A SEMIQUANTITATIVE THEORY OF SYNAPTIC VESICLE MOVEMENTS

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ABSTRACT Under the assumption that vesicles are the anatomic correlate of quantal release, the forces governing the movement of synaptic vesicles inside neurons are analyzed. Semiquantitative calculations are presented to show that a diffuse layer field penetrates a few Debye lengths into the axoplasm. This field binds tightly a monolayer of water to the membrane forming the potential barrier for miniature end-plate potential (mepp) release. The action potential destroys the monolayer and pulls the vesicle to the membrane. The vesicles are brought to the synaptic zone and held there by a Na^+ leak in the synaptic membrane. A stochastic theory of synaptic vesicle release is presented to explain experimental results. The rate of vesicle release is fractionated into a rate of membrane contacts by a vesicle and a rate of vesicle discharge per contact.

INTRODUCTION

The intraneural life of a synaptic vesicle is made up of: (*a*) production, (*b*) movement to the immediate presynaptic zone, (*c*) storage in the presynaptic zone, (*d*) movement to the synaptic membrane for release of contents, (*e*) release of contents outside the cell, and (*f*) a speculated reuse of the vesicle (1). The movement to the synaptic membrane, (*d*) can be subdivided into (*i*) "spontaneous" movement associated with the physiologic observation of spontaneous mepps and (*ii*) synchronous, action potential determined release. A theory is presented here to explain *b*, *c* *d*(*i*), *d*(*ii*). *e* is based on the assumption that the vesicles seen by the electron microscope in presynaptic areas are the anatomic correlates of the mepp quanta recorded electrophysiologically from the postsynaptic cell. This assumption recently received strong support from work in the frog neuromuscular junction (2) and the assumption is used here. No explanation for *a*, *e*, or *f* is attempted.

For an object, the synaptic vesicle, to move through a viscous medium, axoplasm, requires force $F = \mu\, dx/dt$ where μ is the viscosity. Only a limited number of forces can exist at sufficient levels inside the neuron to explain physiological movements. Gravitational, nuclear, and magnetic forces are beyond question not appropriate. This leaves only three possible forces: electric fields, thermal agitation, and chemical

bonds. The vesicle is of a small enough size that it must be subject to significant thermal bombardment and brownian movement (3). Neurons are distinguished in part by the intensity of their electrical behavior. The energy represented by the ionic disequilibrium between the inside and the outside of the cell can and does produce intense electric fields. Finally chemical forces are obviously exceedingly important. One purpose of this paper is to show that thermal and electrical forces are sufficient to explain many of the intraneuronal movements of vesicles. The structural membrane transformations obviously needed to produce the vesicle and for emptying the vesicle into the synaptic cleft almost certainly are chemical bonds and are beyond the scope of this paper.

The theory necessarily deals with an idealized neuron and an idealized synapse. All of the values used in the calculations are "typical" values drawn from the literature or are specifically stated as assumptions. The physiologic reasonableness of the assumptions are evaluated. Nevertheless there is no typical synapse and actual values vary. Therefore the theory can only be semiquantitative and show that within an order of magnitude the values derived from the theory meet known experimental results.

SYMBOLS

q Membrane charge density.
c Membrane specific capacitance.
V Membrane voltage.
$\phi(x)$ Transient electric potential inside the neuron as a function of distance from the membrane.
k Boltzmann's constant.
T Temperature.
e Electron charge.
z Number of electron charges on principal axoplasm ions equal to 1.
L_D Debye length.
A A constant of Gouy-Chapman theory.
ϵ_0 Permittivity of free space which equals 8.8×10^{-12} F/m.
ϵ Relative permittivity of water near the membrane.
c_0 Concentration of axoplasm ions.
G Arbitrary constant introduced to account for membrane geometry.
η Viscosity
Z Number of electron charges on a vesicle.
R Radius of vesicle.
Q Ze which equals charge on a vesicle.
F Force on a vesicle.
m Dipole moment.
U Energy barrier of vesicle release.
P Total probability of vesicle release.
I Total probability of reaching the synapse wall.
M Total probability of membrane releasing a vesicle.
E Intraneuronal electric field generated by membrane leak.

λ Length constant of membrane.
τ Length of time a vesicle will be in a given "state."
N_0 Average number of vesicles in any state.
H Number of water molecules removed from membrane to permit vesicle release.

THEORY

Spontaneous Quantal Release

A vesicle situated in the interior of a neuron which is not firing undergoes brownian movement. Since axoplasm is a good electrical conductor, unless there is a membrane leak or some other current flowing, no electric field will exist (4). The membrane of the neuron is not a good conductor and charge can and does collect there (5). The amount of this charge is estimated from the cable properties of the membrane.

$$q = cV = (10^{-2}\ \mathrm{F/m^2})(10^{-1}\ \mathrm{V}) = 10^{-3}\ \mathrm{C/m^2}.$$

The capacitance used for this estimate is from axon membrane, since no measurements for synaptic membrane exist. Among all neural membranes whose capacitance has been measured, however, less than one order of magnitude variation exists, so the estimate should be reliable.

This negative electric charge generates an electric field which penetrates the axoplasm only a short distance as it decays to zero (6). The shape of this field is given by the Gouy-Chapman equation (7).

$$\phi(x) = \frac{4kT}{ze} \tanh^{-1}\left[\tanh\left(\frac{ze\phi(0)}{4kT}\right)\right] \exp\,(-x/L_D), \tag{1}$$

where x is the distance from the membrane, $\phi(0)$ the value of the generated field at the membrane, and L_D the Debye length. The Gouy-Chapman equation is a theoretical formula based on the thermodynamic equilibrium of mobile charges in response to a fixed charge or field. The axoplasm is a salt solution to which the equation would apply well. The actual fields, however, are known to be significantly altered by details of the geometry, chemical structure, and charge distribution at the surface. There is simply not enough known about these details of membranes to be sure how they deviate from the idealized basis of the equations.

The Debye length is given by

$$L_D = \left(\frac{kT\epsilon\epsilon_0}{8\pi c_0 z^2 e^2}\right)^{1/2}, \tag{2}$$

which for these cases is 5×10^{-8} cm = 5 Å. Therefore for $x \gg L_D$ the effect of this charge vanishes (8). Close to the membrane it can be significant.

$$\phi(0) = \frac{2kT}{ze} \sinh^{-1}\frac{q}{A}. \tag{3}$$

Where A is a constant given by

$$A = \frac{kT\epsilon_0\epsilon}{4\pi z e L_D} = \frac{(1.3 \times 10^{-23}\ \text{J/K})(300\ \text{K})(8.8 \times 10^{-12}\ \text{F/m})(3)}{4\pi(1.6 \times 10^{-19}\ \text{C})(5 \times 10^{-10}\ \text{m})},$$

$$= 8 \times 10^{-5}\ \text{C/m}^2. \tag{4}$$

For the first 5 Å the relative permittivity may be estimated (9) at 3 which in this situation gives $A = 8 \times 10^{-5}$ C/m^2. Therefore

$$\phi(0) = \frac{2(1.3 \times 10^{-23}\ \text{J/K})(300)}{1.6 \times 10^{-19}\ \text{C}} \sinh^{-1}\left(\frac{10^{-3}}{8 \times 10^{-5}}\right),$$

$$= 6 \times 10^{-1}\ \text{V}. \tag{5}$$

Therefore to a good approximation (8)

$$\phi(x) = 6 \times 10^{-1} \exp\ (-x/5 \times 10^{-10}\ \text{m})\ \text{V}.$$

This can be written

$$\phi(x) = \phi(0)e^{-x/L_D},$$

with

$$\frac{d^2\phi(x)}{dx^2} = \frac{\phi(0)}{L_D^2} e^{-x/L_D}. \tag{6}$$

This estimate is almost certainly high. Many factors of local detail would lower the estimate. If the electric charge were deep in the membrane then the field intensity at the axoplasm boundary might be lower than the estimate. If the membrane were 80 Å thick the "inside charge" could be 20–30 Å from the axoplasm. If the mobile charge existed inside the membrane and it were to give a Debye length of 20–30 Å then the potential at the axoplasm surface would have decayed about a factor of 2 or 3. Also, because of the volume near the membrane taken by the vesicles and membrane extensions (10), the effective ionic strength of the axoplasm may be less than bulk axoplasm, thereby raising L_D a small percentage. Local details of geometry could be important. It is unlikely, however, that even if all of the unfavorable assumptions were combined that they would exceed a factor of 0.2. The theory can therefore be continued including a geometry factor (G). Using 0.2 as a lower limit of G gives a value of $\phi(0) \geq 1.2 \times 10^{-1}$ V.

The charge on the vesicle has been studied in two different ways with radically different results (11, 12). The results of direct biochemical isolation of the vesicles and measuring their mobility by electrophoresis in a sucrose gradient are used here. The methods used in the isolation are mechanical and osmotic. These methods should not alter the chemical structure of any charge-bearing portions of the vesicle mem-

brane. Therefore the charge, when measured at physiologic pH, should be correct. The measured mobility of the vesicle is 0.96 μm/s per V/cm. Given a vesicle of measured electrophoretic mobility (v/ξ) and a vesicle radius of 2.5×10^{-6} cm, the charge on the vesicle may be calculated:

$$\begin{aligned} Q = Ze &= 6\pi R\eta\left(\frac{v}{\xi}\right), \\ &= 6\pi(2.5 \times 10^{-8}\ \text{m})(5 \times 10^{-3}\ \text{m}\cdot\text{kg}\cdot\text{s}\cdot\text{P})\left(0.96 \times 10^{-8}\ \frac{\text{m/s}}{\text{V/m}}\right), \\ &= 1.9 \times 10^{-17}\ \text{C} = 120 \text{ electron charges.} \end{aligned} \tag{7}$$

Because the electric field decays rapidly with distance, the force on the vesicle must be estimated by integrating the electric field over the vesicle charge distribution as a function of distance from the front end of the vesicle. Assuming the vesicle to be spherical for approximation (see Fig. 1)

$$\begin{aligned} F &= \int_0^{2R}\int_0^{2\pi} \frac{Q}{4\pi R^2}[\phi(0)e^{-x/L_D}](r\ d\theta)\left(\frac{dx}{\sin\phi}\right), \\ &= \frac{Q}{4\pi R}\int_0^{2R}\int_0^{2\pi} e^{-x/L_D}\ d\theta\ dx, \\ &= \frac{QL_D}{2R}\ e^{-x/L_D}\Big|_0^{2R} \approx \frac{QL_D}{2R}. \end{aligned} \tag{8}$$

The force on the vesicle is the same as if a charge of $QL_D/2R$ were at the front of the vesicle. $L_D/2R \approx 1/80$. This gives a potential barrier, for the vesicle to reach the inside surface of the membrane due to electrostatic repulsion, which is

$$\begin{aligned} U &= Q\left(\frac{L_D}{2R}\right)\phi(0), \\ &= (1.9 \times 10^{-17}\ \text{C})\left(\frac{1}{80}\right)(1.2 \times 10^{-1}\ \text{V}), \\ &= 0.29 \times 10^{-19}\ \text{J} = 0.18\ \text{eV}. \end{aligned} \tag{9}$$

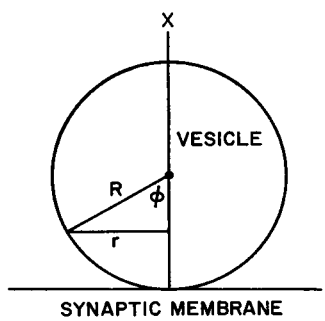

FIGURE 1 Diagrams showing a vesicle touching the membrane. θ is used for rotation about X.

Therefore the contribution of the electrostatic repulsion of the vesicle to the energy barrier-limiting mepp rate is insufficient compared with known values.

The electric field also affects the dipole of the water molecules inside the axon. Water has a fixed dipole of 6×10^{-30} C·m. The force induced by an electric field on a dipole is given by

$$F(x) = m\frac{d^2\phi(x)}{dx^2} = \frac{Gm\phi(0)}{L_D^2}e^{-x/L_D},$$

$$= (6 \times 10^{-30}\ \text{C·m})\left[\frac{1.2 \times 10^{-1}\ \text{V}}{(5 \times 10^{-10}\ \text{m})^2}\right]e^{-x/L_D},$$

$$F(0) = 3 \times 10^{-12}\ \text{N}. \tag{10}$$

To discharge its contents a vesicle membrane must "contact" the synaptic membrane. Then a chemical reaction of membrane rearrangement occurs. It is exceedingly unlikely that the electron transfers of such chemical reactions could occur across a water molecule barrier. Therefore the water monolayer must be removed. Because of the rapid attenuation of the field, the binding energy of the "second layer" may be assumed to be negligible. The energy needed to push one molecule of H_2O from the first layer to the second layer, a distance of about 5 Å, is

$$U = \int_0^{5\text{Å}} F(x)\,dx = \int_0^{\sim L_D} \frac{Gm\phi(0)}{L_D^2}e^{-x/L_D}\,dx = \frac{Gm\phi(0)}{L_D}(1 - e^{-1}),$$

$$\approx 10^{-21}\ \text{J} = 5 \times 10^{-3}\ \text{eV}. \tag{11}$$

Assume that an active site on the vesicle must be brought to within 1 Å of an active site on the membrane. The effective diameter of a water molecule is 4 Å. Then the vesicle must penetrate about 4 Å into the tightly bound monolayer. Using Fig. 1, if $x = 4$ Å and $R = 250$ Å, then $r = 45$ Å. Thus a circle of radius $r = 45$ Å must be denuded of its monolayer of tightly bound water. This is the area covered by about 100 molecules of water. Using the lowered estimate of the voltage involved this takes 10^{-19} J = 0.5 eV. Without any implication of false exactitude this compares reasonably well with the known value of 0.54 eV and 0.79 eV (13, 14). The electrostatic repulsion would contribute part of the difference.

The probability of synaptic vesicle release P (total probability of release per unit time) may be considered in terms of: I, probability of contact with membrane per unit time, and M, probability of release per contact. (I standing for interior and M for membrane.) Although it is not conclusive, there is evidence from the normal distribution rather than Poisson distribution of quantal content for action potential-triggered release that M is not small. Therefore, for spontaneous mepp release, I should be the rate-limiting step. Later calculations show that the action potential so increase I that M becomes the rate-limiting step. Factors such as Ca^{++} and Mg^{++} which affect both spontaneous and triggered release act through M.

The I to be expected from the theory can be calculated. The vesicle concentration near the synaptic membrane is known (1) from electron micrographs to be about 200 μm^{-3}. Since the cross-sectional area of the synaptic membrane has a radius (1, 10) of 0.2 μm and an area of 4×10^{-2} μm^2, the density of vesicles per distance from the membrane is 240/μm. The chemical reaction between the vesicle membrane and the neural membrane which permits transmitter release can only occur when they are no more than a few angstroms apart; therefore the reaction must depend on the concentration in the last 4 Å. Because of the water barrier, however, the vesicles are less likely to be in the last few angstroms than in all the other positions. The relative probability is given by the Boltzmann distribution. Using the monolayer barrier only and ignoring electrostatic repulsion

$$\exp\ (-U/kT) = \exp\ (-0.50\ \text{eV}/0.025\ \text{eV}) = e^{-20} = 2 \times 10^{-9}. \quad (12)$$

Therefore the concentration in the last 4 Å is about 4.8×10^{-11} vesicles/Å. From the ergotic hypothesis, this is the same as the portion of time each of the last 4 Å will be occupied. The frequency with which the state is occupied is the probability of the state divided by the length of time a state lasts. Under brownian movement a particle will move according to the Einstein equation. The time a vesicle occupied 1 Å is

$$\tau = 3Rx^2 = \frac{3(2.5 \times 10^{-8}\ \text{m})(5 \times 10^{-8}\ \text{P}\cdot\text{m}\cdot\text{kg}\cdot\text{s})(10^{-10}\ \text{m})^2}{(1.3 \times 10^{-23}\ \text{J/K})\ 300\ \text{K}} \quad (13)$$

gives

$$\tau = 3 \times 10^{-9}\ \text{s/Å}$$

This gives

$$I = \frac{\text{probability}}{\text{time of state}} = \frac{4.8 \times 10^{-11}\ \text{vesicle/Å}}{3 \times 10^{-9}\ \text{s/Å}} = 0.016\ \text{contacts/s}. \quad (14)$$

The actual frequency would be lower depending on the electrostatic repulsion. The known mepp frequency equals P, typically 0.5–1.0/s at the neuromuscular junction. Since this included 10^2 synaptic endings (15), M must be about 1.

Action Potential-Triggered Release

When an action potential reaches a synapse the intense Na^+ current completely depolarizes the membrane and produces about 10–20 mV of reverse potential. While the time of this overshoot is only a fraction of a millisecond and the whole action potential only about 1 ms, it is quite long compared with the mobility of vesicle across the last few angstrom or compared with the discharge time of the membrane capacitance. Therefore, for approximation purposes, the effect of depolarization and overshoot may be considered a steady-state problem. As soon as the mem-

brane capacitance discharges, which takes only a few microseconds (5), the probability of a vesicle filling that last angstrom would be the same as all the other angstroms at 1.8×10^{-2}. In only 0.5×10^{-3} s this would imply 4×10^{5} contacts.

Even this estimate would be low if one included the force of the transmembrane current pulling the vesicles toward the membrane wall. Of course this number of contacts does not occur. What must happen is that M now becomes saturated and it is the rate-limiting step. Since the delay in release is known to be between 0.5 and 2 ms (16), it must be assumed that no membrane site suitable for vesicle release gets a second chance in any action potential. The number of release sites has been estimated very grossly to 200 ± 100 (1). This then would be the maximum quantal content. If special conditions exist, like two release sites not being usable at the same time, then the quantal content would drop. If Ca^{++} operates from the outside of the membrane to control M, then since in the action potential M is the rate-limiting step, the effect of Ca^{++} on the probability of vesicle release could be expected to be greater (17).

Concentration of Vesicles Near the Synapse

One objection to the concept that the vesicles are free to move under brownian motion is the question as to why they do not then diffuse away from the synaptic ending (1, 3). In fact the problem should be inverted. Since the vesicles *are* subject to brownian forces *something* must be holding them. There are, in fact, only two conceivable somethings. Either there is some matrix on axoplasm binding the vesicles or there is some field generated by the membrane of the synapse. The fact that no matrix extending through the whole presynaptic volume occupied by vesicles has been seen on electron microscopy (18) and that the concentration of vesicles falls off slowly as a function of distance from the synapse argue against the fixed matrix theory. Since gravitational and magnetic forces are again out of the question, the field must be an electric field. Since the axoplasm is a good conductor, there can be no field unless a current is flowing. Some continuous current must flow through the synaptic membrane. To attract the negatively charged vesicles it must be a depolarizing current, positive toward the inside. A likely candidate would be a Na^{+} leak.

Consider that the membrane of the synapse is assumed to be depolarized 10% by a specific Na^{+} leak. The leak would not markedly alter the previous calculations which are concerned with the few angstroms nearest the membrane. The barrier potential would only be reduced 10% and the basic analysis would be unchanged. Only a 10% reduction of the membrane voltage at the synapse would be sufficient to hold the vesicles at the synapse. Deeper inside the cell, where the Gouy-Chapman field has essentially vanished, the effects of a Na^{+} leak would not have vanished. The Na^{+} leaking in would diffuse into the interior of the cell. In the interior the potential would be dominated by the effects of the nonleaky membrane from other parts of the cell (4). Depending on the detail geometry of the synaptic ending, a

volume near the vesicle would be held electropositive compared with the surrounding axoplasm. This effect would attenuate with distance from the synapse according to the membrane constants and the geometry. The chance of a vesicle escaping from the presynaptic zone into the deeper interior of the cell is given by the Boltzmann distribution induced by the electric field of the leak.

$$\exp\ (-Q\Delta\phi/kT) = \exp\ (120 \cdot 0.01\ \text{V}/0.025\ \text{eV}) = \exp\ (-24).$$

The chance of a synapse losing a vesicle to the interior is very slight.

Is such a leak an impossible metabolic burden for a cell to have? A 10 mV depolarization requires a current $I = E/R$, where R is the resistance of a patch of membrane to be depolarized and E is the 10 mV depolarization. If the membrane resistance (assuming it is the same as other neural membrane) is $2 \times 10^3\ \Omega/\text{cm}^2$, then an area

$$4\pi(10^{-1}\ \mu\text{m})^2 = 4\pi \times 10^{-10}\ \text{cm}^2,$$

gives $R = 2 \times 10^{12}\ \Omega$ which gives:

$$I = \frac{E}{R} = \frac{10^{-2}\ \text{V}}{2 \times 10^{12}\ \Omega} = 5 \times 10^{-15}\ \text{C/s},$$

$$= 3 \times 10^4\ \text{Na}^+\ \text{ions/s}. \qquad (15)$$

This is compared with 4×10^{-12} mol/cm^2 for an action potential. Thus if a neuron had an area of only 10^{-3} cm^2 it would lose (5) 2.4×10^7 Na^+ ions/impulse. If that neuron then had 10^3 synaptic endings, the Na^+ loss from these leaks would equal 1 impulse/s.

Movement to the Synaptic Area

Although controversy still rages over the site of production of synaptic vesicles (10), they obviously cannot be produced absolutely *in* the pool of existing vesicles. Therefore, some short-range transport to the presynaptic zone is needed. The Na^+ leak field will do this. Consider how far along a cylinder of membrane the effects of the Na^+ leak would spread. If the length constant of the membrane is λ then the value of the longitudinal electric field at k length constants from the leak is given by

$$E_z = \frac{dV}{dz} = \frac{\Delta V_0}{\lambda} e^{-k}, \qquad (16)$$

for a typical $\lambda = 3 \times 10^{-1}$ m and $K = 1$ gives $E_z = 1.3$ V/m.

Since the decay of the field is relatively slow compared with the vesicle size, the vesicle may be approximated as a point charge. The force on the vesicle is

$$F = QE_z = 120\ (1.6 \times 10^{-19}\ \text{C})(1.3\ \text{V/m}), \qquad (17)$$

$$= 2.2 \times 10^{-17}\ \text{N}.$$

Now for viscous flow

$$v = \frac{F}{\mu} = \frac{F}{6\pi a\eta} = 10^{-8}\ \text{m/s} = 3.6 \times 10^{-3}\ \text{cm/h}. \qquad (18)$$

This rate of transport is not a substitute for axoplasmic flow but it is more than enough to function as a "local delivery" system when the vesicles are in the general area, no matter how they got there.

Distribution of Vesicles

The force on a vesicle is determined by the shape of the electric potential field inside the neuron. This force together with other factors such as the total number of vesicles made available to the synapse will determine the equilibrium distribution of vesicles near the synapse. Details of local geometry will have strong effects. It is therefore not possible to give a general formulation of the cluster distribution of synaptic vesicles which the membrane Na^+ leak would cause. It was shown above that the forces generated by the leak are adequate to move a vesicle into the presynaptic cluster if it should be a short distance away. It was also shown that the probability of vesicle escape is small.

Typical synapses (1) show the vesicles clustered within a few 10ths of a micron of the synaptic membrane. Calculations of the shape of the electric potential (4) show that it will still be strong at this distance.

DISCUSSION

The theory states simply that the charge trapped inside the neuron by the membrane capacitance is sufficient to bind to the membrane a tightly held monolayer of H_2O. This monolayer covers the reactive sites on the synaptic membrane so tightly that only a very rare vesicle is bombarded hard enough by brownian motion to break through and make membrane contact. These few are assumed to produce the mepps. Membrane contact, given the proper Ca^{++}-Mg^{++} content of the external medium, usually leads to discharge. The effect of an action potential is to disrupt and loosen the H_2O monolayer and allow the vesicles through. Also, the transmembrane current creates an intense field drawing the vesicle to the membrane. Finally, to bring and keep the vesicles near the membrane, a slight Na^+ leak is proposed.

The Water Monolayer

The emphasis placed on the water monolayer distinguishes this theory from others that have been presented (1). The theory is compared below with the "calcium hypothesis" and the "thermodynamic theory." The prediction of a water monolayer and the calculation of its binding energy are based on physicochemical properties of the synapse and the Gouy-Chapman equations for diffuse layer fields. While many

local factors could amend the estimate of the binding energy, none of these factors nor all of them together are likely to be very large. An arbitrary 0.2 was introduced to account for these local factors.

It is unfortunate that the estimate must be so inexact. The fact that a crude estimate is so high rather than low, however, is important. This leads certainly to the idea that the monolayer is tightly bound, enough to explain the known energy barrier. A combination of factors which would lower the water barrier below the known energy barriers is not impossible but certainly improbable.

The release of transmitter into the synaptic cleft is generally understood to be based on some chemical reaction between the vesicle membrane and the synaptic membrane. This "chemical reaction" is a temporary reshaping of the membranes so that the interior of the vesicle is open to the synaptic cleft. Such reaction, almost certainly involving covalent bonds, is unlikely to occur across a water barrier. Even a monolayer of water would effectively interfere with the electron transfer and bond formation. Therefore the water must be removed.

The effective diameter of a water molecule is about 5 Å. The packing of the water is tight. Therefore, to clear the final monolayer, some molecules must be moved out against the binding force. This is where the energy of the barrier to mepp release is consumed. This barrier is so effective that it allows only one out of 10^{12} "attempts" through. It limits the access of vesicles to the membrane.

Release Statistics

The importance of the water monolayer permits the second distinctive feature of this theory, the separation of the statistics of release into an interior and a membrane component. It has been shown (19) that the compound probability of release under these conditions is

$$P = \frac{MI}{1 - (1 - M)(1 - I)}, \tag{19}$$

[Vere-Jones uses $p\bar{\alpha}$ for the compound probability with $\bar{\alpha} = \alpha/(1 - q\beta)$, $q = 1 - p$, $\beta = 1 - \alpha \Rightarrow p\bar{\alpha} = p\alpha/[1 - (1 - \alpha)(1 - p)]$. In the terminology used here $p \equiv M$ and $\alpha \equiv I$.] Under resting conditions with $I \ll 1$, $P \approx MI/[1 - (1 - M)] = I$. This means I is the rate-limiting step. When the monolayer is removed $I = 1$, then $P = M$ so that the membrane is the rate-limiting step.

Under resting conditions I is so low that M is usually unsaturated. This is the situation of spontaneous mepp release. The fundamental cause of the low probability of release is in the water monolayer. When the water monolayer is removed (by depolarization, etc.), however, the rate of release can be expected to increase. Rate of release may then be reduced through action on M. This is assumed to be the effect of Ca^{++} and Mg^{++}. There are two different components of release and therefore two essentially different ways to reduce the rate of release.

The statistics of the I component are Poisson. The "number of trials" is high and the probability very low. It is a function of the transmembrane potential but there is no clear reason for it to be sensitive to Ca^{++}. The statistics of M are binomial with a moderate number of trials and not too small a probability. Voltage may have no effect on M while Ca^{++} is an essential cofactor to M.

The theory resembles the thermodynamic type theory of Bass and Moore (20), except that the emphasis is placed on the membrane rather than on the vesicle water. They assume that the vesicle has bound water and that all of that water must be removed for transmitter release. This is as if the whole vesicle must pass through the membrane. If the charge on the vesicle is high, then dehydrating the 1% of the vesicle needed to expose the vesicle surface to the membrane could contribute to the energy barrier, but this contribution would be small.

The M component of the theory is a restricted form of the calcium hypothesis of Katz and Milhedi (16). The restriction of the calcium hypothesis to the membrane frees it of any need to explain the low rate of release when the membrane is polarized.

Comparison with Experiments

The complete formula for the frequency of mepps from this theory is $F = N_0 P$ under resting conditions $\approx N_0 I$ by Eqs. 12 and 14

$$F = N_0 \frac{\exp(-U/kT)}{\tau}, \tag{20}$$

by Eqs. 10 and 11 and following discussion

$$U = Hm \frac{\phi(0)}{L_D} G(1 - e^{-1}), \tag{21}$$

and by Eqs. 3 and 6 and using $\sinh^{-1} q/A \approx q/A$ and $q = cV$ gives:

$$\phi(0) = \frac{2kTcV}{zeA}, \tag{22}$$

therefore,

$$U = Hm \frac{2kTcVG}{zeAL_D}\left(1 - \frac{1}{e}\right). \tag{23}$$

Substituting in Eq. 20, taking the log, and differentiating

$$\begin{aligned}\frac{d \ln F}{dV} &= \frac{2Hm\left(1 - \frac{1}{e}\right)cG}{zeAL_D}, \\ &= \frac{2(100)(6 \times 10^{-30}\ \text{C}\cdot\text{m})(10^{-2}\ \text{F/m}^2)(0.2)\left(1 - \frac{1}{2.7}\right)}{(1)(1.6 \times 10^{-19}\ \text{C})(8 \times 10^{-5}\ \text{C/m}^2)(5 \times 10^{-10}\ \text{m})}, \\ &\approx 250\ \text{V}^{-1}. \end{aligned} \tag{24}$$

This is approximately the same as the experimental result of 160 (V^{-1}) (21).

In presenting the theory it was shown to match reasonably well the known energy barrier. Unless the energy barrier were, itself, a function of temperature this would mean that the theory would match the experimental data. We have:

$$U = Hm \frac{2kTcVG\left(1 - \frac{1}{e}\right)}{ZeAL_D}. \tag{25}$$

This would make it appear that U *was* a function of T. However,

$$AL_D = \frac{kT\epsilon\epsilon_0}{4\pi Ze}. \tag{26}$$

Thus giving

$$U = \frac{8\pi HmcV\left(1 - \frac{1}{e}\right)}{\epsilon\epsilon_0}. \tag{27}$$

Therefore, excepting any effects of temperature on M, the theory fits the experiments of the effect of temperature on the rate of quantal release.

The results of experiments with osmotic pressure (1) are harder to evaluate. Since the barrier is water molecules one would expect it to be sensitive to osmotic effects. It is a few specific water molecules, however, covering the reactive site and not the bulk water. The effects of osmotic pressure on these few water molecules would be an intense function of the local geometry controlling the patterns of water movement. If the membrane monolayer were pulled off by the osmotic pressure the mepp rate might increase by fluid movement carrying vesicles to the membrane. On the other hand, if the H_2O covering the reactive site were pressed even harder in place by osmotic pressure then the mepp rate might decrease. This cannot be systematically analyzed at this time.

The theory also explains recent developments in the statistics of quantal release (22). In this theory, spontaneous release is a Poisson process with a very large number of trials and a very small probability. On the other hand action potential-triggered release has a maximum N determined by the number of release sites, certainly 100 or even less while the probability M is normally high but may be reduced by high Mg^{++}-low Ca^{++}. This would be a normal distribution situation. It is interesting to note that Ca^{++}-Mg^{++} have a larger effect on high mepp rates than on low ones. The theory expects this, since M can only become a rate-limiting factor when I is high.

The Na^+ Leak

This part of the theory is basically independent of the rest. It does relate somewhat because of the necessity to have vesicle storage energy not be too much greater

than the thermal energy kT. If the vesicles were bound too tightly then brownian movement would be stopped.

One can wonder why no such leak has been described yet. Despite its intensity from the point of view of a vesicle, however, a variation of a few millivolts over a distance of about 1 μm very close to the membrane has not been specifically looked for and would offer significant technical difficulties.

The possibility of a Na^+ leak as a mechanism for vesicle storage is an intense function of the charge on the vesicle. It is evident from the completely arbitrary way in which the proposed Na^+ leak was introduced that if the charge on the vesicle is not the $-120\ e$ used here then some other current could be proposed. This would not alter the essential nature of the storage process proposed.

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