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MEMBRANE INSTABILITY INDUCED BY PURIFIED MYELIN COMPONENTS

ITS POSSIBLE RELEVANCE TO EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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Summary

The fusogenic properties of purified myelin components in a system employing chicken erythrocytes were studied. Sulphatides, myelin basic protein and the apoprotein of Folch-Lees proteolipid were capable of individually inducing membrane fusion in the presence of Ca^{2+}. By contrast, cerebrosides or a mixture of sulphatides and myelin basic protein (molar ratio 19 : 1) did not show such effect. The fusogenic ability of sulphatide was correlated to its behaviour in mixed monolayers with phospholipids at the air-water interface. Mixed films of sulphatides with phosphatidylcholine or sphingomyelin but not with phosphatidylethanolamine showed reductions of molecular packing and surface potential similar to those found for other fusogenic compounds. The effects of myelin components described could be of importance in the membrane instability and vesicular disruption of myelin occurring in demyelinative disorders.

Introduction

Animals with experimental allergic encephalomyelitis induced with whole central nervous system showed changes in the contents of brain and spinal cord sulphatides, cerebrosides and esterified cholesterol [1]. Intradermal injection of single myelin components selectively induced some of these alterations. The basic encephalitogenic protein induced alterations of sulphatides, the cerebroside content was changed after injection of lipid haptens and increased

amounts of esterified cholesterol were found after sensitization of animals with the apoprotein of Folch-Lees proteolipid [2—5]. The lipid changes in experimental allergic encephalomyelitis might be the consequence of dissociations of normal interactions among some of the myelin constituents induced by immunological agents [4,5].

Apart from the biochemical alterations several morphological changes appear in demyelinative disorders. One of them is a vesicular disruption of myelin [6,7] which should involve a fusion process of the myelin membrane. To investigate the possibility that isolated myelin components could be involved in processes of membrane fusion we studied the fusogenic ability of some purified myelin components in vitro in a system employing chicken erythrocytes. In addition, the interactions shown by cerebrosides and sulphatides with phospholipids in mixed monolayers at the air/water interface were correlated to their effects on the erythrocyte membrane.

Materials and Methods

Membrane fusion. Induction of membrane fusion was carried out in a system similar to that described by Ahkong et al. [8]. Briefly, washed chicken erythrocytes (approx. 10^8 cells/ml) were incubated at 37°C in a basal medium consisting of a modified Eagle's salt solution containing Dextran 60 C (80 mg/ml) buffered with sodium cacodylate, at pH 7.4. Any myelin component to be tested was ultrasonically dispersed in the basal medium and added to the medium containing the erythrocytes at a final concentration that will be indicated. At different periods of time samples of the incubated cells were examined by phase-contrast microscopy.

Lipid monolayers. Interaction of sulphatides and cerebrosides with phospholipids was studied in mixed monolayers at the air-NaCl (145 mM) interface, pH 5.6. The equipment used, the preparation of the films and the analysis of results was done as described previously [9,24].

Sources of phospholipids and myelin components. Phosphatidylcholine and phosphatidylethanolamine from egg yolk were prepared according to Bangham et al. [10]. Sulphatides, cerebrosides and the myelin proteins from beef brain were prepared as described elsewhere [2,4]. Briefly, purified apoprotein of Folch's proteolipid was obtained according to Mokrasch [11] and was used within two days from when it was rendered soluble in water [12] since these proteins tend to aggregate with time and become insoluble. A crude basic protein fraction was prepared from beef brain myelin by the method of González-Sastre [13] and further purified by Sephadex G-100 according to Deibler et al. [14]. On 7.5% polyacrylamide gel electrophoresis (0.1% sodium dodecyl sulphate) using purified myelin [15] as a control for the position of protein bands, the basic protein migrated as a single band. The apoprotein of Folch's proteolipid showed difficulty in entering the gels but it was possible to detect at least two protein bands running in the corresponding positions of Folch's proteolipids of the whole myelin preparation. The molecular weights of myelin basic protein and the apoprotein of Folch's proteolipid were taken as 17 000 and 25 000, respectively. Beef brain sphingomyelin, crystallized bovine serum albumin and poly-(L-lysine) (mol. wt. 68 000) were purchased from Sigma

Chem. Co. (St. Louis, MO, U.S.A.). Protein determinations were according to Lowry et al. [16] using bovine serum albumin as standard.

Results

Studies employing erythrocytes

Chicken erythrocytes incubated at 37°C in the modified Eagle's medium in the presence of sulphatides at different concentrations showed time-dependent

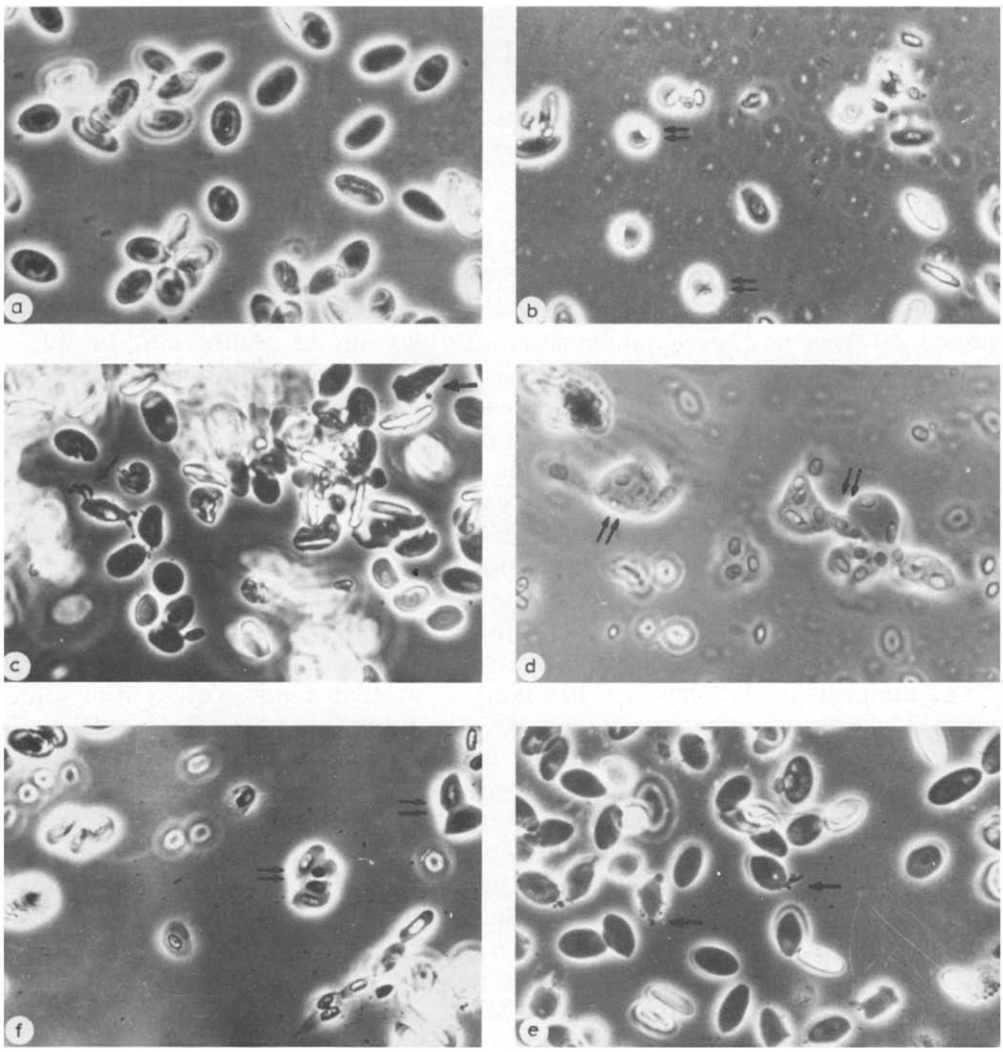

Fig. 1. Morphological changes induced by lipids and proteins of myelin. The erythrocytes were incubated, as described in the text, in presence of: (a) no additions; (b) sulphatides 120 nmol/ml, 60 min of incubation; (c) myelin basic protein, 1.8 nmol/ml, 20 min of incubation; (d) myelin basic protein, 1.8 nmol/ml, 35 min of incubation; (e) apoprotein of Folch-Lees proteolipid, 3 nmol/ml, 15 min of incubation; (f) poly-L-lysine, 0.05 nmol/ml, 15 min of incubation. Phase contrast microscopy: X600. Double arrows indicate bi- or multinucleated cells, single arrows indicate membrane pinching off.

changes in their morphology. At a concentration of 120—240 nmol/ml sulphatides induced the sequential appearance of crenated, rounded and oddly shaped cells, and bi- and multinucleated cells. The latter appeared in about 45—75 min (Fig. 1b). In common with other fusogenic lipids [8—17] sulphatides also increased cell lysis. At a concentration of 60 nmol/ml the changes were less pronounced and developed more slowly. At 20 nmol/ml no fusion was seen up to 180 min of incubation. By contrast, cerebroside, the other major glycosphingolipid of myelin, at a concentration of 120 nmol/ml did not induce changes; the cells remained oval shaped and similar to the controls even after 230 min of incubation.

Cells incubated in the presence of 1.8—2.4 nmol/ml of myelin basic protein showed morphological changes after 15—45 min of incubation. The changes were characterized by the appearance of rather long processes protruding from the cell surface which eventually pinched off into vesicles by membrane fusion (Fig. 1c). During this period it appeared as if cells had fused into bi- and multinucleated cells. However, in contrast to the case of the fusion induced by sulphatides or other fusogenic lipids [8,17] the fused cells induced by the basic protein were not generally rounded but exhibited an irregular shape (Fig. 1d); on occasions this made it difficult to differentiate between intercellular fusion and close cellular attachment. The cells with processes and the fused cells tended to lyse and were rarely seen after 90 min of incubation. In these preparations some cells were aggregated into small clumps presumably due to partial neutralization of their surface negative charge by the basic protein. At a concentration of basic protein of 1.2 nmol/ml the changes developed more slowly, at a concentration of 3.5—6 nmol/ml it was more difficult to observe the changes since the proportion of cells aggregated into clumps increased.

Poly(L-lysine) at 0.05—0.1 nmol/ml also induced the appearance of irregular bi- and multinucleated cells, similar to those found for the myelin basic protein, within 30 min of incubation (Fig. 1f). At higher concentrations large clumps of aggregated cells occurred. Albumin at the concentration of 1.8 nmol/ml did not induce changes up to 240 min of incubation.

The apoprotein of Folch-Lees proteolipid, another major protein of myelin, induced cell rounding and, on a same cell, the formation of multiple vesicles of variable size that rapidly pinched off by membrane fusion and were subsequently seen moving freely in the medium (Fig. 1e). This effect appeared between 5 and 30 min of incubation at a protein concentration of 1—3 nmol/ml and was no longer seen after 120 min. No cell to cell fusion was seen with the apoprotein of Folch's proteolipid up to 180 min of incubation. As with other fusogenic lipids and water-soluble agents [8,17,18] neither sulphatides nor the proteins studied were able to induce membrane fusion in absence of Ca^{2+} or with 3 mM sodium EDTA in the medium (the concentration of Ca^{2+} in the modified Eagle's medium was 1.8 mM).

Since it has been shown that there is a preferential interaction of sulphatides and myelin basic protein [19,20] we studied the possibility that the effects induced by either of these compounds in chicken erythrocytes could be inhibited by the other. In a biphasic system the basic protein forms complexes with sulphatides that acquire a maximal hydrophobicity at a molar ratio (sulphatide : basic protein) of about 19 : 1 [20]. When chicken erythrocytes

were incubated in the presence of a mixture of sulphatides plus myelin basic protein in molar ratio 19 : 1 the changes induced separately, at comparable times, by the basic protein or sulphatides were not seen. The aggregation of cells into clumps induced by the protein at higher concentrations were likewise not present. However, after 150 min of incubation rounded cells similar to those induced by sulphatides alone were seen but no fusion developed up to 180 min of incubation. As the molar proportion of sulphatides in the mixture was increased (sulphatide : basic protein, 38 : 1, 57 : 1, and 76 : 1) the time-dependent pattern of changes was progressively more similar to that described above for sulphatides and cell fusion was observed.

Contrary to the behaviour of the mixture of sulphatide and myelin basic protein in molar ratio 19 : 1 a mixture of sulphatides and poly(L-lysine) at the molar ratio 680 : 1, tested at a sulphatide concentration of 120 nmol/ml, induced before 60 min of incubation the changes found for poly(L-lysine) alone and between 60 and 120 min those described for sulphatides alone. Similarly, when sulphatides (120 nmol/ml) were mixed with albumin at the molar ratio 19 : 1 no inhibition of the changes induced by sulphatides was seen and cell fusion was observed between 60 and 90 min of incubation. On the other hand, cerebroside which was in itself ineffective as fusogenic did not abolish the morphological changes and cell fusion induced by the myelin basic protein.

Lipid monolayers

The occurrence of particular interactions between lipid molecules can be studied in lipid monolayers by inspection of the values of molecular areas and

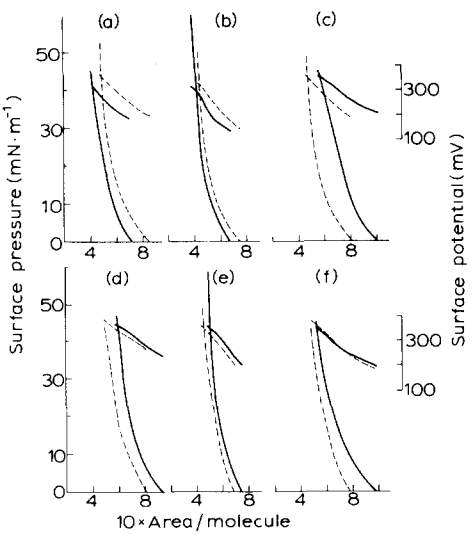

Fig. 2. Interactions of sulphatides and cerebrosides with phospholipids in mixed monolayers. The surface pressure-area and surface potential-area curves were obtained, as described in the text, for mixed monolayers of: (a), (b), (c), sulphatides with phosphatidylcholine, sphingomyelin and phosphatidylethanolamine respectively; (d), (e), (f), cerebrosides with phosphatidylcholine, sphingomyelin and phosphatidylethanolamine respectively. The broken lines represent values for ideal films calculated by the additivity rule [21].

surface potential in mixed films at a given surface pressure compared to the behaviour of a theoretical film in which no interactions are assumed [21].

As for other lipids capable of inducing membrane fusion [22—24] the fusogenic ability of sulphatide could be correlated to its behaviour in mixed monolayers with phospholipids. Thus, the characteristic reductions of molecular area and surface potential were found in mixed monolayers of sulphatides with phosphatidylcholine or with sphingomyelin but not in mixed films of sulphatides with phosphatidylethanolamine. Conversely, cerebroside which is not fusogenic, did not show similar interactions to those of sulphatides with choline-containing phospholipids (Fig. 2).

Discussion

The basic protein of myelin interacts preferentially with sulphatides in a biphasic system [20] and in monolayers at the air-water interface [19] forming lipid-protein complexes. Presumably these complexes also occur in myelin where, even if the structure seems to correspond to a rather fluid liquid-crystalline phase [25], the lipid seem to be immobilized by intermolecular interactions [26,27] probably preventing membrane instability in normal conditions. In the present study the fusion of the membrane of nucleated erythrocytes was used as a model to determine possible effects induced by purified myelin components. Sulphatides, myelin basic protein and the apoprotein of Folch's proteolipid individually induce instability of the erythrocyte membrane leading to Ca^{2+}-dependent membrane fusion. However, the effects induced separately by sulphatides or the myelin basic protein were not found when a mixture of both (molar ratio 19 : 1) was used. By contrast, poly(L-lysine), which does not penetrate into a monolayer of sulphatide [19], did not inhibit the fusogenic action of these lipids. A direct extrapolation of these results to a possible action on the myelin membrane is difficult. However, they support the suggestion that a dissociation of normal interactions between the molecules studied can result in instability of the myelin membrane. It was previously proposed that immunological agents directed against myelin constituents might induce a dissociation of the interactions among them [4,5] and in agreement with this it was recently reported that anti-myelin antibodies can induce vesicular disruption of myelin [28] which must involve processes of membrane fusion. Vesiculation of myelin also occurs in animals with experimental allergic encephalomyelitis and experimental allergic neuritis [6,7]. Furthermore, it was reported that the myelin basic protein is capable of inducing fusion of liposomes [29,30] and of increasing the permeability of liposomes containing sulphatides and other acidic lipids [31,32].

With respect to the molecular mechanisms participating in the effects described, it is known that membrane fusion can be induced in vitro by several lipid agents [8,17,33] and the fusogenic properties of these compounds are closely correlated to their effects on phospholipid monolayers [22—24]. A common feature of their behaviour is to exhibit interactions with choline-containing phospholipids resulting in reductions of the molecular packing and surface potential of the lipid interface. These types of interactions were also found in the present study between sulphatides and phosphatidylcholine or sphingo-

myelin but not for cerebroside, which is not fusogenic. The membrane surface potential is a factor controlling ionic permeabilities [34,35] and it was previously concluded that a reduced electrostatic field perpendicular to the surface of a lipid bilayer may be a key event leading to an increase of the membrane permeability to Ca^{2+} required for membrane fusion [8,18,36]. In this connection, it is relevant that a Ca^{2+} ionophore which induced membrane fusion in chicken erythrocytes [37] is also able to induce vesicular disruption of myelin [38].

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References

1 Maggio, B., Cumar, F.A. and Maccioni, H.J. (1972) J. Neurochem. 19, 1031—1037
2 Maggio, B. and Cumar, F.A. (1974) Brain Res. 77, 297—307
3 Maggio, B. and Cumar, F.A. (1975) Nature 253, 364—365
4 Roth, G.A., Maggio, B., Monferrán, C.G. and Cumar, F.A. (1978) FEBS Lett. 86, 29—32
5 Cumar, F.A., Maggio, B. and Roth, G.A. (1977) Adv. Exp. Med. Biol. 83, 505—511
6 Lampert, P.W. and Kies, M.W. (1967) Exp. Neurol. 18, 210—223
7 Ballin, R.H.M. and Thomas, P.K. (1968) J. Neurol. Sci. 8, 1—18
8 Ahkong, Q.F., Fisher, D., Tampion, W. and Lucy, J.A. (1973) Biochem. J. 136, 147—155
9 Maggio, B., Cumar, F.A. and Caputto, R. (1978) Biochem. J. 171, 559—565
10 Bangham, A.D., Hill, M.W. and Miller, N.G.A. (1974) in Methods in Membrane Biology (Korn, E.D., ed.), Vol. I, pp. 1—68, Plenum Press
11 Mokrasch, L.C. (1971) Prep. Biochem. 2, 1—12
12 Sherman, G. and Folch-Pi, J. (1970) J. Neurochem. 17, 597—605
13 González-Sastre, F. (1970) J. Neurochem. 17, 1049—1056
14 Deibler, G.E., Martenson, R.E. and Kies, M.W. (1970) Biochim. Biophys. Acta 200, 342—352
15 Autilio, L.A., Norton, W.T. and Terry, R.D. (1964) J. Neurochem. 11, 17—27
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
17 Maggio, B., Cumar, F.A. and Caputto, R. (1978) FEBS Lett. 90, 149—152
18 Maggio, B., Ahkong, Q.F. and Lucy, J.A. (1976) Biochem. J. 158, 647—650
19 Demel, R.A., London, Y., Geurtz Van Kessel, W.S.M., Vossenberg, F.G.A. and Van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 311, 507—519
20 Banik, N.L. and Davison, A.N. (1974) Biochem. J. 143, 39—45
21 Gaines, G.L. (1966) Interscience Monographs on Physical Chemistry: Insoluble Monolayers at Liquid-Gas Interfaces (Prigogine, I., ed.), pp. 281—300, Interscience, New York
22 Maggio, B. and Lucy, J.A. (1976) Biochem. J. 155, 353—364
23 Maggio, B. and Lucy, J.A. (1975) Biochem. J. 149, 597—608
24 Maggio, B., Cumar, F.A. and Caputto, R. (1978) Biochem. J. 175, 1113—1118
25 Chapman, D. (1975) Q. Rev. Biophys. 8, 185—235
26 Williams, E.C., Hamilton, J.A., Jain, M.K., Allerhand, A. and Cordes, E.H. (1973) Science 181, 869—871
27 Williams, E.C. and Cordes, E.H. (1976) Biochemistry 15, 5792—5799
28 Saida, T., Saida, K., Silberberg, D.H. and Brown, M.J. (1978) Nature 272, 639—641
29 Smith, R. (1977) Biochim. Biophys. Acta 470, 170—184
30 Stollery, J.G. and Vail, W.J. (1977) Biochim. Biophys. Acta 471, 372—390
31 Gould, R.M. and London, Y. (1972) Biochim. Biophys.Acta 290, 200—218
32 Maggio, B., Mestrallet, M.G., Cumar, F.A. and Caputto, R. (1977) Biochem. Biophys. Res. Commun. 77, 1265—1272
33 Cumar, F.A., Maggio, B. and Caputto, R. (1978) Biochem. Biophys. Res. Commun. 84, 65—69
34 Bangham, A.D. (1968) Prog. Biophys. Mol. Biol. 18, 29—95
35 Gingell, D. (1967) J. Theor. Biol. 17, 451—482
36 Blow, A.M.J., Botham, G.M. and Lucy, J.A. (1978) Biochem. Soc. Trans. 6, 284—285
37 Ahkong, Q.F., Tampion, W. and Lucy, J.A. (1975) Nature 256, 208—209
38 Schlaepfer, W.W. (1977) Nature 265, 734—736