*ELECTROKINETIC MECHANISM OF MINIATURE POSTSYNAPTIC POTENTIALS**

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In 1950, Fatt and Katz[1, 2] discovered the spontaneous subthreshold activity of motor nerve endings and were thereby led to the important concept of quantal release of neuromuscular transmitter. Electron microscopy[3, 4] of presynaptic regions subsequently revealed characteristic vesicles 300–500 Å in diameter which were concentrated sources of acetylcholine.[5] The quantal discharge of acetylcholine was therefore attributed to rupture of individual vesicles at the presynaptic membrane. The miniature end-plate potentials (MEPP) are completely independent events,[6, 7] each associated in almost every instance with the discharge of a single vesicle. We shall outline an electrokinetic model for this process consistent with present experimental data which indicate that $\ln\nu = f(T,P,E)$, where ν is the frequency of MEPP's, T the absolute temperature, P the osmotic pressure of the fluid in the synaptic cleft, and E the polarization of the presynaptic membrane. While the model may not be unique in its consistency with the data, it incorporates electrochemical concepts which are shown to be *quantitatively* appropriate to this new subject matter.

The normal frequency of MEPP's is 1 or 2 per sec, but Liley[8] observed a marked increase in ν with cathodic polarization and his measurements on rat-diaphragm preparations, as replotted by Katz,[9] yielded the empirical relation $d\ln\nu/dE = 1.63 \times 10^2$ [at 20°C], where E is in volts.

Our theoretical treatment is based on the theory of rate processes as applied to electrode phenomena.[10] Let us consider vesicles to be randomly approaching a presynaptic membrane with a total frequency ν_0 [$\sec^{-1}$]. If only those vesicles with an energy greater than some activation barrier U can come into contact with the membrane, discharge transmitter into it, and by a succession of faster processes produce miniature spikes, the discharge frequency will be $\nu_0 \exp(-U/kT)$. The membrane (without fixed charges) may be represented as a capacitor of thickness δ and dielectric constant ϵ'. Its equal and opposite surface charges are each distributed over a thickness of the order of a Debye length κ^{-1}. A positive charge in the presynaptic region must therefore overcome the fraction $\alpha = \epsilon'/\epsilon\kappa\delta$ of the total membrane potential difference in order to reach the membrane, ϵ being the dielectric constant of the intervening medium. Depolarization of the membrane by E decreases the corresponding electrical barrier for a positively charged vesicle by $z|e|\alpha E$ and the frequency increases to

$$\nu = \nu_0 \exp[(-U + z|e|\alpha E)/kT],$$

where $|e|$ is the elementary (electron) charge. Hence,

$$d\ln\nu/dE = z|e|\alpha/kT.$$

Comparison with the empirical result of Liley yields $\alpha z = 4.1$.

The repulsion at a distance κ^{-1} and less acts largely across water molecules hydrating the vesicle. If these were deprived of their entire orientational polariz-

ability by the bond, ϵ would be as small as 3; it is probably somewhat larger. ϵ' is at most that of water ($\epsilon' \leqslant 80$), but capacitance measurements in conjunction with the membrane thickness $\delta \sim 50$ Å indicate that it is rather lower. Taking $\kappa^{-1} \approx 8.6$ Å as below, we find α of the order of unity. If $\alpha \approx 1$ is assumed, then $z \approx 4$ is the average number of positive charges on a synaptic vesicle.

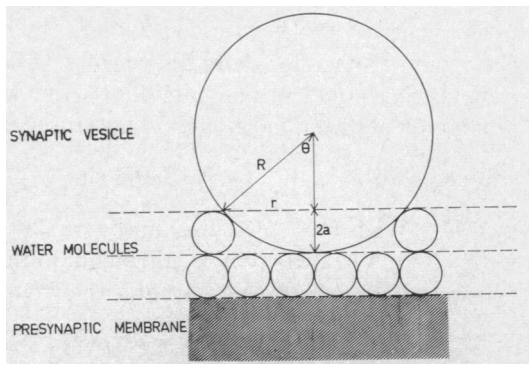

FIG. 1.—Model of rate-determining step in release of transmitter from a synaptic vesicle.

The barrier U can be estimated from the temperature coefficient of ν when E and P are both fixed. Fatt and Katz[2] obtained $Q_{10} = 3$ for the frequency of miniatures at neuromuscular junctions of frog between 8 and 25°C, which corresponds to $U = 0.79$ ev. Temperature-coefficient data on tenuissimus muscle of cat[11] yield $U = 0.54$ ev.

A model for the origin of such a barrier is shown in Figure 1. As a consequence of its electric charge, a vesicle will be hydrated. As such a hydrated vesicle comes into contact with the last monolayer of water separating it from the synaptic membrane, it must lose some or all of its hydrating water molecules before it can coalesce with the membrane and release transmitter into the cleft. Such a dehydration of the vesicle is here assumed to be the rate-determining step in its release of transmitter. According to the Born equation, the energy of hydration B of a sphere of charge $|ze|$ and radius R in a medium of dielectric constant ϵ is

$$B = [(ze)^2/2R]\left[1 - \frac{1}{\epsilon} - \frac{T}{\epsilon^2}\left(\frac{\partial \epsilon}{\partial T}\right)_p\right].$$

With $R = 2.0 \times 10^{-6}$ cm and $\epsilon = 80.1$, the value for free water, we obtain $B = 0.61$ ev. If we take the hydrating water without orientational polarizability ($\epsilon \approx 3$), B is reduced to a minimum value of 0.4 ev. The electrical part of the barrier for $\alpha z = 4$ and a resting potential difference of 70 mv is 0.28 ev. Thus, U is between 0.68 and 0.89 ev. This result compares favorably with experimental $U = 0.54$ and 0.79 ev, but it is sensitive to the charge on vesicles and we have had to use a charge deduced from experiments on rat junctions, whereas the thermal activation barrier was calculated from data on frog and cat junctions.

The Debye length $\kappa^{-1} = 3.04 \times 10^{-8}\, s^{-1/2}$ [cm], where s is ionic strength, about 0.13 in frog nerve.[12] Thus, $\kappa^{-1} = 8.6$ Å, about equal to the diameter of two water molecules. The activation-energy barriers due to electrical repulsion and due to dehydration of the vesicle should therefore nearly coincide, and hence subtraction of the electrical term $z|e|\alpha E$ from the barrier U is justified.

The most striking effect on the frequency ν of MEPP is the marked increase with osmotic pressure. For example, Fatt and Katz observed with frog myoneural junctions that 50 per cent increase in osmotic pressure by addition of sucrose was followed by a reversible 45-fold increase in ν, corresponding to $d\ln\nu/dc = 11.6$, where c is the molar concentration of osmotically active species, about 0.26 in normal frog nerve. Two other measurements gave 10.2 for the coefficient.

In accord with the present model, an increase in osmotic pressure lowers the activation barrier for dehydration of a vesicle. The osmotic pressure P acts over a distance x equal to one half the barrier width. From Figure 1, $x = 2a$, the diameter of a water molecule. The cross-sectional area A of the vesicle which is subjected to P is $A = \pi r^2 = \pi R^2 \sin^2\theta = \pi R^2\left[1 - \left(1 - \frac{2a}{R}\right)^2\right] \cong 4\pi aR$, since $a \ll R$. With $R = 2 \times 10^{-6}$ and $a = 2.0 \times 10^{-8}$ cm, $A = 5.0 \times 10^{-13}$ cm^2. If we let $P = NkT$, where N is additional number of solute molecules/cm^3, the barrier is lowered by $PAx = NkTAx$ and the frequency becomes

$$\nu = \nu_0 \exp(-U/kT) \exp(NAx).$$

Hence, so long as the osmotic pressure persists, the theoretical osmotic coefficient is

$$d\ln\nu/dc = Ax(6.02 \times 10^{20}) = 12.0,$$

in satisfactory agreement with experiments.

It is difficult to obtain a reliable experimental value for the frequency ν_0 per vesicle since it depends on the exact area of end plate that is an active source of MEPP's. The total ν_0 for an end-plate gutter can be obtained from $\nu = \nu_0 \exp(-U/kT)$ and the experimental U as $\nu_0 = 10^{15}$ sec^{-1}. If we assume that the terminal area S is that of a cylinder of radius 1 μ and length 1 mm,[4] $S = 2\pi \times 10^{-5}$ cm^2. If this area is covered with a close-packed layer of vesicles, their number $n = S/\pi R^2 = 5 \times 10^6$. Only a fraction of area is active, however. If $n \approx 10^6$, $\nu_0 \approx 10^9$ sec^{-1}, which is a reasonable pre-exponential factor for a collisional process in solution, although its precise nature cannot be specified at present.

When the frequency of MEPP's is increased by a timed potential pulse, the increase is found to lag behind the pulse by a few msec.[13] From a careful analysis of various possible components of such a synaptic delay, Katz and Miledi[13] concluded that it was mostly in the release of transmitter following arrival of a nerve impulse. It is interesting to note that this time lag is very close to the electrical relaxation time as given by the Maxwell formula $\tau = \epsilon'/4\pi\sigma$. (If one substitutes for σ the ionic conductivity, τ is the familiar Debye-Falkenhagen relaxation time.) We do not have experimental data on σ for the presynaptic end-plate membrane, but for squid axon $\sigma = 3 \times 10^{-9}\ \Omega^{-1}$ cm^{-1} is believed to be a good average value.[14] This yields $\tau = 2.3$ msec if ϵ' is equal to that of free water, or somewhat smaller with a more realistic value of ϵ'. This agreement is consistent with the discharge of acetylcholine through the membrane being preceded by dispersal of the charges of the (dehydrated) vesicle in the membrane. Although this dispersal is slow in absolute terms, it is a fast step in the present model (it could become rate-determining only if the frequency exceeded approximately 10^8 sec^{-1}).

Summary.—A model of the presynaptic mechanism determining the frequency of MEPP's (miniature end-plate potentials) in neuromuscular junctions is constructed in terms of electrochemical rate processes. Quantitative agreement with observations of the effects of depolarization, temperature, and osmotic pressure is obtained, and a quantitative interpretation of the Katz time lag is proposed.

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[1] Fatt, P., and B. Katz, *Nature*, **166**, 597 (1950).

[2] Fatt, P., and B. Katz, *J. Physiol.*, **117**, 109 (1952).

[3] De Robertis, E. D. P., and H. S. Bennett, *Federation Proc.*, **13**, 35 (1954).

[4] Birks, R., H. E. Huxley, and B. Katz, *J. Physiol.*, **150**, 134 (1960).

[5] De Robertis, E. D. P., A. Iraldi, G. Arnaiz, and L. Salganicoff, *J. Neurochem.*, **9**, 23 (1962) (Experimental evidence for presence of acetylcholine in vesicles at neuromuscular junctions is not yet available.)

[6] Del Castillo, J., and B. Katz, *J. Physiol.*, **124**, 560 (1954).

[7] Gage, P. W., and J. I. Hubbard, *Nature*, **208**, 395 (1965).

[8] Liley, A. W., *J. Physiol.*, **134**, 427 (1956).

[9] Katz, B., *Proc. Roy. Soc.* (*London*), **B155**, 455 (1962).

[10] Glasstone, S., K. J. Laidler, and H. Eyring, in *The Theory of Rate Processes* (New York: McGraw Hill Book Co., 1941), p. 575.

[11] Boyd, I. A., and A. R. Martin, *J. Physiol.*, **132**, 61 (1956).

[12] Hodgkin, A. L., *Biol. Rev. Cambridge Phil. Soc.*, **26**, 339 (1951).

[13] Katz, B., and R. Miledi, *Nature*, **207**, 1097 (1965).

[14] Floyd, W. F., in *Modern Aspects of Electrochemistry*, ed. J. Bockris (London: Butterworth Scientific Publications, 1954), vol. 1, p. 290.

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*ON THE GENETICS OF ENZYME LOCATIONAL SPECIFICITY**

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In the years since the pioneering work of Beadle and Tatum[1] that led to the one gene:one enzyme concept, the cell has not generally been treated as a highly integrated system. Nevertheless, biochemical mutants without defects in the primary structure or quantity of enzymes are known. In such instances, the *in vivo* function of an enzyme may be altered by a defect in its structural integration into a multienzyme system.[2] The apparent dichotomy between the two classes of enzyme alteration, structural and integrational, is resolved through the hypothesis of Mitchell,[3] who proposed that certain amino acids of an enzyme may be essential for binding to specific intracellular sites. Such "locational specificity" may not necessarily involve amino acids at or near the active center of the enzyme. Thus, mutations causing amino acid replacements at the "locational site" of the enzyme could alter enzyme function *in vivo* as a consequence of distorted conformation due to irregular binding. Hence, such a structurally altered enzyme may exhibit little or no modification of catalytic properties *in vitro* but may be functionally defective *in vivo*. Conversely, genetic alteration of the structure of a protein at a specific intracellular site may lead to *in vivo* malfunction of enzyme(s) bound to that protein.

This report describes experiments concerned with locational specificity of *Neurospora* malate dehydrogenase. In this system, two nonlinked structural genes code for the amino acid sequence of the α and β polypeptide subunits, respectively, of the enzyme protein of the quaternary structure, $\alpha\alpha\alpha\beta$.[4-8] Although mutations