Clinica Chimica Acta, 80 (1977) 55—59

CCA 8773

AN IMMUNOLOGICAL STUDY OF NINE PROTEINS IN CSF AND SERUM OF A GROUP OF EPILEPTIC PATIENTS

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(Received March 10th, 1977)

Summary

The concentrations of nine proteins, alpha-1-acid glycoprotein, antitrypsin, prealbumin, transferrin, albumin, IgG, ceruloplasmin, IgA and alpha-2-macroglobulin, have been determined in the serum and CSF of two groups of patients, one control and one experimental, by an immunological method. The experimental group were patients suffering from grand mal epilepsy. The control group showed no detectable neurological disorder.

In the group of grand mal epileptics, only prealbumin showed a significant elevation in CSF when compared with the control group. In contrast, the rest of the proteins are decreased with respect to the controls except for α1-acid glycoprotein and transferrin.

The results from this study also suggest that something more than an ultrafiltration process dependent upon molecular weight, is important in determining the concentration of some serum proteins in the CSF.

Introduction

The quantification of individual proteins in serum and CSF in several neurological disorders has been the aim of a number of reports [1—3]. Many of these studies however, have involved a wide range of different neurological entities and few have been concerned with a well defined group of patients. Mora et al. [4] have studied the oxidase and immunological properties of one of the serum proteins, ceruloplasmin, in grand mal epileptics, and Rosenthal and Soothill [1] have studied several proteins in the CSF and serum of five cases of post-

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traumatic epilepsy. In the present paper, we report the first quantitative study in a well defined group of grand mal epiletics, of nine different proteins in serum and CSF.

Materials and methods

All blood and CSF samples were taken from two groups of six male hospitalized patients. The group of epileptics were clinically diagnosed as grand mal epileptics prior to the begining of the experiment and although under chronic treatment with phenobarbital and diphenylhydantoin they reported intermittent grand mal attacks.

The control group were residual psychotic patients who now lived and worked in the hospital but did not show any detectable neurological disorder. The age in both groups ranged from 20 to 55 years.

Samples were always taken at least 30 days before or after an epileptic attack. The CSF samples (10—15 ml) were taken by lumbar puncture. The blood sample (20 ml) were taken from the cubital vein, from 7.30 to 8.30 in the mornining under basal conditions. After the blood was centrifuged and the serum decanted, all the samples were divided into two aliquots for analysis of total and individual proteins. They were then coded and kept at $-20°C$ until the analysis was performed. During the analysis it was not known which samples were the controls. Total protein was measured using the Biuret method [5]. For the analysis of individual proteins, the CSF samples were lyophilized and redissolved in 0.5 ml of normal saline. The concentration factor, depending upon the initial volume, averaged twenty to thirty times.

The nine proteins measured were; alpha-1-acid glycoprotein, mol. wt. 44 000; antitrypsin, mol wt. 45 000; prealbumin, mol. wt. 61 000; transferrin, mol. wt. 88 000; albumin, mol. wt. 68 000; Immunoglobulin G, mol. wt. 150 000; ceruloplasmin, mol. wt. 151 000; Immunoglobulin A, mol. wt. 165 000; alpha-2-macroglobulin, mol. wt. 820 000. The proteins were quantified using the immunological diffusion method of Mancini et al. [6]. Serum proteins were immunologically identified for both groups by the precipitation method of Ouchterlony [7]. Details of both methods have been described previously [8]. In parallel to this, immunoelectrophoresis was performed on each sample according to the method of Grabar and Williams [9]. The antisera and serum standard were supplied by Behringwerke A.G. (G.F.R.).

Results

Table I gives the average concentrations (mean ±S.E.) for all the proteins measured in both control and epileptic groups. In this table we have not included the results for alpha-2-macroglobulin because concentrations in the CSF were too low to be detectable in most cases.

Table II presents for both groups the average value (mean ± S.E.) of the concentration of each protein in the CSF expressed as a percentage of the plasma concentration of the same protein. Values for the control and epileptic groups were statistically compared using a Man Witney U test. The '*P*' values are pre-

TABLE I

CONCENTRATION (MEAN ±S.E.) OF SERUM AND CSF PROTEINS AND TOTAL PROTEINS (mg/100 ml) IN PATIENTS WITH NO NEUROLOGICAL DISORDERS AND WITH GRAND MAL EPILEPSY

	Controls		Epileptics	
	CSF conc. (mg%)	Serum concn. (mg%)	CSF concn. (mg%)	Serum concn. (mg%)
α_1-Acid glycoprotein	0.41 ± 0.13	106.50 ±± 40.26	0.28 ± 0.03	77.50 ±± 5.30
Antitrypsin	1.01 ± 0.24	281.33 ± 59.00	0.74 ± 0.08	284.00 ± 19.47
Prealbumin	1.06 ± 0.15	28.54 ± 4.86	1.50 ± 0.11	24.29 ± 6.30
Transferrin	1.04 ± 0.12	185.66 ± 14.76	0.85 ± 0.14	123.66 ± 8.31
Albumin	16.46 ± 2.25	2733.33 ± 241.75	12.33 ± 0.68	2841.66 ± 81.05
IgG	1.57 ± 0.23	982.00 ± 160.70	1.00 ± 0.23	993.00 ± 68.76
Ceruloplasmin	0.16 ± 0.01	57.50 ± 0.01	0.13 ± 0.01	62.30 ± 6.62
IgA	0.21 ± 0.04	253.00 ± 27.86	0.16 ± 0.01	265.00 ± 25.06
Total proteins	30.00 ± 1.52	6350.00 ± 444.78	34.33 ± 2.67	6750.00 ± 210.95

TABLE II

[CSF]/SERUM] % VALUES FOR EIGHT PROTEINS IN BOTH CONTROL AND EPILEPTIC GROUPS

Protein	Mol. wt.	Control	Epileptic	*P* value
α_1-Acid glycoprotein	44 000	0.43 ± 0.046	0.38 ± 0.069	>0.1
Antitrypsin	45 000	0.33 ± 0.032	0.26 ± 0.04	<0.1
Prealbumin	61 000	3.93 ± 0.4	7.8 ± 1.24	<0.01
Transferrin	88 000	0.59 ± 0.094	0.67 ± 0.09	>0.1
Albumin	68 000	0.59 ± 0.027	0.43 ± 0.027	<0.05
IgG	150 000	0.17 ± 0.025	0.106 ± 0.026	<0.05
Ceruloplasmin	151 000	0.29 ± 0.02	0.22 ± 0.03	<0.05
IgA	165 000	0.08 ± 0.01	0.06 ± 0.007	<0.1

sented in the table. Five of the eight proteins in the epileptic group show a significant decrease in the CSF/serum concentration ratio when compared to controls; prealbumin shows a very markedly significant increase in the ratio; and for transferrin and alpha-1-glycoprotein the value are not significantly different from controls.

Discussion

When comparing the CSF/serum concentration ratio for the epileptic group with those of the control group two points emerge; there is a decrease in the ratio for antitrypsin, alpha-1-acid glycoprotein, albumin, IgG, IgA, and ceruloplasmin in the epiletic group, and a significant increase in the same ratio for prealbumin. The essentially uniform decrease in the CSF/serum ratio when compared to be controls for most of the proteins would suggest a common factor, the origin of which is at present unknown, although the pharmacological treatment that these patients had chronically received could partially account for this phenomena. Phenobarbital for instance is known to produce a slight decrease in blood pressure when administered chronically to epiletic patients

[10] and this could be reflected to some extent in a decreased rate of filtration of proteins from serum to CSF. It is difficult to see, however, how this treatment might produce an increase in the relative concentrations of transferrin (111%) and in particular prealbumin (200%). It is interesting that for these proteins there is now considerable evidence which suggests that at least part of the CSF content has its origin within the CSF or the ventricles [11 and 15]. It could be then, that the increased concentrations found for transferrin and mainly prealbumin in the CSF of the epipeltic group are in some way linked with grand mal epilepsy, itself, although a non-specific drug effect cannot be definitely ruled out.

Part of our study has also been concerned with the relationship between CSF/serum concentration ratio and the molecular weight for each of the proteins in both the epileptic and control group. It has been suggested that proteins in the CSF are derived from plasma by an ultrafiltration process dependent upon the molecular weight [13,14 and 1]. This suggestion has however been questioned in recent years [15,16,12 and 17]. In the data presented in this paper there are features of the CSF protein composition which suggest that something more than an ultrafiltration process is involved. The data presented by Rosenthal et al. for instance show a selectivity in favour of small protein molecules being ultrafiltered from serum. Our results indeed agree with those of Rosenthal when the same group of proteins are compared. However, when as in this study, a larger spectrum of proteins is measured, a more complicated view of the picture emerges. Proteins such as α_1 acid glycoprotein and antitrypsin with low molecular Wts. do not follow that general trend. It could be that for the glycoproteins, this anomaly is due to their high carbohydrate content [18,19]. Alpha-1-acid glycoprotein for instance, is know to show anamalous behaviour on gel electrophoresis, and although undertermined, this seems to be due to a difference in the three dimensional structure of the variants [18]. For these protein it could be that the complex carbohydrate group [19] restricts passage across the blood brain barrier. We did not find any difference between the control groups and epiletics when comparing the CSF/serum concentration ratio against the molecular weight for each of the proteins.

Pathological factors other than neurological disorders are of extreme importance in determining CSF protein ratios [20]. In this respect we have been cautious in ensuring that in both groups there was no indication of any physiological disorder other than grand mal epilepsy. It is important in this respect to mention that serum protein values in both groups fell with the range of normal values quoted by several authors [21].

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