Electrokinetic Properties of Isolated Cerebral-Cortex Synaptic Vesicles

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Synaptic vesicles isolated from guinea-pig cerebral cortex had an electrophoretic mobility of $-3.55 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$ in saline-sorbitol, pH 7.2, at 25°C (ionic strength 0.015 g-ions/l). The mobility was pH-dependent, varied with ionic strength and indicated that the vesicular surface contained weak acidic functions with a pK_a in the range 3.0–3.8. Although the vesicular surface was determined to be highly negatively charged, treatment with neuraminidase had no effect on mobility and indicated that the relatively strong carboxyl groups of sialic acid do not contribute significantly to vesicular electrokinetic properties. Treatment of synaptic vesicles with trypsin or trypsinized concanavalin A resulted in increases in mobility, but treatment with ribonuclease, deoxyribonuclease, chondroitinase ABC or hyaluronidase had no significant effect on mobility. Mn^{2+} or Ca^{2+} was more effective in decreasing vesicle mobility than was Mg^{2+} , Sr^{2+} or Ba^{2+} . The electrokinetic properties of the synaptic vesicle surface are discussed and contrasted with the properties of the synaptosomal membrane.

The energetics of the presumed interaction between synaptic vesicles and the synaptic membrane can be approximately expressed as a function of geometric, electrostatic and London-van der Waal parameters of the interacting surfaces (Ambrose, 1965; Heard & Seaman, 1960), but the theoretical and experimental development of this general type of approach is rudimentary. In the present study, the technique of particle micro-electrophoresis has been used to determine some of the electrokinetic properties of isolated synaptic vesicles. Alterations of these electrokinetic properties by changes in bulk solution pH or ionic strength, enzymatic action and adsorption of various bivalent cations and other agents provide information about the biochemical micro-environment of the vesicular surface. Analysis of these data leads to estimation of the zeta potential and average charge density at the surface. As has been pointed out (Ambrose, 1965), electrophoretic measurements cannot indicate precise arrangements of ionogenic groups.

Recently the electrokinetic properties of the plasma membrane of synaptosomes isolated from guinea-pig cerebral cortex have been reported (Bosmann, 1972a; Bosmann & Carlson, 1972). In many respects this membrane is similar to the plasma membranes of other mammalian cells (Ambrose, 1965; Mehrishi, 1972) with terminal *N*-acetylneuraminic acid groups of surface glycoconjugates contributing to the electrophoretic mobility of the synaptosome. The mechanism(s) of neurotransmitter release and the functional role of synaptic vesicles are matters of considerable con-

troversy (Holtzman *et al.*, 1973). An exocytotic mechanism of release would, by definition, involve two membranes, the synaptosomal (nerve ending; presynaptic) plasma membrane and the synaptic vesicle membrane, and it was deemed important to investigate the electrochemical nature of the synaptic vesicle membrane. An exocytotic process of neurotransmitter release would almost certainly involve complex control mechanisms for the correct alignment of the synaptic vesicle membrane with the inner surface of the synaptosome plasma membrane, for membrane fusion, transmitter release and the re-synthesis or recycling of synaptic vesicle membranes (Heuser & Reese, 1973; Ceccarelli *et al.*, 1973). Thus the nature of the interacting surfaces, perhaps as modulated by intra- and extra-cellular events, may in part mediate or control such release. The present paper demonstrates that the electrokinetic properties of the vesicular surface are markedly different from the properties of the synaptosome surface and indeed from other mammalian cell and cell organelle surfaces. These unusual surface characteristics may contribute to the yet undefined role of synaptic vesicles in the process of neurotransmitter storage and release.

Experimental

Materials

Neuraminidase (EC 3.2.1.18; 0.57 unit·mg⁻¹, where 1 unit of activity equals 1 μmol of sialic acid released/min at 37°C and pH 5.0; derived from *Clostridium perfringens*), trypsin (EC 3.4.4.4; derived

from bovine pancreas), ox pancreas RNAase* (EC 2.7.7.16), bovine pancreas DNAase (EC 3.1.4.5) and hyaluronidase (EC 3.2.1.35; 320 U.S.P. units/mg, derived from bovine testes) were all purchased from Worthington Biochemical Corp. (Freehold N.J., U.S.A.). Chondroitinase ABC (EC 4.2.-.-; derived from *Proteus vulgaris*) was purchased from Miles Laboratories (Kankakee, Ill., U.S.A.). Concanavalin A and albumin (crystalline, bovine serum) were purchased from Calbiochem (La Jolla, Calif., U.S.A.). Density-gradient sucrose was purchased from Mann Research Laboratories Inc. (New York, N.Y., U.S.A.). D-Sorbitol and soya-bean-trypsin inhibitor were purchased from Sigma Chemical Corp. (St. Louis, Mo., U.S.A.). All other biochemicals or chemicals used were purchased from either Fisher Scientific Co. (Pittsburgh, Penn., U.S.A.) or Sigma Chemical Corp.

Methods

Solutions. Physiological saline was a solution of 0.145M-NaCl, made 0.3mM with respect to NaHCO_3 , $\text{pH} 7.2 \pm 0.1$. Saline-sorbitol medium of low ionic strength was 4.5% (w/v) sorbitol, 0.0145M-NaCl and 0.3mM- NaHCO_3 , $\text{pH} 7.2 \pm 0.1$. These solutions and iso-osmotic solutions of NaOH and HCl for adjusting solution pH were prepared as described by Heard & Seaman (1960).

Isolation of synaptic vesicles. Sub-synaptosomal particle fractions were prepared exactly by the method of Whittaker and co-workers (Whittaker & Sheridan, 1965; Whittaker, 1965; Gray & Whittaker, 1962), as previously described (Bosmann & Hemsworth, 1970), at 0–4°C from guinea-pig cerebral cortex (brain stem transected between superior and inferior colliculi). Guinea pigs (250–300g), which had been starved 16h before being killed, were the source of cerebral cortex. Fraction D was removed by suction, diluted with physiological saline, and then centrifuged at 50000g for 10min. Washing with saline was performed twice and the resultant pellet was subjected to the various experimental procedures described below.

Protein determination. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin used as a standard.

Sialic acid. Released or free sialic acid was measured by the method of Warren (1959) and total sialic acid was determined by the procedure of Svennerholm (1958). *N*-Acetylneuraminic acid was used as a standard.

Treatment with trypsinized concanavalin A. Concanavalin A was trypsinized by a modified method

of Burger & Noonan (1970). Concanavalin A (25 mg) was dissolved in 2.4ml of 0.2M-phosphate, $\text{pH} 7.0$, 0.1ml of 2.5% (w/v) trypsin was added and the solution was incubated at 37°C for 5h. The trypsinization was stopped by the addition of 2.5ml of 1% (w/v) trypsin inhibitor. A portion (0.9ml) of vesicle suspension in saline containing about 0.1–1.0mg of protein was incubated with 0.1ml of trypsinized concanavalin A solution at 37°C for 20min. After incubation, samples were diluted with saline-sorbitol medium and centrifuged at 50000g for 10min. The pellets were washed twice with the saline-sorbitol medium and then resuspended in 7ml of saline-sorbitol for measurement of electrophoretic mobility.

Enzymic treatment of isolated synaptic vesicles. Fraction D washed pellets containing about 0.1–1.0mg of protein were treated with various concentrations of neuraminidase, trypsin, hyaluronidase, chondroitinase ABC, DNAase or RNAase dissolved in 1ml of physiological saline. pH was adjusted to 6.5 for treatment with neuraminidase or hyaluronidase and to 6.5–7.2 for treatment with trypsin, DNAase, RNAase or chondroitinase ABC. Tubes were incubated in a gently rocking water bath at 37°C for 20min. After incubation the samples were diluted with saline-sorbitol and spun at 50000g for 10min. The resulting pellets were washed two more times in saline-sorbitol and then resuspended in 7ml of saline-sorbitol for the determination of electrophoretic mobility.

Electrophoretic mobility. Measurements were made at $25 \pm 0.1^\circ\text{C}$ in a horizontal cylindrical chamber of small volume equipped with reversible, blackened platinum electrodes (Heard & Seaman, 1960; Bosmann, 1972a; Bosmann & Carlson, 1972). The chamber was viewed by dark-field illumination in the apparatus obtained from Rank Brothers, Bottisham, Cambs., U.K. Mobilities of the particles were calculated in $\mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$ ($\pm \text{S.E.M.}$); each independent value was obtained by timing the movement of at least 20 particles over a distance of $166\mu\text{m}$ with a reversal of polarity after each measurement. Alignment of the apparatus was checked by the method of Heard & Seaman (1960). Determinations of the mobility of washed human erythrocytes for calibration purposes were made in saline-sorbitol medium. Normal blood was taken into sodium citrate-citric acid-dextrose (ACD) medium, immediately washed in saline and then in saline-sorbitol, and assayed in the electrophoretic apparatus before each experiment. Erythrocyte mobility was compared with the value of $-2.78 \pm 0.08 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$ reported by Heard & Seaman (1960). Mobilities obtained in saline-sorbitol were corrected to the unit bulk viscosity of standard saline at 25°C . Data were analysed with the aid of a PDP-8 computer obtained from Digital Equipment Corp. (Maynard, Mass., U.S.A.).

* Abbreviations: RNAase, ribonuclease; DNAase, deoxyribonuclease.

Theoretical

Debye-Hückel parameter. At 25°C the effective thickness of the ionic double layer (in ångströms) can be calculated from the equation given by Heard & Seaman (1960):

$$\chi = 3.05I^{-1/2}$$

Zeta potential. The generalized Helmholtz-Smoluchowski equation for the electrophoresis of non-conducting solid spheres can be expressed as

$$\zeta = m6\pi N_0/D_0 RF$$

where ζ = zeta potential, m = electrophoretic mobility, N_0 = bulk viscosity (0.01 poise) and D_0 = bulk dielectric constant (80). As is discussed by Davies & Rideal (1963), the size and shape of particles can, in some cases, appreciably affect electrophoretic mobility. R is a correction factor allowing for ionic relaxation effects and F corrects for the interaction of the applied field with the membrane surface and interphase. The main assumptions of this equation (Abramson *et al.*, 1964) are that inertia terms in the hydrodynamic equations used in its derivation are negligible, that slip between solvent and migrating particles does not occur and that ions behave as point charges. Correction factors for the finite size of ions have not been used and it is recognized that use of bulk-phase values of D and N is not strictly correct.

Average charge density. The application of the Gouy equation to small particles with curved surfaces is hardly justified, except for purposes of discussion. At 25°C in a uni-univalent electrolyte solution, this equation reduces (Heard & Seaman, 1960) to

$$\sigma = 3.52 \times 10^4 \times I^{1/2} \times \sin h(\zeta/51.3)$$

where σ = charge density in e.s.u. · cm⁻². This equation presumes that the surface potential is identical with the zeta potential and assumes that the membrane interphase is impenetrable to counterions, that the counterions behave as point charges and that the charge is distributed uniformly. Although in practice the assumptions often partly cancel one another (Davies & Rideal, 1963), results obtained with this equation should be used for comparative and not for exact quantitative purposes.

For calculation of vesicle surface area, a diameter of 40 nm (400 Å) was chosen from electron micrographs of the fraction. It should be emphasized that this is a representative value and one chosen only for calculation purposes.

Significance tests were carried out by using Student's *t* test, rejecting the null hypothesis at the 0.05 level.

Results

Electrophoretic mobility of untreated synaptic vesicles

Table 1 gives the electrophoretic mobility of isolated synaptic vesicles which were washed twice in saline-sorbitol medium and then resuspended in the same medium for measurement. The value of $-3.55 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$ is the average of eight independent experiments (25 or more measurements per experiment) and contrasts sharply with the previously determined mobility of isolated synaptosomes (Bosmann, 1972a; Bosmann & Carlson, 1972). Vesicles incubated in saline at 37°C for 30 min, and then washed and assayed in saline-sorbitol, gave identical mobility values. The data given in Fig. 1 indicate that essentially a monomodal distribution of mobilities of isolated synaptic vesicles obtained. The absence of sizeable additional peaks suggests that either a homogeneous population of vesicles was being examined or that a heterogeneous population of particles had quite similar mobilities. Furthermore, in all control measurements all particles in the field had a similar mobility, as indicated by the small standard error, and it is unlikely that adsorption of varying amounts of contaminating particles or macromolecules to vesicular surfaces was occurring in these experiments. Finally, it should be noted that even in medium of high ionic strength the electrophoretic mobility is extremely great; indeed, it is probably the highest recorded to date for either a mammalian cell or a cell organelle (Bosmann, 1972a; Bosmann *et al.*, 1972; Seaman & Uhlenbruck, 1963; Mehrishi, 1972).

Treatment of isolated guinea-pig cerebral-cortex synaptic vesicles with neuraminidase and/or trypsin

Fig. 2 and Table 2 indicate that treatment with various concentrations of neuraminidase had no

Table 1. *Electrophoretic mobility of isolated guinea-pig synaptic vesicles and synaptosomes*

Experiments were performed as described in the Experimental section at $25 \pm 0.1^\circ\text{C}$ in solutions of 0.0145 M-NaCl, 0.3 mM-NaHCO₃ and 4.5% (w/v) sorbitol, pH 7.2 ± 0.1 . Data for electrophoretic mobility are means $\pm$ S.E.M. The value for synaptic vesicles is a mean determined from 375 observations obtained in eight independent experiments. The value for isolated synaptosomes was taken from previous data (Bosmann & Carlson, 1972) and confirmed in additional experiments.

Fraction	Mobility ($\mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$)
D:synaptic vesicles	-3.55 ± 0.04
B:synaptosomes	-1.86 ± 0.03

Fig. 1. Distribution of electrophoretic mobilities of isolated guinea-pig synaptic vesicles

Three hundred and seventy-five observations from eight independent experiments are plotted, where N equals the number of observations having the indicated electrophoretic mobility. All electrophoretic mobilities were rounded up to the nearest tenth. Experiments were performed as given in the Experimental section. The solution for measurement contained 0.0145M-NaCl, 0.3mM-NaHCO₃ and 4.5% (w/v) sorbitol, pH 7.2±0.1, 25±0.1°C.

effect on the electrophoretic mobility of cerebral cortex synaptic vesicles. The values for neuraminidase-treated vesicles, vesicles treated with boiled enzyme, or vesicles incubated in saline alone, were virtually identical. Parallel studies with human erythrocytes resulted in a sharp decrease in mobility after neuraminidase treatment, a well-known finding (Seaman & Cook, 1965; Bosmann, 1972b).

Incubation of the vesicle fraction with increasing concentrations of trypsin resulted in increasing electrophoretic mobilities, whereas incubation with boiled enzyme or enzyme inactivated by soya-bean-trypsin inhibitor had no effect. Brief exposure to trypsin resulted in either no effect on electrophoretic mobility or an increase in mobility. The finding of increased mobility after treatment

Fig. 2. Electrophoretic mobility of isolated guinea-pig synaptic vesicles after treatment with trypsin or neuraminidase

Synaptic vesicles were incubated with the indicated amount of trypsin (○) or neuraminidase (●) for 20 min at 37°C and mobilities determined at 25±0.1°C as given in the Experimental section. Solutions were 0.0145M-NaCl-0.3mM-NaHCO₃, pH 7.2±0.01. 'Boil' refers to neuraminidase and trypsin (both 60 μg/mg of vesicle protein) which had been boiled for 10 min before use in the experiment; Δ, trypsin (60 μg/mg of vesicle protein) treated with a tenfold excess of soya-bean-trypsin inhibitor. Vertical lines indicate S.E.M.

with trypsin is not an isolated or anomalous finding, a similar finding having been reported for guinea-pig cerebral cortex synaptosomes (Bosmann, 1972a) and for chicken erythrocytes (Seaman & Uhlenbruck, 1963).

Attempts to demonstrate biochemically the release of sialic acid during treatment of vesicles with either neuraminidase or trypsin were repeatedly unsuccessful. Chemically the total synaptic vesicle fraction was found to contain 0.011±0.001 (mean±S.E.M.) μmol of sialic acid/mg of synaptic vesicle protein.

Other treatments

Effect of enzymes. A slight but significant increase in mobility was found when untreated vesicles were treated with trypsinized concanavalin A. After prior trypsinization of vesicles, treatment with

Table 2. Electrophoretic mobility of isolated guinea-pig synaptic vesicles treated successively with neuraminidase and trypsin

Synaptic vesicles were treated with neuraminidase or trypsin for 20min, with trypsin for 20min and then with neuraminidase for 20min, or by the reverse sequence as detailed in the Experimental section. Measurements of electrophoretic mobilities were made at $25\pm0.1^{\circ}\text{C}$ in 0.0145M-NaCl, 0.3mM-NaHCO₃ and 4.5% (w/v) sorbitol, pH7.2 $\pm$ 0.1. Data for mobilities are means $\pm$ s.e.m.

Treatment (μg of enzyme/mg of vesicle protein)	Mobility ($\mu\text{m}\cdot\text{s}^{-1}\cdot\text{V}^{-1}\cdot\text{cm}$)
None	-3.55 ± 0.04
Neuraminidase (60) alone	-3.56 ± 0.06
Trypsin (60) alone	-4.35 ± 0.09
Neuraminidase (60) and then trypsin (60)	-4.32 ± 0.07
Trypsin (60) and then neuraminidase (60)	-4.40 ± 0.11

Table 3. Electrophoretic mobility of guinea-pig synaptic vesicles after treatment with concanavalin A, trypsin and then concanavalin A or with hyaluronidase

Synaptic vesicles were treated for 20min at 37°C as detailed in the Experimental section. Measurements of electrophoretic mobilities were made at $25\pm0.1^{\circ}\text{C}$ in 0.0145-M-NaCl, 0.3mM-NaHCO₃ and 4.5% (w/v) sorbitol, pH7.2 $\pm$ 0.1. Data for mobilities are means $\pm$ s.e.m. Amount of enzyme is given as $\mu\text{g}/\text{mg}$ of synaptic vesicle protein.

Treatment	Mobility ($\mu\text{m}\cdot\text{s}^{-1}\cdot\text{V}^{-1}\cdot\text{cm}$)
None	-3.55 ± 0.04
Concanavalin A alone	-3.73 ± 0.04
Trypsin (25 μg) alone	-3.98 ± 0.04
Trypsin (25 μg) and then concanavalin A	-4.12 ± 0.06
Hyaluronidase (60 μg)	-3.46 ± 0.06
Chondroitinase ABC (60 μg)	-3.45 ± 0.07
DNAase (60 μg)	-3.54 ± 0.04
RNAase (60 μg)	-3.54 ± 0.07

trypsinized concanavalin A led to a slight further, insignificant, increase in mobility, as shown in Table 3.

Treatment with RNAase, DNAase, chondroitinase ABC or hyaluronidase had no significant effect on synaptic vesicle electrophoretic mobility, as shown in Table 3. However, concentrations of these

Fig. 3. Electrophoretic mobility of isolated guinea-pig synaptic vesicles as a function of bulk pH

Experiments were performed as given in the Experimental section at $25\pm0.1^{\circ}\text{C}$ in solutions of 0.0145M-NaCl-4.5% (w/v) sorbitol. The pH was adjusted with iso-osmotic NaOH or HCl. Vertical lines indicate s.e.m.

enzymes likewise had no effect on synaptosomal electrophoretic mobility.

Effects of pH and ionic strength. The variation in electrophoretic mobility with bulk pH at a constant ionic strength of 0.0145, shown in Fig. 3, indicates the presence of a mono-ionic group with a pK_a between 3.0 and 3.8. Mobility is essentially constant at $-3.55\mu\text{m}\cdot\text{s}^{-1}\cdot\text{V}^{-1}\cdot\text{cm}$ above pH5.0 (Fig. 3).

The data of Fig. 4 demonstrate that the electrophoretic mobility of synaptic vesicles at pH7.2 is a function of the bulk ionic strength of the suspending media.

Effects of bivalent cations. Table 4 gives the results of studies of the decrease in the mobility of synaptic vesicles by bivalent cations in the measuring medium. Mn^{2+} and Ca^{2+} were significantly more effective in decreasing vesicle mobility than was Mg^{2+} , Sr^{2+} or Ba^{2+} . Further studies are in progress to determine selectivity sequences and the effect which macro-environmental influences such as pH and ionic strength may have on these sequences (Diamond & Wright, 1969).

Fig. 4. *Electrophoretic mobility of isolated guinea-pig synaptic vesicles as a function of ionic strength*

Experiments were performed as given in the Experimental section at $25 \pm 0.1^\circ\text{C}$ in 4.5% (w/v) sorbitol–0.3 mM NaHCO_3 , pH 7.2 ± 0.1 ; ionic strength adjustments were made with NaCl and for very low ionic strengths the NaHCO_3 was omitted and the measurements were made in water containing 4.5% (w/v) sorbitol. Vertical lines indicate S.E.M.

Discussion

The data presented show that the membranes of isolated guinea-pig synaptic vesicles are strongly negatively charged at near-physiological and physiological values of pH and ionic strength. In saline medium the mobility was determined to be $-1.35 \pm 0.04 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$. This corresponds to an uncorrected zeta potential of -17.3 mV . Neglecting relaxation effects, but including a correction for field interactions necessitated by the small size of vesicles, results in a slightly larger zeta potential, -18.9 mV . In saline–sorbitol medium, above pH 5.0, mobility was essentially constant at $-3.55 \pm 0.04 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$, corresponding to an uncorrected zeta potential of -45.6 mV . Neglecting relaxation effects, which in this instance should influence results only slightly (Davies & Rideal, 1963), but using a sizeable correction for field interactions, results in an estimated zeta potential of -54.3 mV . The magnitude of the mobility of synaptic vesicles can be

Table 4. *Effect of various bivalent cations on electrophoretic mobility of isolated guinea-pig synaptic vesicles*

Mobilities were determined as detailed in the Experimental section at $25 \pm 0.1^\circ\text{C}$ with the chloride salt of the bivalent cation present at 0.010 M; ionic strength was constant at 0.145 made up with NaCl. Solutions were 0.3 mM NaHCO_3 –4.5 (w/v) sorbitol, pH 7.2 ± 0.1 . Data for mobilities are means $\pm$ S.E.M.

Bivalent cation	Mobility ($\mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$)
None	-1.35 ± 0.04
Mn^{2+}	-0.75 ± 0.04
Ca^{2+}	-0.90 ± 0.03
Mg^{2+}	-1.04 ± 0.02
Sr^{2+}	-1.04 ± 0.05
Ba^{2+}	-1.05 ± 0.06

appreciated by a comparison with other mammalian cell and organelle surfaces. Synaptosomes derived from guinea-pig cerebral cortex, for example, have a mobility of $-1.86 \pm 0.006 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$ (Bosmann, 1972a) measured in saline–sorbitol; rat cerebral-cortex mitochondria, $-2.88 \pm 0.01 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$ (Bosmann *et al.*, 1972); human erythrocytes, $-2.78 \pm 0.08 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$ (Heard & Seaman, 1960). Numerous other mammalian membrane surfaces are characterized by electrophoretic mobilities much lower than that found for synaptic vesicles. The chromaffin granule membrane is an important exception to this generalization (Matthews *et al.*, 1972) and will be discussed below.

The variation of electrophoretic mobility with pH at constant ionic strength (Fig. 3) indicates the involvement of a dissociable acid function with a pK_a in the range of 3.0–3.8, suggestive of weak carboxyl groups. This value seems larger than the pK_a 2.0–2.5 expected for phosphate groups (Bangham *et al.*, 1958), and the rather surprising finding that neuraminidase treatment had no effect on mobility makes it unlikely that the relatively strong carboxyl groups of sialic acids contribute significantly to vesicular electrokinetic properties. Neuraminic acids may well be present in vesicles (Wesemann *et al.*, 1971), but are either refractory or inaccessible to neuraminidase attack as judged by the inability to demonstrate released sialic acid after neuraminidase treatment and, as indicated, must be present in small amounts.

Treatment of vesicles with hyaluronidase or chondroitinase (Table 3) did not result in any significant alteration of mobility. Taken together, these data suggest that glycosaminoglycans may not contribute importantly to the vesicular electrokinetic surface. The sulphated mucopolysaccharides

reported by Åborg *et al.* (1972) to be present in vesicular fractions derived from rat, cat and dog brain and postulated by Uvnäs (1973) to be involved in transmitter storage and release, if present, may be in more interior regions of the vesicle and probably are not important in determining vesicle surface properties. Possible species differences, however, require that the present results be confirmed for other synaptic vesicle preparations.

While this study was in progress Matthews *et al.* (1972) published the results of electrokinetic studies of isolated bovine chromaffin granules. In 0.16M-KCl at a pH above 6.5, granule mobility was determined to be $-1.12 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$, compared with the present value of $-1.35 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$ found for synaptic vesicles in a slightly different medium. The granule average surface charge density was calculated to be $1.38 \times 10^{-6} \text{C} \cdot \text{cm}^{-2}$ or one electronic charge per 11.7nm^2 ($1167 \text{\AA}^2$). In saline medium the average surface charge density of the vesicular surface computes to $1.70 \times 10^{-6} \text{C} \cdot \text{cm}^{-2}$ or one charge per 9.4nm^2 ($943 \text{\AA}^2$). As was mentioned in the Experimental section, use of the Gouy equation to calculate an exact numerical value for the average charge density of small spheres is not strictly justified, but it is apparent that the values obtained for the granule and synaptic vesicle membrane are of similar magnitude; both surfaces are highly negatively charged.

Matthews *et al.* (1972) calculated a value of 3.11 for surface pK_a , and although neuraminidase treatment did decrease the mobility of chromaffin granules somewhat, the authors concluded that their data were most consistent with the idea that the net charge of the granule membrane at the surface of shear was due largely to non-sialic acid carboxyl functions. The present data for synaptic vesicles indicate that an even higher pK_a value obtains, that neuraminic acid carboxyl groups are not a major source of negativity at the shear surface, and that the vesicle membrane may be even more negatively charged than the granule membrane.

Experiments with bivalent cations indicated that under the conditions given Mn^{2+} and Ca^{2+} were significantly, if not dramatically, more effective than was Mg^{2+} , Sr^{2+} or Ba^{2+} in decreasing the electrophoretic mobility of synaptic vesicles. As selectivity patterns and pK_a are a function of site field strength (Diamond & Wright, 1969), any alterations of the chemical environment near sites can be expected to alter these parameters. Thus it would be of some interest to determine selectivity sequences for both univalent and bivalent cations and the influence on the apparent pK_a of a wide variety of environmental conditions. The spread of excitation into nerve terminal regions is undoubtedly paralleled by transient perturbations of the nerve-ending micro-environment. *A priori*,

these perturbations may be expected to influence the energetics of any interaction between synaptic vesicles and the synaptic membrane, affecting the field strength of various interacting sites. Alternatively, or in addition, changes in the molecular micro-environment may bring about a re-orientation of sites, again leading to altered field properties capable of influencing membrane-membrane interactions. The present data are consistent with the notion that Mn^{2+} and Ca^{2+} under the conditions given for the experiment were more effective than were Mg^{2+} , Sr^{2+} and Ba^{2+} in decreasing the average net negative charge of the synaptic vesicle membrane. These preliminary results are encouraging when the critical role of Ca^{2+} in neurotransmitter release is considered and are an impetus for further study.

This laboratory has previously published the results of similar studies of the electrokinetic properties of isolated guinea-pig synaptosomes (Bosmann, 1972a; Bosmann & Carlson, 1972) and a comparison of their properties with those of synaptic vesicles is of some interest. Synaptosomes in saline-sorbitol media had an electrophoretic mobility of $-1.86 \pm 0.04 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$, compared with the unusually high value of $-3.55 \pm 0.04 \mu\text{m} \cdot \text{s}^{-1} \cdot \text{V}^{-1} \cdot \text{cm}$ for synaptic vesicles. Neuraminidase treatment of synaptosomes decreased mobility by 20% and did so in a time- and concentration-dependent manner. Release of synaptosomal sialic acid closely paralleled the decrease in mobility and the calculation of a pK_a of 2.7 for an acid function at the surface of shear was consistent with the conclusion that sialic acid is present at the nerve-ending periphery and significantly contributes to the electrokinetic properties of the synaptosomal surface. The mobility of synaptic vesicles, as discussed previously, was unaffected by neuraminidase treatment over a wide range of concentrations. In addition, whereas treatment of synaptosomes with trypsinized concanavalin produced a definite decrease in mobility, similar treatment of vesicles led to a slight increase in mobility. Thus the electrokinetic properties of isolated synaptosomes and the properties of synaptic vesicles are markedly different, not only in the magnitude of the charge at the shear plane but also in the nature of the groups contributing to that charge. This fact may relate to the mechanism of neurotransmitter release.

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