SIALIC ACID ON THE SYNAPTOSOME SURFACE AND EFFECT OF CONCANAVALIN A AND TRYPSIN ON SYNAPTOSOME ELECTROPHORETIC MOBILITY*

H. Bruce BOSMANN**

Department of Pharmacology and Toxicology, University of Rochester, School of Medicine and Dentistry, Rochester, New York 14642, USA

Received 15 February 1972

1. Introduction

The *in vitro* subcellular fraction the "synaptosome" (nerve ending particle) prepared from the cerebral cortex of the guinea pig retains the neurotransmitter content and morphology of the nerve ending *in vivo* [1–6]. Recently synaptosome glycoprotein and glycolipid have been recognized as important with respect to both synthesis and degradation [6–12] and structure [13–16]. Very recently, this laboratory [17] has determined that terminal sialic acid residues are present at the nerve ending surface and has determined the electrophoretic mobility of isolated purified synaptosomes. The present communication describes electrokinetic properties of the synaptosome surface with respect to trypsin digestion, pH and ionic strength and records the effects of concanavalin A and albumin on the elctrophoretic mobility of the synaptosomes.

2. Methods

2.1. *Solutions*

Saline–sorbitol media of low ionic strength was 4.5% sorbitol, 0.0145 M NaCl and 6×10^{-4} M $NaHCO_3$; this solution was utilized for all measurements unless noted and was at pH 7.2 ± 0.1. Purified neuraminidase from Worthington Biochemical Corp. had an activity of 0.7 units/mg, where 1 unit of activity equals 1 μmole of *N*-acetyl neuraminic acid released per min at 37°, pH 5.0. Purified trypsin was purchased from Worthington Biochemicals.

2.2. *Electrophoretic mobilities*

Measurements were made at 25 ± 0.1° in a horizontal cylindrical chamber of small volume equipped with reversible, blacked platinum electrodes [17, 18]. The alignment of the apparatus was checked by the method of Heard and Seaman [19], and iso-osmotic solutions of HCl and NaOH for the pH:mobility curves were prepared as described [19].

2.3. *Particle isolation*

Purified synaptosomes were prepared exactly by the method of Whittaker et al. [1–4] at 0–4° from guinea pig cerebral cortex; fraction B was utilized in all experiments as described [5, 6].

Electrophoretic mobilities of particles in saline–sorbitol media were corrected to the viscosity of standard saline at 25° [17].

2.4. *Protein*

Protein was determined by the method of Lowry et al. [20], utilizing bovine serum albumin as standard.

2.5. *Neuraminidase or trypsin treatment before clumping*

The procedure used [17] was to treat unclumped synaptosomes with neuraminidase or trypsin and to measure the electrophoretic mobility on clumped synaptosomes (clumped in the washing of the treated synaptosomes).

Synaptosomes, removed with as little excess sucrose solution as possible, corresponding to 100 mg (as protein) were treated with 1 ml of various concentrations

* Supported in part by grants No CA-13220 and No. GM-15190 from the National Institutes of Health

** Research Career Development Awardee of the National Institute of General Medical Sciences.

of neuraminidase or trypsin dissolved in physiological saline. The pH was adjusted to 6.5–7.0 and the tubes were incubated in a gently rocking water bath at 37° for 30 min. The synaptosomes were centrifuged at 7,000 *g* for 10 min, washed 3 times with physiological saline, centrifuged for 10 min at 2500 *g*, and finally washed in saline–sorbitol of low ionic strength. The washed synaptosome clumps were resuspended in 1 ml saline–sorbitol, and 4 drops of this solution were added to 30 ml saline–sorbitol to obtain a dilute mixture for observation of electrophoretic mobility. The particles were timed successively in both directions at 60 V for 2 grids (166 μ). The chamber was routinely washed with dichromate, distilled water, and saline–sorbitol between runs. The pH of the solution was kept at 7.2 ± 0.1.

2.6. *Treatment with trypsinized Concanavalin A or albumin*

Concanavalin A was trypsinized by a modified method of Burger and Noonan [21]. 25 mg of Concanavalin A (Calbiochem) was dissolved in 2.4 ml of 0.2 M phosphate, pH 7.0. 0.1 ml of 2.5% trypsin was added, and the solution was incubated at 37° for 5 hr. The trypsinization was stopped by adding 2.5 ml of 1% trypsin inhibitor (Sigma). 0.5 ml of synaptosome suspension was incubated with 0.1 ml trypsinized Concanavalin A solution, albumin solution, or control solution plus 0.4 ml of physiological saline for 30 min. The particles were centrifuged at 7000 *g* for 10 min and washed twice with physiological saline. Suspensions for the observation of electrophoretic mobility were made and measurements were taken as outlined above.

3. Results and discussion

3.1. *Electrophoretic mobility and neuraminidase treatment*

As previously determined [17] the electrophoretic mobility of isolated guinea pig cerebral cortex synaptosomes was −1.860 μ/sec/V/cm. Treatment with neuraminidase reduced this mobility to −1.50 μ/sec/V/cm (fig. 1), indicating the presence of sialic acid on the nerve ending periphery. It has been previously [17] shown biochemically that such neuraminidase treatment results in the loss of sialic acid from the guinea pig cerebral cortex synaptosomes.

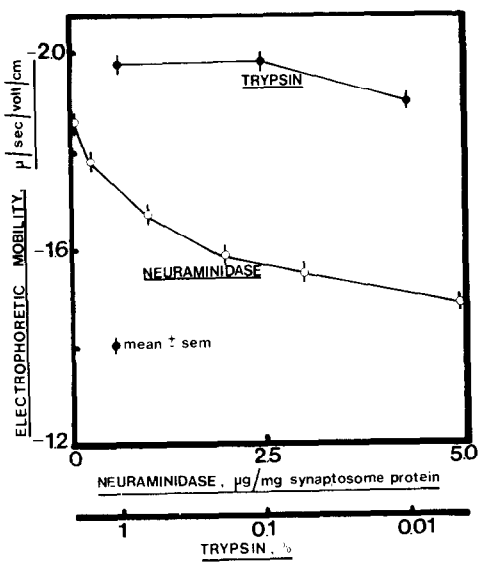

Fig. 1. Electrophoretic mobility of isolated guinea pig cerebral cortex synaptosomes after trypsin or neuraminidase treatment. Synaptosomes (Fraction B) were incubated with the indicated amount of neuraminidase or trypsin and electrophoretic mobilities determined as given in Materials and methods.

3.2. *Electrophoretic mobility and trypsin treatment*

In an attempt to demonstrate that the electrokinetic properties of the synaptosome were due to glycoprotein sialic acid and not glycolipid sialic acid, the synaptosomes were treated with trypsin, as shown in fig. 1. The synaptosome electrophoretic mobility unexpectedly increased, instead of decreasing with the trypsin treatment (fig. 1). This is not an isolated or anomolous finding, however, since erythrocytes of the chicken have been demonstrated to increase in electrophoretic mobility after trypsin treatment even though sialic acid has been demonstrated to be important in the chicken erythrocyte electrokinetic behaviour [22]. Therefore in the synaptosome or at the nerve ending periphery, even though sialic acid residues are terminal and probably present in glycoprotein as well as glycolipid, the groups unmasked by trypsin digestion or preferentially allowed to remain after trypsin treatment result in a higher net negative electrophoretic mobility.

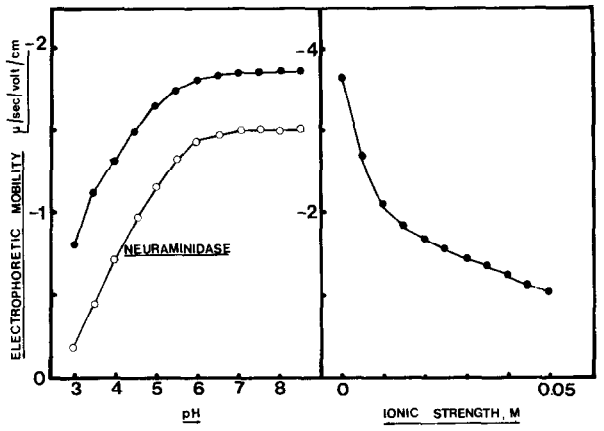

Fig. 2. Electrophoretic mobility of isolated guinea pig cerebral cortex synaptosomes as a function of pH or ionic strength. The pH:electrophoretic mobility curves were generated at an ionic strength of 0.0145 M NaCl in the saline–sorbitol solution. The (o–o–o) synaptosomes treated with 5 μg of neuraminidase per mg synaptosome protein before measurement of electrophoretic mobility at the indicated pH. The ionic strength:electrophoretic mobility curve was generated at pH 7.2 ± 0.1 utilizing varying NaCl concentrations in the saline-sorbitol solution and omitting $NaCO_3$ at very low ionic strengths.

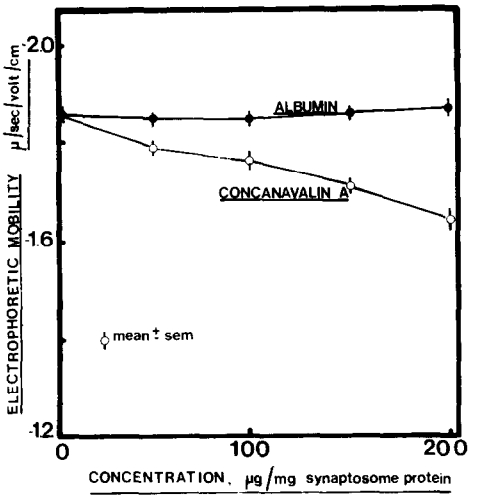

Fig. 3. Electrophoretic mobility of isolated guinea pig cerebral cortex synaptosomes after treatment with albumin or trypsinized Concanavalin A. Synaptosomes (Fraction B) were incubated with the indicated amounts of trypsinized Concanavalin A or albumin and electrophoretic mobilities determined as given in Materials and methods.

3.3. *Effect of pH on the electrophoretic mobility of normal or neuraminidase-treated guinea pig cerebral cortex synaptomes*

The data of fig. 2 demonstrate that the electrophoretic mobility of isolated synaptosomes was stable over a pH range of 6–8 and also shows the lowered electrophoretic mobilities of the synaptosomes after neuraminidase treatment.

3.4. *Effect of ionic strength on the electrophoretic mobility of guinea pig cerebral cortex synaptosomes*

The data of fig. 2 demonstrate that the electrophoretic mobility of the synaptosome is a function of the ionic strength of the suspending media. These data and the pH-mobility relationship indicate that the electrokinetic behaviour of the isolated guinea pig cerebral cortex synaptosome arise in part from one ionogenic group: a carboxyl group of a sialic acid with pK ∿2.7. It should be emphasized that the electrophoretic mobility of any cell or cell particle is the net result of the summation of many ionogenic groups on the surface or near the plane of shear, and the sialic acid group implicated herein may in fact be only a partial contributor to the net surface charge of the synaptosome.

3.5. *Electrophoretic mobility of isolated guinea pig cerebral cortex synaptosomes in the presence of albumin*

The data of fig. 3 demonstrate that albumin at the concentrations studied did not affect the synaptosome electrophoretic mobility and therefore probably was not bound by the isolated synaptosomes.

3.6. *Electrophoretic mobility of isolated guinea pig cerebral cortex synaptosomes in the presence of trypsinized Concanavalin A*

In contradistinction to the data for albumin, the data of fig. 3 show that Concanavalin A lowered the synaptosome electrophoretic mobility. These data are of interest since this plant lectin is thought to selectively agglutinate tissue culture cells transformed by oncogenic viruses or chemical carcinogens [23], although recently [24, 25] the amount of bound Concanavalin A has been shown not to be the determinant of agglutination (i.e., "normal" and "transformed"

cells bind about the same amount of Concanavalin A). The present results can be explained by two alternatives: 1) Concanavalin A binds to synaptosome plasma membrane and in binding to synaptosome plasma membrane either exposes more electropositive groups or masks electronegative groups yielding a lower net negative electrophoretic mobility or 2) binding of Concanavalin A to the synaptosome surface itself adds electropositive ionogenic groups, thus decreasing the net negative electrophoretic mobility.

Acknowledgements

I thank Mr. Richard Ball, Mr. Kenneth R. Case, and Mrs. Delena DeHond for excellent technical assistance.

References

[1] E.G. Gray and V.P. Whittaker, J. Anat. 96 (1962) 76.
[2] V.P. Whittaker and M.N. Sheridan, J. Neurochem. 12 (1965) 363.
[3] V.P. Whittaker, I.A. Michaelson and R.J.A. Kirkland, Biochem. J. 90 (1964) 293.
[4] V.P. Whittaker, Prog. Biophys. Mol. Biol. 15 (1965) 39.
[5] H.B. Bosmann and B.A. Hemsworth, Biochem. Pharmacol. 19 (1970) 133.
[6] B.A. Hemsworth, K.I. Darmer and H.B. Bosmann, Neuropharmacology 10 (1971) 109.
[7] H.B. Bosmann, and B.A. Hemsworth, J. Biol. Chem. 245 (1970) 363.
[8] H.B. Bosmann, J. Neurochem. 19 (1972) 763.
[9] L.A. Autilio, S.H. Appel, P. Pettis and P.L. Gambetti, Biochemistry 7 (1968) 2615.
[10] H.B. Bosmann, J. Biol. Chem. 247 (1972) 130.
[11] M.W. Gordon and G.G. Deanin, J. Biol. Chem. 243 (1968) 4222.
[12] H.B. Bosmann and B.A. Hemsworth, Biochim. Biophys. Acta 242 (1971) 152.
[13] K.J. Ryan, H. Kabant, and L. Thomas, J. Cell Biol. 49 (1971) 235.
[14] H.B. Bosmann, Neurobiology, 1 (1971) 144.
[15] O.Z. Sellinger, R.N. Barens and L.M. Nordrum, Biochim. Biophys. Acta 173 (1969) 185.
[16] H.B. Bosmann, K.R. Case and M.B. Shea, FEBS Letters 11 (1970) 261.
[17] H.B. Bosmann, Exp. Cell Res., in press.
[18] H.B. Bosmann, Nature 233 (1971) 566.
[19] D.H. Heard and G.V.F. Seaman, J. Gen. Physiol. 43 (1960) 635.
[20] O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 234 (1951) 1971.
[21] M.M. Burger and K.D. Noonan, Nature 228 (1970) 512.
[22] G.V.F. Seaman and G. Uhlenbruck, Arch. Biochem. Biophys. 100 (1963) 493.
[23] M. Inbar and L. Sachs, Nature 223 (1969) 710.
[24] M.J. Cline and D.C. Livingston, Nature New Biol. 232 (1971) 155.
[25] B. Ozanne and J. Sambrook, Nature New Biol. 232 (1971) 157.