Biochimica et Biophysica Acta, 436 (1976) 613–616

BBA 77348

A SIMPLE MODEL FOR CALCIUM INDUCED EXOCYTOSIS

JAMES E. HALL[a] and SIDNEY A. SIMON[a, b]

[a]*Department of Physiology and* [b]*Department of Anesthesiology, Duke University Medical Center, Durham, N. C. 27710 (U.S.A.)*

(Received January 12th, 1976)

SUMMARY

We have developed a simple model showing how the presence or absence of Ca^{2+} can determine whether an uncurved or curved membrane surface is favored energetically. The model shows why fusion of vesicles with the presynaptic membrane is favored in the presence of calcium and why the budding off of vesicles is favored in the absence of calcium inside of the presynaptic membrane. The model accurately predicts the radius of a synaptic vesicle using known properties of lipids and suggests consequences of temperature change, varied stimulation rate and addition of calcium by artificial means on rates of transmitter release.

In chemical synapses, membrane depolarization initiates transmitter release. The immediate cause of this event is probably a transient increase of calcium inside the cell [1]. This increase in intracellular calcium is thought to increase the probability that the vesicles will fuse with the plasma membrane and release their contents [2].

At the neuromuscular junction [2], as well as in other systems [3], the vesicular material is apparently recycled during this process. In a recent paper Heuser and Reese [4] demonstrated that the total area of synaptic membrane, cisternal membrane, and surface membrane remains constant. They found upon stimulation that although the amount of membrane in synaptic vesicle form decreases, the amount in the form of plasma and cisternal membrane increases accounting for the synaptic vesicle loss. These authors as well as others suggest that these results imply that the vesicles are recycled [3]. The scheme they suggest is as follows: synaptic vesicles fuse to plasma membrane; plasma membrane buds to form vesicles; coated vesicles fuse to cisternae; cisternae divide to synaptic vesicles.

We propose a simple model which can account semiquantitatively for the budding event in this sequence. The model makes predictions which can experimentally be tested.

We assume that the vesicles and the plasma membrane contain significant amounts of negatively charged lipid and that there is an efficient mechanism for rapidly reducing the intracellular calcium concentration in the nerve terminal either by expulsion or sequestration. Both of these assumptions are justified by experimental evidence [1, 5–7].

We propose that the surface energy changes produced by entry and exit of calcium provide the driving force for the fusion and budding off of synaptic vesicles. The model can be made quantitative by a few simple considerations. For a bilayer in equilibrium, the attractive forces are balanced by compressional resistance or surface pressure [8]. At a given temperature this condition may be written:

$$\Pi = -K\frac{\Delta A}{A_0} \tag{1}$$

where, Π = surface pressure (dynes/cm); K = compressibility modulus of bilayer (dynes/cm); $\Delta A/A_0$ = area dilation of bilayer.

A bilayer consists of two monolayers apparently unconnected [9]. If the surface pressure of one monolayer changes relative to that of the other, the bilayer will respond by curving. For a bilayer that has spherical symmetry as a result of curvature, Evans has shown [9] that

$$\frac{1}{R^{\mathrm{i}}} + \frac{1}{R^{\mathrm{o}}} = \left(\frac{\Delta\Pi^{\mathrm{i}}}{K^{\mathrm{i}}} - \frac{\Delta\Pi^{\mathrm{o}}}{K^{\mathrm{o}}}\right)\frac{1}{d} \tag{2}$$

where R^{i} and R^{o} are the radii of curvature of the inside and outside bilayer; d = membrane thickness. If for simplicity, we let $R^{\mathrm{i}} \simeq R^{\mathrm{o}}$ and $K^{\mathrm{i}} = K^{\mathrm{o}}$ then Eqn. 2 becomes

$$\frac{2}{R} = \frac{\Delta\Pi^{\mathrm{i}} - \Delta\Pi^{0}}{Kd} \tag{3}$$

To get a quantitative estimate of the radius of curvature of budding vesicles, we need to know both $(\Delta\Pi^{\mathrm{i}} - \Delta\Pi^{0})$ the change in suface pressure difference and, the surface compressibility K, (assuming $d \approx 30$ Å). To relate the change of surface pressure difference to entry of calcium, we assume the surface pressure of each surface is given by the Davies equation [12] which relates surface pressure to surface charge:

$$\Delta\Pi = \frac{2kT}{e}\Delta\sigma \tag{4}$$

where T is temperature, k Boltzmann's constant, e the electronic charge, and $\Delta\sigma$ the surface charge. We further assume that the change in surface charge is produced by a change in Ca^{2+} concentration. This in turn alters the amount of Ca^{2+} available to screen or bind negative charges added to the membrane.

Since the calcium concentration is nearly constant outside the terminal, the change in surface pressure of the outside surface will be negligible compared to that of the inside surface. We can thus assume that no alteration of the outside surface pressure occurs and can consider only the change of the interior monolayer surface pressure. Combining Eqn. 3 and Eqn. 4 for the inside surface then gives

$$R = \frac{Kde}{kT}\frac{1}{\Delta\sigma^{\mathrm{i}}} \tag{5}$$

We assume that the negative charge density before entry of calcium arises from negative lipids and that Ca^{2+} entry reduces the charge to nearly zero by binding and screening.

If no negative lipids are available, there will thus be no change of charge with calcium entry.

We can now describe a cycle of fusion and budding according to our model. If the nerve has been unstimulated for some time, vesicles will be fusing with the presynaptic membrane infrequently producing the well known phenomenon of miniature end plate potentials. Now the nerve is stimulated resulting in a transient Ca^{2+} influx. In our model this has two consequences. First Ca^{2+} screens a negative charge on the interior of the presynaptic terminal reducing electrostatic repulsion between vesicles and membrane. Close contact between vesicle and membrane now becomes a more likely event as suggested by Van der Kloot and Kita [16]. Second the vesicles' negative lipids now bind the newly available calcium. This reduces the molecular area of the vesicle's outside monolayer and increases the energy of the highly curved vesicles' configuration (Eqn. 5). This energy can be reduced only by flattening, but the vesicle can flatten only by fusing with the presynaptic membrane! Details of the actual fusion process may be very complex, but the prefusion and post-fusion states are well defined. Our model shows how Ca^{2+} alters the relative energy of these states without having to consider the complicated transition states involved in the actual fusion process. The presence of calcium thus accomplishes two things which favor fusion. It promotes close approach of vesicles to the presynaptic membrane and lowers the energy of the flat state relative to the curved state.

If stimulation of the nerve stops, so will calcium entry, and the cell's mechanisms of calcium sequestration and expulsion will reduce the calcium concentration inside the terminal [4]. Now the negatively charged lipids*, newly added to the inside of the presynaptic membrane, will have their negative charges unscreened, and the energy of the curved state of presynaptic membrane will be reduced with respect to that of the flat. The membrane will thus begin to pucker inward and, if enough negative lipid has been added, will bud off into vesicles passing presumably through a complex series of states of inverse fusion. Again our model only describes the energy difference between the well defined end points.

We could calculate the expected radius of vesicles budding off from the membrane from Eqn. 5 if we knew the change in surface charge and the compressibility of the monolayers. We can take $\Delta\sigma^i$ equal to about one electronic charge per 50 $Å^2$. The surface compressibility can be estimated from lipid monolayer studies at about 50 dynes/cm (for a close-packed monolayer at an oil-water interface [12]). If we take $T = 300$ °K and $d = 30$ Å, Eqn. 5 gives $R = 180$ Å, a value in good agreement with observation [2].

Thus our model predicts that in regions of the plasma membrane where the surface charge density reaches a value of about one electronic charge every 50 $Å^2$, vesicles will begin to bud off when calcium is removed. The result depends on the

---

* The synaptic vesicles are negatively charged and have a diameter of about 300 $Å^2$ [2]. Vesicles of this size have been found to have about twice as many negatively charged lipids on the outside surface than the inside [10]. The topology of fusion then requires that more negative lipid go to the inside of the membrane than the outside. In addition any negative lipid going to the outside will be immediately neutralized by screening or binding of external calcium [11].

We also note that calcium interacts with most negatively charged lipids more strongly than magnesium [15].

actual properties of lipids of the presynaptic membrane and should be considered only as a semiquantitative estimate.

We can make several specific predictions which may be tested experimentally. First as the temperature is decreased, the amount of stimulation necessary to produce an appreciable rate of vesicle budding should increase. That is the plasma membrane should increase in area more for a given amount of transmitter released. Secondly, the addition of calcium (or other divalent ions) at a rate which cannot be overcome by the available elimination mechanisms should result in failure of the terminal to bud off vesicles and consequently replenish its supply of loaded synaptic vesicles. This could be accomplished by use of calcium ionophore [13]. Thirdly, any processes which would increase the ionic strength of the interior of the presynaptic membrane, such as increasing the osmolality [14] extracellularly, should increase the rate of fusion and decrease the rate of vesicles budding off.

This model is of course speculative, but it shows how the processes of fusion and budding off of vesicles may be driven by the entry and removal of calcium in a simple semiquantitative way**.

This work was supported by NIH grants HL-12157 and HL-17961-01 (to JEH) and ONR contract No. N0014-67-A-0251-0022.

REFERENCES

1 Katz, B. (1969) The Release of Neural Transmitter Substances p. 60 Liverpool University Press
2 Hubbard, J. I. (1970) in Mechanism of Transmitter Release. Progress in Biophysics and Molecular Biology. (Butler, J. A. V. and Noble, D. eds.) Vol. 21, pp. 33–123 Pergamon Press
3 Douglas, W. W. (1968) Br. J. Pharmacol. 34, 451–474
4 Heuser, J. E. and Reese, T. S. (1973) J. Cell Biol. 57, 315–344
5 Vos, J., Kuriyama, K. and Roberts, E. (1968) Brain Res. 9, 224–230
6 Gilbert, D. L. and Ehrenstein, G. (1970) J. Gen. Physiol. 55, 822–835
7 Tjioe, S., Bianchi, C. P. and Haugaard, N. (1970) Biochem. Biophys. Acta 216, 270–273
8 Evans, E. A. and Simon, S. A. (1975) J. Colloid Int. Sci. 51, 266–271
9 Evans, E. A., (1974) Biophys. J. 14, 923–931
10 Isrealachvilli, J. N. (1973) Biochim. Biophys. Acta 323, 659–663
11 McLaughlin, S. G. A., Szabo, G., Eisenman, G. and Ciani, S. (1970) Proc. Natl. Acad. Sci. U.S. 67, 1268–2175
12 Davies, J. T. and Rideal, E. K. (1961) Interfacial Phenomena, p. 231 Academic Press, New York
13 Kita, H. and Van der Kloot, W. (1974) Nature 250, 658–660
14 Hubbard, J. I., Llinas, R. and Quastel, D. M. J. (1969) Electrophysiological Analysis of Synaptic Transmission, p.123, The Williams and Wilkins Company
15 Dawson, R. M. C. and Hauser, H. (1970) Calcium and Cellular Function, (Cuthburt, A. W., ed.) pp. 17–43, St. Martins Press, New York
16 Van der Kloot, W. and Kita, H. (1973) J. Membrane Biol. 14, 365–382

** We are aware of several complications including the possibility that some of the vesicular material may retain an identifiable shape as it moves through the fusion-budding sequence. We also did not address the question of how vesicles m ght receive coats. Although these observations may be incorporated into the model with a simple ad hoc assumption, we will refrain from doing so because of possible artifactual origin of these observations.