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A QUANTITATIVE DESCRIPTION OF END-PLATE CURRENTS

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SUMMARY

1. End-plate currents have been studied in glycerol-treated frog sartorius nerve–muscle preparations with the voltage clamp technique.

2. The effects of temperature on the decay rate of end-plate currents were investigated over a temperature range from 10 to 30·5° C. The Q_{10} for the decay constant of end-plate currents depends somewhat on membrane potential; at -100 mV the decay constant has a Q_{10} of 2·7.

3. Peak end-plate current depends non-linearly on membrane potential with a decreasing slope conductance associated with hyperpolarization.

4. The 'instantaneous' voltage–current relationship for end-plate channels was determined by causing step changes in membrane potential during end-plate current flow. This relationship appears to be linear.

5. The interaction of acetylcholine with its receptor is viewed as being analogous to the first step in enzymic catalysis. On this view, acetylcholine binds to its receptor and induces a conformational change which is responsible for opening end-plate channels. By analogy to the first steps in the catalytic sequence of enzymes, the binding step is very rapid, almost diffusion-limited, and the conformational change is rate-limiting.

6. Equations describing this process have been derived. Expressions for the rate constants have also been derived by considering changing dipole moments of the transmitter-receptor complex associated with the conformational change. As the transmitter-receptor complex is in the membrane field, different conformational states have different energies, and the rate of conformational change thus depends on membrane potential. The equations thus derived are shown to account adequately for the time course of end-plate conductance change.

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INTRODUCTION

The end-plate currents of frog sartorius muscle decay with a simple exponential time course, and the rate constant characterizing this decay depends exponentially on membrane potential (Magleby & Stevens, 1972): end-plate current decay is slowed approximately twofold for each 100 mV of hyperpolarization. In this paper we develop a quantitative description for the time course of end-plate currents and their voltage dependence. Although sufficient information about end-plate physiology and structure is not yet available to permit a rigorous derivation of equations describing the end-plate currents, we do provide what is intended to be a plausible physical basis for our description.

Of the various different physical interpretations possible for the equations to be described here, we favour the following: acetylcholine is viewed as binding to its receptor, and inducing a conformational change which opens an end-plate channel. This conformational change follows first-order kinetics and is supposed to be the rate limiting process so that the exponential decay of end-plate currents reflects the relaxation of the receptor back to its normal, closed, conformation. Further, the receptor-acetylcholine complex, since it is embedded in the post-synaptic membrane, is in an electric field produced by the end-plate membrane potential; a 50 Debye (D) change in the dipole moment of this receptor-transmitter complex associated with the shift from an open to a closed conformation is adequate to account for the voltage sensitivity of the end-plate currents' declining phase.

In addition to the quantitative analysis of end-plate current time course presented here, we also include further experiments intended to give better definition to end-plate properties. Manipulation of temperature has revealed that the time constant for the declining phase of end-plate currents has a Q_{10} of approximately 3. We have confirmed Kordaš's (1969) observation that peak end-plate current often is non-linearly related to membrane potential, with a flattening of the voltage–current relationship for membrane potentials more negative than about -80 mV, but have presented evidence that the 'instantaneous' voltage–current relationship may well be linear.

METHODS

The methods used here are the same as those described previously (Magleby & Stevens, 1972). For experiments in which temperature was manipulated, cooling was accomplished by circulating coolant through coils in thermal contact with the base plate upon which the experimental chamber was mounted, the temperature being regulated by controlling the rate of coolant flow and its temperature.

The equations describing end-plate conductance changes (eqns. (1), (12) and (9c))

were solved with an analogue computer, and the solutions (Fig. 6) displayed on an oscilloscope. The driving function $W(t)$ (continuous curve in Fig. 4) was realized by applying a brief square voltage pulse to a suitable passive circuit.

RESULTS

Part I

The end-plate current decay constant has a Q_{10} of about 3

In order to specify better the properties of end-plate currents, and particularly to determine if their temperature sensitivity is that expected for simple diffusion or instead for some more complicated process, we have examined the effects of temperature over the range from 10 to 30·5° C. Graphs of the rate constant (α) for end-plate current decay as a function of membrane potential are presented in Fig. 1 for two preparations. From these data it is clear that cooling both prolongs end-plate currents and increases the slope of the log α *vs.* membrane potential relationship. As we have shown previously (Magleby & Stevens, 1972), currents decay exponentially with a rate constant α which depends exponentially on membrane potential (V)

$$\alpha(V) = Be^{AV}. \tag{1}$$

For one preparation (filled circles in Fig. 1), $A = 0{\cdot}93 \times 10^{-2}$ mV^{-1} and $B = 1{\cdot}55$ msec^{-1} at 22·5° C and $A = 1{\cdot}25 \times 10^{-2}$ mV^{-1} and $B = 0{\cdot}585$ msec^{-1} and 10° C, corresponding to a Q_{10} of 2·9 for α at -100 mV; for a second preparation (squares in Fig. 1), $A = 0{\cdot}64 \times 10^{-1}$ mV^{-1} and $B = 2{\cdot}37$ msec^{-1} at 30·5° C and $A = 1{\cdot}09 \times 10^{-2}$ mV^{-1} and $B = 1{\cdot}33$ mV^{-1} at 20° C, corresponding to a Q_{10} for α of 2·7 at -100 mV. Because the slope of the log α *vs.* membrane potential curves depends on temperature, the Q_{10} calculated for α also varies with voltage. Our values of Q_{10} would agree more closely with that reported by Takeuchi & Takeuchi (1959) if the calculation had been carried out for a membrane potential closer to zero.

Peak end-plate current depends non-linearly on voltage

Kordaš (1969) has reported that the peak end-plate current is non-linearly related to membrane potential, and this phenomenon must of course be considered in any quantitative treatment of end-plate currents. We have confirmed Kordaš's observation, as shown by the data presented in Fig. 2; in this experiment, for example, the slope conductance decreased from 1·3 μmhos at 0 mV to 0·85 μmhos at -100 mV. Associated with the decreasing slope conductance, the time to peak end-plate current increased slightly for more negative membrane potentials. For the cell which yielded the data in Fig. 2, the end-plate currents reached their peak value about 100 μsec later at -100 mV than at 0 mV, a latency increase of about 15%. Although this effect was small, it was observed consistently in our experiments, and also seems to be present in Kordaš's published records.

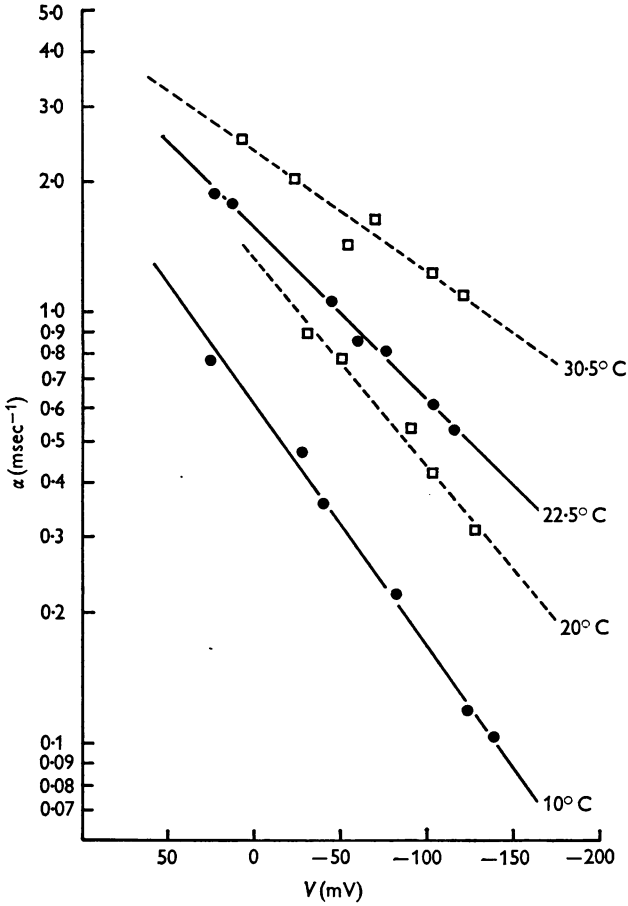

Fig. 1. Semilogarithmic plot of the end-plate current decay constant α as a function of membrane potential for various temperatures. Temperature is indicated with each curve. These data were obtained from two different preparations, one indicated by open squares and the other by filled circles.

The 'instantaneous' end-plate voltage–current relationship is linear

We wish to formulate our description of end-plate currents in terms of the underlying permeability changes, but in order to do so it is necessary to have information about the voltage–current relationship for ensembles of end-plate channels. One might be tempted to attribute rectifying properties to the end-plate membrane from data like those presented in Fig. 2, but such curves of peak end-plate current as a function of membrane potential do not necessarily reflect the voltage–current characteristics of the membrane accurately. The difficulty in using the peak current *vs.* voltage curve to infer channel properties is that the time course of the

underlying permeability changes depends itself upon membrane potential, so that there is no guarantee that the end-plate membrane is in the same state at the time of peak current flow for each membrane potential. For example, considering hypothetical two-state channels which can be either open or closed, the data shown in Fig. 2 can be accounted for either (*a*) by assuming that the same number of channels are open at the point of peak current flow and each such channel has a non-linear voltage–current relationship; or (*b*) by supposing that individual channels have a linear

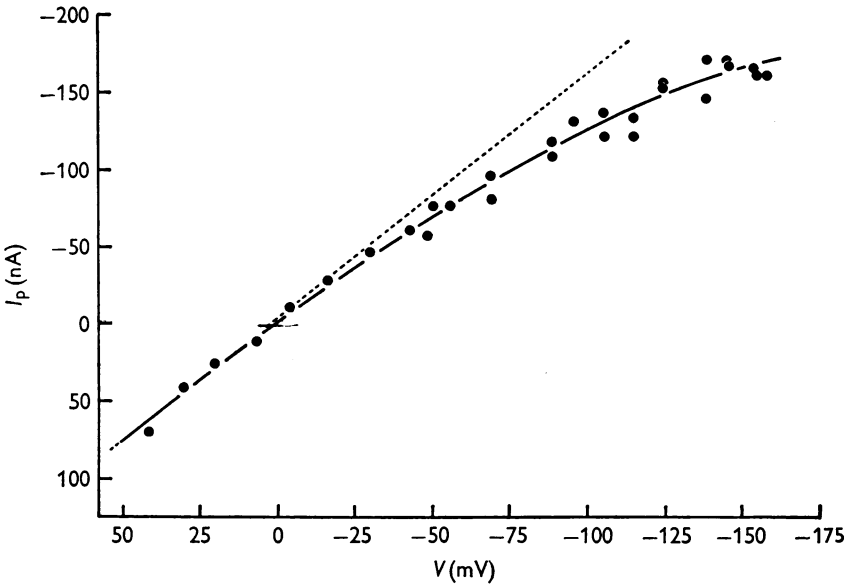

Fig. 2. Peak end-plate current as a function of membrane potential. Other data from this end-plate appear in Figs. 5 and 6*A*. The continuous curve above was calculated from eqns. (1), (12), and (9*c*) with the driving function that appears in Fig. 4 (continuous curve) and the values of constants given on page 189. Negative values denote inward currents. The interrupted straight line is included to indicate the departure from a linear peak current–voltage relationship.

voltage–current relationship, and (because of the opening and closing kinetics of these channels) fewer channels are in the open state at the time of peak current flow when the membrane is hyperpolarized than when it is near 0 mV.

The appropriate way of measuring permeability properties of the end-plate membrane would be to determine the 'instantaneous' voltage–current relationship with step-wise changes of the membrane potential at various times during end-plate current flow. This experiment is, unfortunately, impractical because the responses of non-end-plate membrane

obscure those of the end-plate channels under investigation, and because of complications arising from longitudinal membrane potential gradients. With the type of experiment used previously to distinguish between memory and no-memory mechanisms, however, it is possible to make estimates of 'instantaneous' voltage–current relations during the decaying phase of end-plate currents as α in eqn. (1) relaxes instantaneously (on the experimental time scale) to the value appropriate for its new membrane potential (Magleby & Stevens, 1972). The technique, then, is to cause step changes in membrane potential during the declining phase of end-plate currents, subtract out the contributions from non-end-plate membrane,

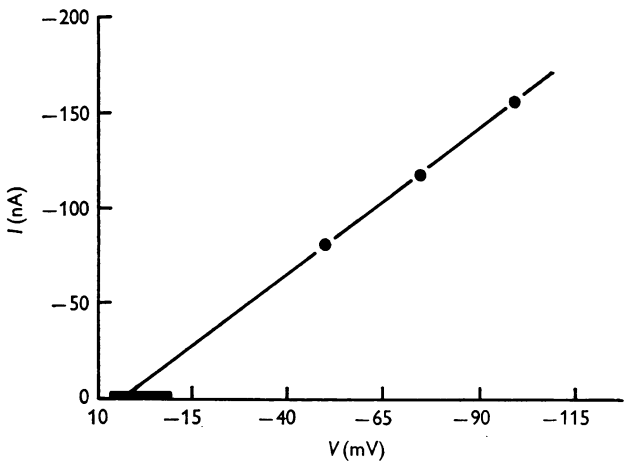

Fig. 3. 'Instantaneous' voltage–current relationship for end-plate channels, obtained as described in the text. The equilibrium potential measurement for this end-plate was uncertain but falls within the range indicated by the bar near the origin of the voltage axis.

and extrapolate the exponentially declining currents back, over the several hundred microsecond time period where observations are not available, to find the current flowing immediately after the step. To obtain the data presented in Fig. 3, the membrane potential was held at -50 mV and, 400 μsec after the time of peak current flow, was stepped (in less than 80 μsec) to -75 or -100 mV. The current flows were extrapolated back to the time of the step, yielding the linear voltage–current plot shown in Fig. 3.

An alternative approach to measuring the 'instantaneous' voltage–current relation is to cause step changes in membrane potential near the time of peak current flow in a prostigmine-treated preparation. In this situation, because of the rather broad peak produced by prostigmine, one can make a measurement of current before there has been appreciable (less

than about 10 %) relaxation of permeability away from its value at the time the membrane potential jump occurred. This experiment also gave a linear voltage–current curve not essentially different from that shown in Fig. 3. Although these experiments are inherently difficult, with many sources of error, the results are consistent with the hypothesis that the voltage–current curve for end-plate channels is linear.

Part II

Although many features of synaptic transmission are quite clear, a sufficient number of gaps exist in current knowledge to make impossible a rigorous derivation of equations describing end-plate processes. Nevertheless, with some simplifying assumptions, it is possible to arrive at a general description of end-plate current flows which should be adequate to provide a context for the following discussion. After developing such a description, we examine the various terms in the resulting equations to identify ones which may be most plausibly neglected; we wish to arrive finally at equations which can account for the end-plate currents we have observed in experiments.

Basic hypothesis

Following the depolarization of the nerve terminal, the concentration of acetylcholine in the synaptic cleft rapidly increases because of transmitter release, and then decreases with a time course determined by diffusional and hydrolytic losses, and by binding to and unbinding from receptor and possibly other molecules fixed within the cleft region. The acetylcholine also interacts with receptor molecules on the post-synaptic membrane and induces increases in permeability to sodium and potassium ions; these permeability increases give rise to the measured end-plate currents. As the receptor appears to be a membrane-bound protein with considerable specificity in ligand binding (Changeux, Kasai & Lee, 1970; Changeux, Meunier & Huchet, 1971; Miledi, Molinoff & Potter, 1971), it seems most natural to view the interaction of acetylcholine with its receptor as analogous to an enzymatic process. Following the binding of substrate to enzyme, many enzyme-substrate complexes are thought to undergo a conformational change as the first step in the catalytic sequence (Eigen & Hammes, 1963; Hammes, 1968*a*, *b*; Chock, 1971; Gutfreund, 1971); by analogy, acetylcholine would bind to its receptor, and the succeeding conformational change would allow ionic transport through the end-plate channels. Thus,

$$T + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} T \cdot R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} T \cdot R^*, \qquad (2)$$

where T represents acetylcholine, R represents the receptor, $T \cdot R$ is the transmitter-receptor complex associated with a closed channel, and $T \cdot R^*$ is the 'open' conformation of this complex; k_1, k_2, α and β are the rate constants for the indicated processes. Note that it has been assumed that one acetylcholine molecule reacts with a single receptor, and that co-operative interactions between receptors do not occur.

Equations for end-plate currents

The preceding description can be formalized as follows: suppose the end-plate region under consideration contains N receptors of which y are complexed with transmitter and in the closed conformation, and x are complexed with transmitter and in the open conformation. It will be assumed that the conductance is proportional to x. Specifically

$$g = \gamma x, \tag{3}$$

where g is the end-plate conductance and γ is the conductance of one open channel. According to scheme (2), the rate at which the number of complexes in the open conformation changes is given by

$$\frac{dx}{dt} = -\alpha x + \beta y. \tag{4}$$

Similarly, from (2) the number of complexes in the closed configuration changes according to

$$\frac{dy}{dt} = \alpha x + k_1 c(t)(N - x - y) - (\beta + k_2)y, \tag{5}$$

where $c(t)$ is the local concentration of acetylcholine. Because the dimensions are generally small, it will be assumed that the cleft volume V containing the receptors under consideration is thoroughly mixed by diffusion so that partial differential equations do not result. The rate at which the number of molecules in this volume changes is thus given by the conservation equation

$$\overline{V}\frac{dc(t)}{dt} = \underbrace{f(t)}_{\text{release}} - \underbrace{\int_0^t G(t-\tau)c(\tau)\,d\tau}_{\text{diffusion}} - \underbrace{k_E c(t)}_{\text{hydrolysis}}, \tag{6}$$

where $f(t)$ is the function describing release from the nerve terminal, $G(t)$ is the kernel characterizing diffusion out of the cleft volume $\overline{V}$, and k_E is the rate constant for enzymatic hydrolysis of acetylcholine. Terms which describe buffering of cleft concentration by the binding of acetylcholine to receptor and other molecules have, for simplicity, been neglected. The function $G(t)$ which characterizes diffusional loss of transmitter will

depend upon the properties and geometry of the cleft region outside of the volume under consideration. The rate of acetylcholine loss from the cleft volume due to cholinesterase hydrolysis is taken to be simply proportional to acetylcholine concentration here because we have assumed that the number of cholinesterase molecules is sufficiently large that appreciable saturation does not occur; this assumption is not essential for the arguments that follow, and it is made here merely to simplify the equations.

The simultaneous eqns. (3), (4), (5) and (6) constitute (to the accuracy of our approximations) a general description of end-plate currents. Before considering the magnitude of contributions made by the various terms in these equations, it will be convenient, although not essential, to further simplify matters by introducing two additional approximations. The binding of substrate to enzyme, while specific, is generally so rapid as to be almost diffusion-limited (Eigen & Hammes, 1963; Hammes, 1968*b*; see also Peller & Alberty, 1959). The usual approximation, then, for enzyme-substrate systems is to assume that the binding reaction is essentially at equilibrium; in our case, this is equivalent to saying that, on the time scale determined by k_1 and k_2, y is slowly varying so that $\mathrm{d}y/\mathrm{d}t$ in eqn. (5) nearly vanishes. To this approximation, eqn. (5) becomes

$$y = \frac{k_1 c(t) N + x(\alpha - k_1 c(t))}{\beta + k_2 + k_1 c(t)}. \tag{7}$$

This equation is further simplified by noting that k_1 and k_2 are generally large compared to α and β, the rate constants for conformational change and by assuming that N is large compared to x (that is, only a small fraction of the total number of possible channels is open at a given time). With these simplifications, eqn. (7) finally becomes

$$y = \frac{C(t) N}{K + c(t)}, \tag{8}$$

where K is equal to k_2/k_1, the equilibrium constant for the binding reaction. Eqn. (8) may be used to eliminate y from eqn. (4) to give

$$\frac{\mathrm{d}x}{\mathrm{d}t} = -\alpha x + \beta \frac{c(t) N}{K + c(t)}. \tag{9a}$$

With the aid of (3) this becomes

$$\frac{\mathrm{d}g}{\mathrm{d}t} = -\alpha g + \gamma\beta \frac{c(t) N}{K + c(t)}, \tag{9b}$$

which may be written

$$\frac{\mathrm{d}g}{\mathrm{d}t} = -\alpha g + \beta W(t), \tag{9c}$$

with $W(t)$ denoting

$$\gamma \frac{c(t)N}{K+(c)t}.$$

Eqn. (9*a*) then specifies the number of receptors in the open conformation and equations (9*b*, *c*) give the additional conductance due to the action of the transmitter.

A simple exponential decay of end-plate conductance would in general be obtained from a system described by eqns. (9*c*) and (6) under one of two conditions: first, it is possible that the hypothesized conformational change occurs rapidly compared to the change in cleft transmitter concentration described by eqn. (6). In this case, (2) would always be close to equilibrium, and dg/dt in eqn. (9*c*) would nearly vanish so that conductance would be approximately proportional to concentration

$$g(t) = \frac{\beta}{\alpha} W(t).$$

The observed exponential decay of end-plate currents would then reflect an exponential decay of cleft acetylcholine concentration. Secondly, the receptor conformational change may be slow compared to the changes in transmitter concentration governed by eqn. (6). According to this alternative, the terms on the right of eqn. (6) would be such to produce a rapid rise and fall of cleft acetylcholine concentration, and the exponential decay of end-plate conductance would follow from eqn. (9*b*) when $c(t)$ has decayed nearly to zero. Briefly, we have selected the second of these alternatives because terms on the right of eqn. (6) do not have the properties required to account for the decay of conductance, while macromolecular conformational changes do frequently have rate constants comparable to those of the observed decay.

According to the first alternative above, the time course of end-plate currents essentially reflects the acetylcholine concentration, so that the exponential decay of end-plate currents must arise from one or more of the terms on the right of eqn. (6). For this alternative to be acceptable, then, the properties of the processes in eqn. (6) must be compatible with the observed properties of end-plate currents. Because the release function $f(t)$ is a very rapid transient declining at 10° C, for example, with a rate constant (Katz & Miledi, 1965; E. F. Barrett & C. F. Stevens, unpbl. obs.) of approximately 2 $msec^{-1}$ (compared to the 0·2 $msec^{-1}$ rate constant for decline of end-plate currents at 10° C and -90 mV; see Fig. 1), this factor seems unlikely to make an appreciable contribution to the declining phase of end-plate currents. Nor does the second term in eqn. (6), the diffusional loss of acetylcholine, appear to be able to account for the decaying phases of end-

plate currents. Not only does iontophoresis of acetylcholine by end-plate currents fail to yield the required voltage sensitivity (Magleby & Stevens, 1972), but the rate constant which characterizes the decay of end-plate currents has a Q_{10} of approximately 3, while the Q_{10} of diffusion should be close to 1. If we wished to maintain the first alternative, then, the decline of cleft acetylcholine concentration would have to be mainly governed by the remaining term in eqn (6), the hydrolytic loss of transmitter. A Q_{10} of 3 is plausible for enzymic catalysis, and acetylcholinesterase molecules closely associated with the post-synaptic membrane could well be voltage sensitive, but we have previously presented evidence against the hypothesis that the decaying phase of end-plate currents is determined by the rate of acetylcholine hydrolysis (Magleby & Stevens, 1972). As the processes described in eqn. (6) apparently do not have properties which are compatible with those for the decaying phase of end-plate currents, we have rejected the notion that end-plate conductance is a simple reflexion of cleft acetylcholine concentration.

According to the second alternative, the processes described by eqn. (6) are more rapid than the hypothesized conformational change and the decay of end-plate conductance is thus effectively determined by α in eqn. (9*c*). It does seem possible that diffusional and hydrolytic losses from the receptor area could be as rapid as required by this view (see Eccles & Jaeger, 1958), and the rates reported for conformational changes in enzymes could easily be as slow as indicated by the approximately 1 $msec^{-1}$ decay constants observed for the decaying phases of end-plate currents. The most rapid conformational changes reported for enzymes described by scheme (2) occur at rates of around 10 $msec^{-1}$ (Erman & Hammes, 1966; Holler, Rupley & Hess, 1969; del Rosaria & Hammes, 1970; Hammes & Simplicio, 1970), and range down to rates of about 0·01 $msec^{-1}$ (Kirschner, Eigen, Bittman & Voigt, 1966; Halford, Bennett, Trenthan & Gutfreund, 1969; Janin & Iwatsubo, 1969); values close to the 1 $msec^{-1}$ found for end-plate currents seem most common, however (Hammes, 1968*a*, *b*; Chock, 1971; see also references cited in Gutfreund, 1971). Altogether then, the evidence at hand seems to favour the notion that the decay of end-plate currents is determined by the rate of conformational change, and not by the decline of cleft transmitter concentration. We shall use this conformational change hypothesis as a basis of our treatment of end-plate currents; it must be emphasized, however, that while our proposals can provide a plausible explanation for the observed results, firm conclusions about the mechanisms we have discussed must await more complete information about the processes underlying end-plate currents.

Calculation of rate constants in end-plate current equations

According to our proposal, then, acetylcholine which enters the synaptic cleft is rapidly removed by diffusion and by cholinesterase activity, so the cleft concentration rapidly approaches zero. When transmitter concentration becomes low, $W(t)$ in eqn. (9*c*) approaches zero and the conductance is approximately governed by the equation

$$\frac{dg}{dt} = -\alpha g.$$

Since α in this equation has a specified physical significance, we can predict the form of its dependence on membrane potential. Specifically, α should be given by (Glasstone, Laidler & Eyring, 1941; Vineyard, 1957; Prigogine & Bak, 1959)

$$\alpha(V) = \nu \exp\left(\frac{-U(V)}{kT}\right), \tag{10}$$

where ν is an effective vibration frequency, k is Boltzmann's constant, T is the temperature (°K) and U is the Helmholtz free energy difference between the receptor molecule in its open conformation and its transition state.

As the molecule changes its conformation, in general its dipole moment in the direction normal to the membrane surface will change because the relative position of charges is altered, and also perhaps because the number of ionized groups changes (Schwartz, 1967; 1970; Schwartz & Seelig, 1968; Seelig & Schwartz, 1969; see however, Marchal, 1971). For a changing dipole moment in an electrical field the energy difference between two states is given by $U(V) = U_0 - E\mu$; here $U(V)$ is the force energy difference between two states as a function of membrane potential, U_0 is the free energy difference between the states in the absence of field, E is the field strength, and μ is the difference between the two states in dipole moment normal to the field direction. Membrane potential is taken to be proportional to field strength, with the proportionality constant M being the effective thickness of the membrane

$$E = \frac{V}{M}.$$

Thus the equation for $\alpha(V)$ becomes

$$\alpha(V) = \nu \exp \frac{-U_0}{kT} \exp\left(\frac{V\mu(V)}{MkT}\right).$$

In general, μ depends upon field strength (as well as differences in dipole moment resulting from conformational change) because dipoles tend to

align in the direction of the field (Debye, 1929). However, in the case of a membrane-bound protein whose dipole moment is almost entirely determined by ionized groups (cf. Schlecht, 1969), molecular movement is greatly restricted so that alignment with the field should be minimal. For the receptor, then, μ should be approximately field-independent, and $\alpha(V)$ should be given by

$$\alpha(V) = \nu \exp \frac{-U_0}{kT} \exp V\left(\frac{\mu}{MkT}\right) \tag{11}$$

(with μ constant). If $A = \mu/MkT$ and $B = \nu \exp(-U_0/kT)$, the result becomes

$$\alpha(V) = Be^{AV}. \tag{1}$$

This is of course the empirically determined form of α.

Eqn. (11) accurately predicts the dependence of α on membrane potential, but can be considered a plausible explanation for this dependence only if reasonable values for dipole moment difference μ result from the data. If an effective membrane thickness of 50 Å is selected, the average value of A ($0{\cdot}00795 \pm 0{\cdot}00043$ (S.E.) mV^{-1}) determined previously (Magleby & Stevens, 1972) gives a μ of $48{\cdot}4 \pm 2{\cdot}6$ (S.E.) D. Since dipole moments for proteins are often in the range of 200–500 D (McClellan, 1963), the calculated value for μ requires only a change in dipole moment equal to about 10–25 % of the total dipole moment, an amount that seems reasonable. The quantity $\alpha(0)$ was found previously to be $1{\cdot}67 \pm 0{\cdot}04$ (S.E.) msec^{-1} (Magleby & Stevens, 1972), a value quite comparable to those found for enzymes (Hammes, 1968*a*, *b*; Chock, 1971; also references cited by Gutfreund, 1971). Therefore, U_0 must also have a value typical of energy differences between enzyme conformational states. Altogether then, the available data are consistent with the physical interpretation of eqn. (11).

Time course of the cleft acetylcholine concentration

If the preceding analysis of the declining phase of end-plate conductance change is accurate, eqn. (7) should describe the entire course of this conductance change, not just its decaying phase. Specifically, β should depend exponentially on voltage as does α, and this dependence, together with that of α and the voltage-independent function

$$W(t) = \frac{\gamma c(t) N}{K + c(t)}$$

should predict the end-plate conductance change for all membrane potentials.

Unfortunately, we have in the experiments reported here no method for independently measuring or controlling the function $W(t)$. The form of this function can, however, be inferred from the end-plate currents by using

eqn. (9c) to find, for one particular voltage, $\beta(V)W(t) = dg/dt + \alpha g(t)$. The result of multiplying currents by α and adding their derivative is shown in Fig. 4; the $W(t)$ functions determined in four preparations all decayed approximately exponentially, although rather large departures from exponential decay might not have been detected, with time constants of 200 μsec. Poisoning with prostigmine 1·5 μg/ml. raised this time constant to 260 μsec in one preparation and 280 μsec in another. Note that $W(t)$ can be determined only up to an arbitrary multiplicative constant because the value of $\beta(V)$ is unknown.

Rate constant for opening channels

Because the product $\beta(V)W(t)$ appears in eqn. (9c), it is not possible to find absolute values for $\beta(V)$, as $W(t)$ is not under direct control in our experiments. Relative values of β may be found, however, by making use of the fact that, according to our theory, β is a function of voltage, but not of time, while W is a function of time but not voltage. Thus, β can be determined, up to a multiplicative constant, by finding the maximum

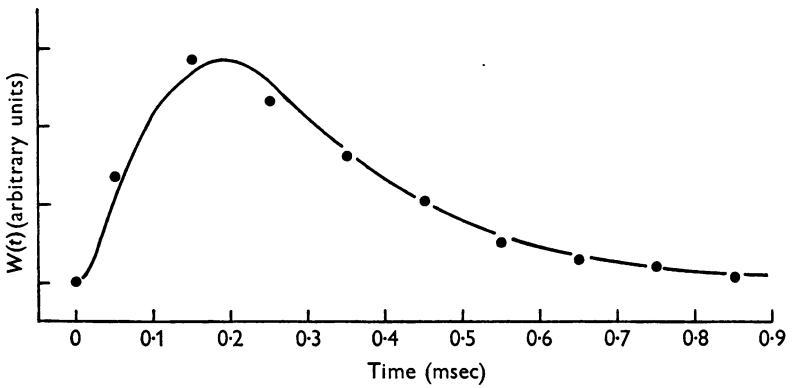

Fig. 4. Driving function $W(t)$ for eqn. (9c) as a function of time, obtained as described in the text (filled circles). The continuous curve is the approximation used in solving eqn. (9c) to yield the predictions shown in Fig. 6.

amplitude of the product $\beta(V)W(t)$. This is most conveniently done by solving eqn. (9c) with the empirically determined $\alpha(V)$ and adjusting the amplitude of the driving function $\beta(V)$ so that the calculated peak conductance matches the observed peak conductance. Because eqn. (9c) is a linear differential equation, the peak amplitude of the solution $g(t)$ is proportional to the peak amplitude of the driving function $\beta(V)W(t)$; the voltage dependence of β is thus established from the amplitude of the product $\beta(V)W(t)$ required to produce the observed peak $g(t)$. Fig. 5 presents semilogarithmic plots of β as a function of membrane potential

determined in this way with the $W(t)$ shown by the smooth curve in Fig. 4; it is apparent that β does indeed depend approximately exponentially on membrane potential with the form

$$\beta(V) = be^{aV}. \tag{12}$$

The value of a in the Figure is $0{\cdot}00315\ \mathrm{mV}^{-1}$, which is equivalent to a dipole moment difference between the transition state and closed state of the receptor molecule of 19·2 *D*.

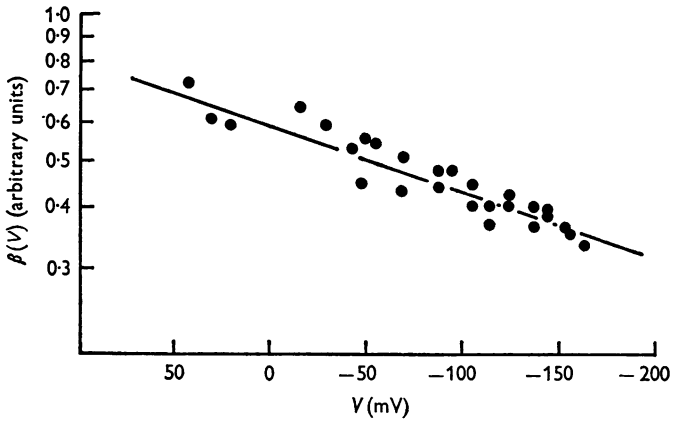

Fig. 5. The rate constant β plotted semilogarithmically as a function of membrane potential. Values of β were estimated as described in the text, and the straight line approximation to the data points was used in the solution of eqn. (9*c*) to yield the predicted end-plate currents shown in Fig. 6*A*. Experimental points were calculated from data on the same end-plate that yielded the records shown in Fig. 6*A*.

The theory accurately predicts end-plate currents

Eqns. (1), (12) and (9*c*), together with the empirically determined driving function $W(t)$ (Fig. 4), constitute a complete description of the end-plate conductance change; these equations should thus predict the observed end-plate currents for any voltage. We now wish to test the accuracy of our description by using the equations and empirically determined constants A, B (eqn. (1)) and a (eqn. (12)) to compare predicted and observed end-plate currents.

The continuous curve in Fig. 2 is the predicted peak amplitude of end-plate current as a function of membrane potential. This curve was computed by solving the equations

$$\alpha(V) = Be^{AV}, \tag{1}$$

$$\beta(V) = be^{aV}, \tag{12}$$

$$\frac{dg(t)}{dt} + \alpha(V)g(t) = \beta(V)W(t), \tag{9c}$$

with the driving function shown by the smooth curve in Fig. 4, $A = 0{\cdot}00582$ mV^{-1}. $B = 1{\cdot}57$ $msec^{-1}$, and $\beta(V)$ as given by the straight line in Fig. 5 ($a = 0{\cdot}00315$ mV^{-1}). The non-linear relationship between peak end-plate current and membrane potential is thus adequately described by the theory and can be viewed as arising from the different dipole moments of the

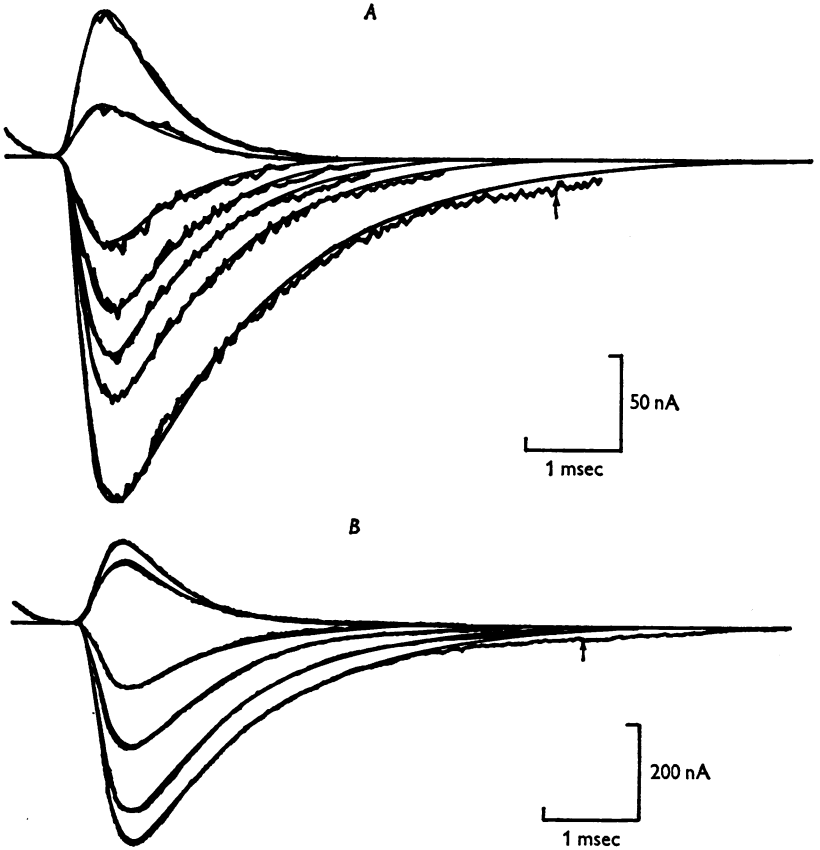

Fig. 6. Comparison of observed and predicted end-plate currents for two preparations. Predictions were made with eqns. (1), (12), and (9c), the driving function shown in Fig. 4 (continuous curve), and the constants given in the text. Arrows indicate departures of observed currents from simple exponential decay. *A*, peak current as a function of membrane potential for this end-plate appeared in Fig. 2, and estimates of β as a function of membrane potential in Fig. 5. The driving function estimated for this end-plate appears in Fig. 4. Membrane potentials associated with the exhibited end-plate currents (top to bottom) are +32, +20, −30, −56, −82, −106 and −161 mV. *B*, the end-plate current records shown here are the same as those in Fig. 1 of Magleby & Stevens (1972), and the values of α obtained from this end-plate appear in Fig. 3 of that paper. Associated membrane potentials are +38, +22, −40, −70, −95, and −120 mV.

receptor molecule in its open and closed states relative to that of the transition state.

That the entire time course of end-plate currents is also well described by these relations is shown in Fig. 6, where predicted and observed end-plate currents are superimposed. Because the amplitude of end-plate current fluctuates from trial to trial, the predicted and observed amplitudes do not agree perfectly on any one trial, as can be seen from Fig. 2. In order to facilitate comparison between theory and experiment, the predicted end-plate currents in Fig. 6 have been scaled to match the amplitude of the observed end-plate current on the particular trial. The curves in Fig. 6*A* were computed with the same constants which yielded the smooth curve in Fig. 2, except that the driving function $W(t)$ shown in Fig. 4 was adjusted in amplitude to appropriately scale the predicted peak current. The predicted end-plate currents in Fig. 6*B* were computed with the driving function illustrated in Fig. 4, $\alpha(V)$ as indicated by the straight line in Fig. 3 of Magleby & Stevens (1972) ($A = 0{\cdot}00626\ \mathrm{mV}^{-1}$; $B = 1{\cdot}43\ \mathrm{msec}^{-1}$), and $a = 0{\cdot}00095\ \mathrm{mV}^{-1}$ in eqn. (12). From Figs. 2 and 6 it is apparent that the equations presented here give a satisfactory formal description of end-plate currents for the range of membrane potentials investigated.

DISCUSSION

The physical interpretation we have given the formal description embodied in eqns. (9), (1) and (12) is not, of course, unique and any picture with certain essential features would lead quite naturally to the same equations. Any first-order process which determined by itself the time course of conductance change could give an equation like (9), and if this physical process were taking place in association with the post-synaptic membrane, one might expect the energy barriers to depend, as in eqn. (11), linearly on membrane potential. For example, if hydrolysis of acetylcholine by cholinesterase did in fact determine the time course of end-plate conductance, and if this cholinesterase were incorporated in the post-synaptic membrane, arguments like those given previously might well predict the rate-limiting step of hydrolysis to depend on membrane potential with a rate constant of the type observed. Even though really plausible alternatives may not spring readily to mind, the point to be emphasized is that, since we have made no direct observations of conformational changes in receptors or any of other molecules, the theory presented here must remain hypothetical until additional independent tests of it can be devised.

A feature of our model which we find most disturbing is the necessity for departing from the traditional view of anticholinesterase activity.

Although it is well recognized (Koelle, 1970) that anticholinesterases can exert effects other than through the inhibition of acetylcholine hydrolysis, the generally accepted view that the decreased rate of transmitter destruction and prolonged transmitter action accounts for the increased duration of end-plate currents is very attractive. According to our analysis, however, diffusional and hydrolytic losses of acetylcholine from the synaptic cleft are so rapid as to play virtually no role in the declining phase of end-plate currents, and the prolongation of this phase by anticholinesterases is mediated through effects on the receptor molecules: in terms of our physical model, anticholinesterases would have to act either through (see eqn. (11)) the vibration frequency ν, the height of the energy barrier U_0, or perhaps by binding on or near receptors to alter the local electrical field density. Anticholinesterases do of course decrease the rate of acetylcholine loss from the synaptic cleft, and according to our calculations, prostigmine 1·5 μg/ml. prolongs transmitter action by about 50% (see page 186). According to this view, then, the increased time to peak end-plate current observed with anticholinesterase treatment (Takeuchi & Takeuchi, 1959; Magleby & Stevens, 1972) is a reflexion of the prolonged transmitter action.

We favour this non-traditional view of anticholinesterase mechanism for the reasons discussed previously (Magleby & Stevens, 1972). Nevertheless, while we feel that, on balance, transmitter-receptor conformational changes seem the most likely rate-limiting step, it is clear that the role of cholinesterase in end-plate activity and the mechanisms through which this activity is modified by anticholinesterases requires additional study.

A number of assumptions have been made in developing the various equations presented in the text, and it is important to indicate which of these assumptions are essential for our argument and what effects might result from violations of them. Eqn. (6) is an ordinary rather than partial differential equation because we supposed that diffusional mixing through the entire cleft region of interest was instantaneous on the experimental time scale. Eccles & Jaeger (1958) made this same approximation in their treatment of diffusional losses from synaptic cleft, and C. R. Anderson (personal communication) has verified with computer solutions for the problems that this is an accurate approximation. In our case, rather more complicated equations would result if we did not make this assumption, but it seems unlikely that diffusional mixing could be so slow as to produce really important variations from eqn. (6). Enzymic hydrolysis, for simplicity, has been considered to proceed by a first-order reaction in the same equation, but we have made no essential use of this assumption. Buffering of the cleft concentration by binding to receptors and other mole-

cules has been assumed negligible, an assumption which receives some support from the observation that curare does not alter the values of $\alpha(V)$ (Magleby & Stevens, 1972). Altogether, none of these assumptions have been of central importance, and their precise nature would become crucial only if the step in question were considered rate-limiting for the processes under investigation.

We have argued that cleft concentration should decline rapidly but a rather prolonged small tail might be expected on several grounds. The transmitter release function $f(t)$ in eqn. (6) does indeed decay rapidly from its peak, but residual transmitter release persists for a number of milliseconds (Katz & Miledi, 1967; E. F. Barrett & C. F. Stevens, unpubl. obs.). Unless the influence function $G(t)$ in eqn. (6) is a delta function, diffusional loss of transmitter would be non-exponential and might have a prolonged tail. Finally, buffering of cleft concentration by receptors or other molecules would also be expected to prolong the final removal of transmitter from the cleft. Slight departures from exponential decay of end-plate currents have been observed (see Fig. 6 and Fig. 2*B* of Magleby & Stevens, 1972), and a prolonged tail of transmitter from the sources just noted might account for at least a portion of these departures from simple exponential decay.

To arrive at eqns. (9), we have had to rely upon the analogy to the first steps in enzyme catalysis, and the extent to which this analogy is valid is, of course, not known. Other alternatives are possible, and a number of variations on scheme (2) could lead to similar equations. For example, we have assumed that acetylcholine cannot dissociate from the receptor when the channel is in its open state, but viewing the transmitter-receptor interaction as a trigger for the conformational change, with the complex lasting only long enough to produce the open state, can be formally identical. Under some assumptions, the same formal description could also result from treating the binding step as slow and the conformational change as rapid. Until more is known about receptor properties and receptor-transmitter interactions, however, it seems most natural to rely upon the analogies with better studied systems.

To obtain equations (9) we have in addition supposed that neighbouring receptors do not interact. Although several authors have suggested the existence of co-operative interactions between acetylcholine receptors (Changeux *et al.* 1967; Karlin, 1967), evidence on this point for the frog neuromuscular junction is at present very scanty. Consistent with the notion of co-operativity is the observation by Katz & Thesleff (1957) that the graph (their Fig. 9) relating depolarization to acetylcholine concentration has an unexplained positive curvature in the low concentration region; this observation has been confirmed in an unpublished voltage-clamp study cited by Rang (1971). On the other hand, two types of results

seem to indicate the absence of strong co-operativity at the frog neuromuscular junction. First, if the data in Fig. 9 of Katz & Thesleff (1957) are converted from depolarization to end-plate conductance change Δg, the slope of the Hill plot (log $[\Delta g/\Delta g_{max} - \Delta g]$ *vs.* log (acetylcholine concentration)) differs from unity less than about 5 %. Secondly, the observed exponential decays of end-plate currents would not be expected were there significant cooperativity, although weak channel–channel interactions could produce the small deviations from exponential decay of end-plate currents we have sometimes observed. Altogether, then, the small amount of evidence currently available on the presence or absence of co-operative interactions between receptors is inconclusive. Since our treatment assumes independence of receptors, it would require modification if significant co-operative effects in fact exist at the frog end-plate.

The final assumption made in arriving at eqn. (9*a*) was that only a small fraction of the receptors are complexed with acetylcholine at any time. Had this assumption not been made, the equation

$$\frac{dx}{dt} = -\left(\alpha - \frac{(\alpha - k_1 c)\beta}{\beta + k_2 + k_1 c}\right)x + \frac{\beta k_1 c N}{\beta + k_2 + k_1 c}$$

would have resulted. The last terms on the right of this equation are more complicated than those designated by $W(t)$ in eqn. (9*c*), but since we have not measured cleft acetylcholine concentration directly, the difference would not have been detected. Further, once the cleft concentration became negligible, the expression multiplying x in the above equation would reduce to that of eqn. (9*a*) On the other hand, if

$$[(\alpha - k_1 c(t))\beta]/[\beta + k_2 + k_1 c(t)]$$

were ever significant compared to α for actual values of $c(t)$, the solutions for the above equations would depart from those of eqn. (9*c*), so that both equations cannot simultaneously account for the observed end-plate currents. Since eqn. (9*c*) provides a good description of end-plate currents, the assumption that only a small fraction of the receptors is complexed at any one time becomes an important one, and the demonstration that this assumption is false would be a strong argument against the interpretation presented here.

The development of α's dependence on membrane potential (eqn. (11)) from our physical picture contained a number of assumptions, some of which were implicit. For example, the dipole of our hypothetical gating molecule was treated as though it were isolated and did not interact with neighbouring molecules; also, the energy of the dipole was calculated as though it were a point dipole when in fact the charge separations giving rise to the dipole moment are almost certainly not small compared to

distances over which considerable changes in field strength occur. Further, field was taken as proportional to membrane potential which is equivalent to assuming field strength within the membrane is constant, where it doubtless varies dramatically over small distances. These difficulties are avoided in a more detailed treatment which is too long and complicated to present here, especially in view of the final results' simplicity. This more detailed treatment is carried out with a master equation (van Hove, 1957; Zwanzig, 1964) of the same form as eqn. (9), and an analysis of molecular interactions similar to that in the statistical mechanical theory of dielectrics (Kirkwood, 1939). An equation like (11) appears in this development, but quantities termed 'effective dipole moments' replace the free-solution dipole moments of the treatment given here. Although we have been unable to detect departures from eqn. (1), with large enough membrane potentials the more refined treatment predicts that energy barriers should depend on a term that increases as the square of the voltage (see Debye, 1929).

As a formal description the theory presented here seems generally adequate to account for the data we have obtained. Our theory is, however, an incomplete one in at least several regards, for we provide no explanation for desensitization (Katz & Thesleff, 1957) or the effects of procaine and similar local anaesthetics (Furukawa, 1957). It has already been suggested (Steinbach, 1968) that the action of local anaesthetics involves an altered state of the receptor molecule, and it may be possible to extend the present description by including say, one or more additional conformations. The existence of an additional ('desensitized') conformational state of the receptor is, incidentally, another possible explanation for the failure of some end-plate current tails to decay with a simple exponential time course.

The physical processes considered here are inherently stochastic, and the more detailed treatment referred to previously is an explicitly probabilistic one. On this view, the length of time a channel remains open is a random variable, with the mean time open given by α^{-1}. Because the opening and closing processes are random, the model presented here predicts the existence of fluctuations in conductance around the mean value, and permits the statistical structure of these fluctuations to be calculated. For example, with a constant acetylcholine concentration (and neglecting the problem of desensitization), the spectrum of conductance fluctuations should be given by (Stevens, 1972)

$$S(f) = \frac{S(0)}{1+(2\pi f/\alpha(V))^2}, \tag{13}$$

where $S(f)$ is the spectral density at frequency f, and α depends upon membrane potential as described by eqn. (1).

Katz & Miledi (1971) have presented noise spectra obtained by focal recording which should be approximately proportional to the spectrum for conductance fluctuations given above, and the data in their Fig. 2 seem adequately fit by eqn. (13) with an α equal to about 0·6 msec^{-1}. This value of α is somewhat smaller than typical for membrane potentials near -90 mV at 25° C, but not outside of the range of α's we have encountered. The α required for the data in Fig. 4 of Katz & Miledi would be about 0·9 msec^{-1}, a value closer to what might be expected from our experience.

An obvious test of eqn. (13) is to investigate the dependence of spectrum on membrane potential, anticholinesterases, and on other factors which cause α to vary. Failure of eqn. (13) would of course be evidence against our physical picture, but, unfortunately, the success of eqn. (13) would not be a strong confirmation of our model. In fact, because we feel a fluctuation-dissipation-like theorem (Kubo, 1957) should apply in this situation, the spectrum of conductance fluctuations given by eqn. (13) should be predicted simply from the exponential relaxation of end-plate currents and the empirical relation given by eqn. (1) for a variety of physical mechanisms.

Gage & Armstrong (1968) have proposed that the variation of end-plate current time course with membrane potential reflects the different kinetics of two separate sets of channels, one for sodium and one for potassium. Kordaš (1969) has offered a number of arguments against the two-channel hypothesis, and our observations also do not support this two-channel view. The two-channel hypothesis could not, unless the separate channels were also voltage-sensitive, give a simple exponential decay at all voltages. Furthermore, if the channels were insensitive to voltage, the duration of end-plate currents should not be further prolonged once the membrane potential was more negative than the potassium equilibrium potential, whereas we find the most dramatic effects at the most hyperpolarized voltages. Our observations do not bear directly on the question of whether sodium and potassium ions pass through the same or separate channels at the end-plate, but they do further confirm Kordaš's conclusion that two voltage-insensitive channels cannot account for the dependence of end-plate current time course on membrane potential.

Because the neuromuscular junction has proved to be a good model for central synapses, it can be anticipated that voltage sensitivity of post-synaptic mechanisms will be discovered in the central nervous system as well. If central post-synaptic membranes were to exhibit pronounced voltage sensitivity, this property could have implications for neuronal information processing. For example, inhibitory post-synaptic potentials (PSPs) could be self-re-inforcing, and inhibition time course could be graded by the magnitude of the excitatory drive. The presence or absence of voltage sensitivity for the long-lasting IPSPs characteristic of many

central neurones should not be particularly difficult to determine, and these PSPs would seem to offer a good possibility for the use of such a phenomenon in information processing.

If the interpretation we have given here were correct, it would be of significance not only for synaptic physiology but also possibly for excitable membranes, as we would have accounted for the voltage sensitivity of a gating molecule. Indeed, a number of similarities between our treatment and the Hodgkin–Huxley equations (Hodgkin & Huxley, 1952) are apparent: our eqns. (9) describing the opening and closing of transmitter-receptor complexes have the same form as the Hodgkin–Huxley rate equations for their accessory variables m, n, and h, and the behaviour of our α's and β's with membrane potential bears similarities to that of the Hodgkin–Huxley rate constants. If one were to attempt a similar description of excitable membranes, however, the dipole moment changes would have to be much larger, and they could no longer be independent of voltage. Such a description could perhaps be made plausible, since alignment of very high dipole moment molecules in the large field of the membrane might well be expected.

Whatever the defects in the theory we have presented, it is specific and in principle testable. Now that the isolation of receptor molecules is underway, transmitter-receptor interaction and subsequent possible conformational changes may be subject to direct investigation.

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REFERENCES

CHANGEUX, J. P., KASAI, M. & LEE, C. Y. (1970). Use of a snake venom toxin to characterize the cholinergic receptor protein. *Proc. natn. Acad. Sci. U.S.A.* **67**, 1241–1247.

CHANGEUX, J. P., MEUNIER, J. C. & HUCHET, M. (1971). Studies on the cholinergic receptor protein of *Electrophorus electricus*. I. An assay *in vitro* for the cholinergic receptor site and solubilization of the receptor protein from electric tissue. *Molec. Pharmacol.* **7**, 538–553.

CHANGEUX, J. P., THIERY, J., TUNG, Y. & KITTEL, C. (1967). On the cooperativity of biological membranes. *Proc. natn. Acad. Sci. U.S.A.* **58**, 335–341.

CHOCK, P. B. (1971). Relaxation methods and enzymology. *Biochimie*, **53**, 161–172.

DEBYE, P. (1929). *Polar Molecules*. New York, N.Y.: Dover Publications.

DEL ROSARIO, E. J. & HAMMES, G. G. (1970). Relaxation spectra of ribonuclease VII. The interaction of ribonuclease with uridine 2′,3′-cyclic phosphate. *J. Am. chem. Soc.* **92**, 1750–1753.

ECCLES, J. C. & JAEGER, J. C. (1958). The relationship between the mode of operations and the dimensions of the junctional regions at synapses and motor end organs. *Proc. R. Soc.* B **148**, 38–56.

EIGEN, M. & HAMMES, G. G. (1963). Elementary steps in enzyme reactions studied by relaxation spectrometry. *Adv. Enzymol.* **25**, 1–38.

ERMAN, J. E. & HAMMES, G. G. (1966). Relaxation spectrum of ribonuclease. IV. The interaction of ribonuclease with cytidine 2′,3′-cyclic phosphate. *J. Am. chem. Soc.* **88**, 5607–5614.

FURUKAWA, T. (1957). Properties of the procaine endplate potential. *Jap. J. Physiol.* **7**, 199–212.

GAGE, P. W. & ARMSTRONG, C. M. (1968). Miniature end-plate currents in voltage clamped muscle fibers. *Nature, Lond.* **218**, 363–365.

GLASSTONE, S., LAIDLER, K. J. & EYRING, H. (1941). *Theory of Rate Processes.* New York, N.Y.: McGraw-Hill, Inc.

GUTFREUND, H. (1971). Transients and relaxation kinetics of enzyme reactions. *A. Rev. Biochem.* **40**, 315–344.

HALFORD, S. E., BENNETT, N. G., TRENTHAN, D. R. & GUTFREUND, H. (1969). A substrate-induced conformation change in the reaction of alkaline phosphatase from *Escherischia coli. Biochem. J.* **114**, 243–251.

HAMMES, G. G. (1968*a*). Relaxation spectrometry of biological systems. *Adv. Protein Chem.* **23**, 1–58.

HAMMES, G. G. (1968*b*). Relaxation spectrometry of enzymatic reactions. *Acc. Chem. Res.* **1**, 321–329.

HAMMES, G. G. & SIMPLICIO, J. (1970). Relaxation spectra of pyruvate kinase. *Biochim. biophys. Acta* **212**, 428–433.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544.

HOLLER, E., RUPLEY, J. A. & HESS, G. P. (1969). Kinetics of lysozyme substrate interactions. *Biochim. Biophys. Res. Comm.* **37**, 423–429.

JANIN, J. & IWATSUBO, M. (1969). The threonine-sensitive homoserine dehydrogenase and aspartokinase activities of *E. coli.* K12. Relaxations of the allosteric equilibrium. *Eur. J. Biochem.* **11**, 530–540.

KARLIN, A. (1967). On the application of a 'plausible model' of allosteric proteins to the receptor for acetylcholine. *J. theor. Biol.* **16**, 306–320.

KATZ, B. & MILEDI, R. (1965). The measurement of synaptic delay, and the time course of acetylcholine release at the neuromuscular junction. *Proc. R. Soc.* B **161**, 483–496.

KATZ, B. & MILEDI, R. (1967). The release of acetylcholine from nerve endings by graded electric pulses. *Proc. R. Soc.* B **167**, 23–38.

KATZ, B. & MILEDI, R. (1971). Further observations on acetylcholine noise. *Nature, New Biol.* **232**, 124–126

KATZ, B. & THESLEFF, S. (1957). A study of the 'desensitization' produced by acetylcholine at the motor endplate. *J. Physiol.* **138**, 63–80.

KIRKWOOD, J. G. (1939). Dielectric polarization of polar liquids. *J. chem. Phys.* **7**, 911–919.

KIRSCHNER, K., EIGEN, M., BITTMAN, R. & VOIGT, B. (1966). The binding of nicotinamide-adenine dinucleotide to yeast D-glyceraldehyde-3-phosphate dehydrogenase: temperature-jump relaxation studies on the mechanism of an allosteric enzyme. *Proc. natn. Acad. Sci. U.S.A.* **56**, 1661–1667.

KOELLE, G. B. (1970). Anticholinesterase agents. In *The Pharmacological Basis of Therapeutics*, IV, edn., ch. 22, pp. 442–465, ed. GOODMAN, L. S. & GILMAN, A. New York: MacMillan Company.

KORDAŠ, M. (1969). The effect of membrane polarization on the time course of the end-plate current in frog sartorius muscle. *J. Physiol.* **204**, 493–502.

KUBO, R. (1957). Statistical mechanical theory of irreversible processes. I. General theory and simple application to magnetic and conduction problems. *J. phys. Soc. Japan* **12**, 570–586.

MAGLEBY, K. L. & STEVENS, C. F. (1972). The effect of voltage on the time course of end-plate currents. *J. Physiol.* **223**, 151–171.

MARCHAL, E. (1971). Dielectric relaxation measurements as a tool for studying the kinetics of the helix-coil transition of poly(γ-benzyl L-glutamate). *Biopolymers* **10**, 417–419.

MCCLELLAN, A. L. (1963). *Tables of Experimental Dipole Moments*. San Francisco: W. H. Freeman.

MILEDI, R., MOLINOFF, P. & POTTER, L. T. (1971). Isolation of the cholinergic receptor protein of *Torpedo* electric tissue. *Nature, Lond.* **229**, 554–557.

PELLER, L. & ALBERTY, R. A. (1959). Multiple intermediates in steady state enzyme kinetics. I. The mechanism involving a single substrate and product. *J. Am. chem. Soc.* **81**, 5907–5914.

PRIGOGINE, I. & BAK, T. A. (1959). Diffusion and chemical reaction in a one-dimensional condensed system. *J. chem. Phys.* **31**, 1368–1370.

RANG, H. P. (1971). Drug receptors and their function. *Nature, Lond.* **231**, 91–96.

SCHLECHT, P. (1969). Dielectric properties of hemoglobin and myoglobin. II. Dipole moment of sperm whale myoglobin. *Biopolymers* **8**, 757–765.

SCHWARZ, G. (1967). On dielectric relaxation due to chemical rate processes. *J. phys. Chem.* **71**, 4021–4030.

SCHWARZ, G. (1970). Acid-base catalysis of dielectric relaxation of zwitterions. *J. phys. Chem.* **74**, 654–658.

SCHWARZ, G. & SEELIG, J. (1968). Kinetic properties and the electric field effect of the helix-coil transition of poly(γ-benzyl L-glutamate) determined from dielectric relaxation measurements. *Biopolymers* **6**, 1263–1277.

SEELIG, J. & SCHWARZ, G. (1969). Concentration dependence of dielectric relaxation due to the helix-coil transition of poly(γ-benzyl L-glutamate). *Biopolymers* **8**, 429–431.

STEINBACH, A. B. (1968). A kinetic model for the action of xylocaine on receptors for acetylcholine. *J. gen. Physiol.* **52**, 162–180.

STEVENS, C. F. (1972). Inferences about membrane properties from electrical noise measurements. *Biophys. J.* (in the press).

TAKEUCHI, A. & TAKEUCHI, N. (1959). Active phase of frog's end-plate potential. *J. Neurophysiol.* **22**, 395–411.

VAN HOVE, L. (1957). The approach to equilibrium in quantum statistics. A perturbation treatment to general order. *Physica* **23**, 441–480.

VINEYARD, G. H. (1957). Frequency factors and isotope effects in solid state rate processes. *J. Phys. Chem. Solids* **3**, 121–127.

ZWANZIG, R. (1964). On the identity of three generalized master equations. *Physica* **30**, 1109–1123.