14}C]oleoyl lecithin. No appreciable difference in enzyme activity was noted between samples of CSF derived from either child or adult patients, so only the mean values for the three groups are given. It can be seen that the level of lecithin:cholesterol acyl transferase activity of CSF under optimal conditions is somewhat less than the calculated value for serum diluted to the same protein concentration. The values obtained using labelled cholesterol and lecithin represented only 41% and 54%, respectively, of the corresponding values for the diluted serum. The much higher esterification of [4-^{14}C]cholesterol by CSF found in the presence of heat-inactivated serum was not due to residual lecithin:cholesterol acyl transferase activity present in the heat-treated serum because the blank values for controls using water in place of CSF were negligible. The lecithin:cholesterol acyl transferase activities of both CSF and diluted serum were also found to be reduced by freezing the samples, some 30–35% of the original activity being lost by storage at −12° for 2 weeks.

In view of the report that some esterase activity is present in CSF[15], the possibility that hydrolysis of newly formed cholesterol esters could occur was investigated. Biosynthetically prepared samples (200 nmoles) of [4-^{14}C]cholesterol esters (specific activity 104 disint./min per nmole) were dispersed in 1 ml of 0.05 M Tris-HCl buffer

(pH 7.4) containing 1.5 mg sodium deoxycholate and 0.5 ml of concentrated CSF added. After incubation for 3 h at 37° the lipids were extracted and carriers added. Free and esterified cholesterol were resolved by thin-layer chromatography. In no case was any activity detected in the free cholesterol fraction, thereby demonstrating that cholesterol esterase activity is absent from human CSF.

Bearing in mind the known presence of high-density lipoprotein in CSF[16-18] and the association of lecithin: cholesterol acyl transferase with plasma high-density lipoprotein, it appears that the activity detected in concentrated human CSF is derived from plasma after filtration through the choroid plexuses. The marked stimulation of lecithin: cholesterol acyl transferase activity in CSF in the presence of heat-inactivated serum indicates the lack of suitable substrate in normal CSF.

This situation arises from at least two principal factors. Firstly, human lecithin: cholesterol acyl transferase shows a preference for lecithins containing linoleate[6,12]. The very low proportion of this acid (4% of total fatty acids) present in CSF lecithin[19] compared to that (25%) of serum lecithin would favour a slower utilization of CSF lecithins for cholesterol esterification.

However, this difference of 6-fold concentration in linoleic acid is unlikely to account on its own for the nearly 10-fold stimulation in enzymic activity following the addition of the dilute heat-inactivated serum. The second factor would appear to involve the relative concentrations of lecithin: cholesterol acyl transerase and high-density lipoprotein cholesterol and lecithin in CSF compared to the values for serum. The level of lecithin: cholesterol acyl transferase activity in serum has been shown to be proportional to the quantity of high-density lipoprotein present[21]. Thus CSF which has lower concentrations of high-density lipoprotein, cholesterol and lecithin per mg protein than serum[18,22] would be expected to have a lower absolute level of lecithin: cholesterol acyl transferase assuming that high-density lipoprotein and its associated lecithin: cholesterol acyl transferase permeated equally well through the choroid plexuses. The finding, however, that the enzyme was operating at only 1:10th its optimal capacity in CSF was unexpected. CSF would appear to contain a surplus of free lecithin: cholesterol acyl transferase for the amount of high-density lipoprotein normally available there.

The results establish, however, that CSF possesses appreciable cholesterol ester synthesising activity provided there is appropriate juxtapositioning of the lecithin and cholesterol as in the lipid component of high-density lipoprotein. Such a situation might occur in lipoproteins of brain tissue cell membranes around cholesterol molecules given greater degrees of freedom of movement by the depletion of some neighbouring phospholipid molecules.

D.R.I. received financial support from the Medical Research Council.

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BBA 63487

Some properties of abnormal red blood cell pyruvate kinase

Erythrocyte pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) deficiency is a metabolic disorder in which a great variety in the parameters of enzymatic activity [V and K_m (phosphoenol pyruvate)] has been found. Three types could be distinguished based on these kinetic parameters. In most cases the parameters are measured on the crude hemolysate. MUNRO AND MILLER[1] concluded for a mutant enzyme that the ADP and PEP (phosphoenol pyruvate) sites show a greater cooperation than the normal enzyme. However they did not calculate the extent of cooperation, for instance, expressed as the Hill coefficient.

This paper deals with three unrelated pyruvate kinase-deficient patients from whom the enzymes are partially purified to stage 4, according to the method of STAAL *et al.*[2]. The enzymes were finally dialysed overnight against 0.01 M Tris–malate containing 50% (v/v) glycerol (pH 8.0). The enzymatic activity of pyruvate kinase was measured according to the method of BÜCHER AND PFLEIDERER[3].

Fig. 1 shows the $1/v$–$1/[PEP]$ plots at various ADP concns. of the normal, (highly purified) enzyme and the mutant enzymes. In contrast to the normal enzyme (Fig. 1A), the enzyme of patient J. v/d K. (Fig. 1B) gives straight lines. This was also found with the enzyme of an unrelated patient (N.S.) with pyruvate kinase deficiency (figure not shown). However, the enzyme of the third patient (B.H.) shows the same picture as the normal enzyme. The K_m values for PEP at $[ADP] = \infty$ are, within experimental error, identical. Moreover, in all $1/v$ *versus* $1/[ADP]$ plots of the data of these patients, straight lines are obtained (not shown) and the K_m (ADP) at $[PEP] = \infty$ are also normal. From these data it can be concluded that the enzymes of patients J. v/d K. and N.S., in contrast to normal enzyme and the enzyme of patient B.H. have lost their allosteric properties.

Further evidence for this conclusion is found in Fig. 2. This experiment shows