

Towards a Molecular Model of Nerve Excitability*

EBERHARD NEUMANN**

Max-Planck-Institut für Biophysikalische Chemie, 3400 Göttingen-Nikolausberg, Postfach 968, Federal Republic of Germany

With 7 Figures

1. Introduction

Bioelectricity and excitability, universal properties of all higher organisms, are already encountered in algae and lower animals. It is, however, most convenient to study bioelectrical phenomena in specialized neuronal tissue evolved for the absorption, processing and transmission of environmental information and for the coordination and regulation of higher organismic function. Such specialized nerve tissue, particularly suited for electrophysiological studies, are the giant axons of certain squids. The coupling of biochemical events and electrical parameters in excitable membranes are readily demonstrable at most neuromuscular junctions or with the isolated electroplax of the electric eel *Electrophorus electricus*.

Although numerous experimental data have been accumulated in the various disciplines working on bioelectricity, the mechanism of nerve excitability and synaptic transmission is still an unsolved problem. Many mechanistic interpretations of nerve behavior and the various molecular and mathematical models cover only a part of the known facts, are thus selective and of only limited value. According to AGIN [1], there is no need for further (physically) unspecific mathematical models (such as for instance the Hodgkin-Huxley scheme for squid giant axons). "What is needed is a quantitative theory based on elementary physicochemical assumptions, and which is detailed enough to produce calculated membrane

* Financial support of the ALFRED P. SLOAN Foundation and the Stiftung Volkswagenwerk is gratefully acknowledged.

** Dedicated to the memory AHARON KATZIR-KATCHALSKY (1913—1972).

behavior identical with that observed experimentally. At the present time such a theory does not exist. Its construction will be an important advance, for like all theories its lifetime will be short, and in the process of destroying it we will learn things which are new and exciting".

The first proposal of a *physically specific* mechanism for the control of bioelectricity is due to NACHMANSOHN [2, 3]. The chemical hypothesis of NACHMANSOHN remained on a qualitative level, and until recently there was no detailed link between the numerous biochemical data on excitability and the various electrophysiological observations. A first attempt at integrating some basic data of biochemical and pharmaco-electrophysiological studies has been initiated by the late AHARON KATCHALSKY, and a first report has been recently given [4].

The present account is a further step towards a specific quantitative physico-chemical model for the control of ion flows during excitation. After an introductory survey on fundamental electrophysiological and biochemical observations, our integral model is developed and some basic electrophysiological excitation parameters are formulated in terms of specific membrane processes.

2. Problems in Biomembrane Electrochemistry

It is well known that bioelectricity and nerve excitability are electrically manifested in stationary membrane potentials and the various forms of transient potential changes such as, *e.g.*, the action potential [5, 6]. Although these electrical properties reflect membrane processes, bioelectricity and excitability are intrinsically coupled to the metabolic activity of the excitable cells. Now, inherent to all living cells is a high degree of coupling between energy and material flows. But already on the subcellular level of membranes intensive chemo-diffusional flow coupling occurs, and apparently time-independent properties reflect balance between active and passive flows of cell components.

The specific function of the excitable membrane requires the maintenance of nonequilibrium states [7]. The intrinsic nonequilibrium nature of membrane excitability is most obviously reflected in the asymmetric ion distributions across excitable

membranes. These nonequilibrium distributions are metabolically mediated and maintained by "active transport" (*e.g.*, Na^+/K^+ exchange-pump). A fundamental requirement for such an active chemo-diffusional flow coupling is spatial anisotropy of the coupling medium [8], including the membrane structure [7].

Anisotropy is apparently a characteristic property of biological membranes. Furthermore, cellular membranes including nerve membranes may be physico-chemically described in terms of a layer structure. For instance, it was found that the internal layer of the axonal membrane contains proteins required for excitability. Proteolytic action on this layer causes irreversible loss of this property [9].

The membrane components of excitable membranes include ionic constituents: fixed charges (some of them may serve to facilitate locally permselective ion diffusion), mono- and divalent metal ions, especially Ca^{2+} -ions, and in particular ionic side groups of membrane proteins directly involved in the control of electric properties. Local differences in the distribution of fixed charges may create membrane regions with different permeability characteristics, and (externally induced) ionic currents may amplify either accumulation or the depletion of ions within the membrane. Structural inhomogeneity of excitable membranes may thus lead to the observed nonlinear dependencies between certain physical parameters, *e.g.* between current intensity and potential [10]. A well known example of this nonlinearity is the current rectification in resting stationary states of excitable membranes. Correspondent to current rectification, there is a straightforward dependence of the membrane potential on the logarithm of ion activities only in limited concentration ranges [11, 12]. Thus, various chemical and physical membrane parameters show the intrinsic structural and functional anisotropy of excitable membranes. This recognition reveals the approximate nature of any classical equilibrium and nonequilibrium approach to electrochemical membrane parameters on the basis of linearity, and involving the assumption of a homogeneous isotropic membrane. Since, however, details of the membrane anisotropy are not known, any exact quantitative nonequilibrium analysis of membrane processes and ion exchange currents across excitable membranes faces great difficulties for the time being.

3. Stationary Membrane Potentials

It is only in the frame of a number of partially unrealistic, simplifying assumptions that we may approximately describe the electro-chemical behavior of excitable membranes, within a limited range of physico-chemical state variables [13]. Explicitly, we use the formalism of classical irreversible thermodynamics restricted to linearity between flows and driving forces. The application of the Nernst-Planck equation to the ion flows across excitable membranes represents such a linear approximation, widely used to calculate stationary membrane potentials.

There is experimental evidence that large contributions to the resting stationary membrane potential, $\Delta\psi_r$, are attributable to ion selectivities. Indeed, it appears that excitable membranes have developed dynamic structures which are characterized by permselectivity for certain ion types and ion radii. This property may be described by "Nernst" terms which, however, are strictly valid only for electrochemical equilibria. These Nernst terms may be calculated by application of the Nernst-Planck equation relating the ion flow J_i to the gradient of the electrochemical potential $\nabla\tilde{\mu}_i$ of the ion type i ,

$$J_i = U_i \cdot C_i \nabla(-\tilde{\mu}_i) \quad (1)$$

where U_i is the ionic mobility and C_i is the molar concentration. The electrochemical potential of ion i of valency Z_i is defined by

$$\tilde{\mu}_i = \mu_i^0 + RT \ln a_i + Z_i \cdot F \cdot \psi. \quad (2)$$

In Eq. (2), μ_i^0 is the standard chemical potential and a_i the thermodynamic activity which in dilute solution may be approximated by $a_i \cong C_i$; RT is the (molar) thermal energy, F is the Faraday constant, and ψ is the electrical potential.

In the frame of the linear model, the flux component $J_i(x)$ perpendicular to the membrane surfaces, at the point x within the membrane is given by

$$J_i(x) = U_i(x) \cdot C_i(x) \cdot \frac{d}{dx}(-\tilde{\mu}_i). \quad (3)$$

Integration of Eq. (3) within the membrane boundaries ($x = 0$ and $x = d$, the membrane "thickness") in a closed form requires restrictive assumptions. Despite the experimentally suggested inhomogeneity of excitable membranes, the following approximations are

used: (1) the ion mobilities are the same throughout the membrane and (2) space charge effects are negligible, so that we may assume approximate microscopic electroneutrality. Thus, $\nabla^2\psi \cong 0$ within the membrane. The Laplace equation $\nabla^2\psi = 0$ is equivalent to the constant field condition that usually is not an appropriate physical assumption [13, 14].

For the condition of stationarity, *i.e.* at zero net membrane current ($I_m = 0$), the uniform model treatment yields the Nernst contributions

$$(\Delta\psi_N)_{I_m=0} = \frac{RT}{F} \sum_i \frac{t_i}{Z_i} \ln \frac{a_i^{(o)}}{a_i^{(i)}} \quad (4)$$

where t_i is the transference number representing the fraction of membrane current carried by ion type i . (The fraction t_i may vary between 0 and 1.)

Although stationary states of excitable membranes always reflect balances between active transport processes and passive "leakage" fluxes, we may safely neglect flow contributions associated with active transport as long as the time scale considered does not exceed the millisecond range.

The electrical potential difference of an excitable membrane under "resting" conditions (stationary case of $I_m = 0$) may thus be approximated

$$\Delta\psi_r = (\Delta\psi_N)_{I_m=0}. \quad (5)$$

This relationship is useful for a limited number of practical cases.

It should be realized that the measured potential difference (measured for instance with calomel electrodes in connection with salt bridges) cannot be used to calculate the exact value for the average electric field, \bar{E} across the permeation barrier of thickness d . \bar{E} may be defined by

$$\bar{E} = -\frac{1}{d} \Delta\psi'. \quad (6)$$

The electrical potential difference $\Delta\psi'$ associated with the permeation barrier results from different sources. Although these sources are mutually coupled, we may formally separate the various contributions. Due to the presence of fixed charges at membrane surfaces [15] there are Donnan potentials $\Delta\psi_D^{(o)}$ and $\Delta\psi_D^{(i)}$ from the inside (i) and outside (o) interfaces between membrane and environ-

ment. Furthermore, there are interdiffusion potentials $\Delta\psi_r$ arising from differences in ionic mobilities within ion exchange domains of intramembraneous fixed charges (TASAKI, 1968). We may sum up these terms to $\Delta\psi_{DU} = \Delta\psi_D^{(o)} + \Delta\psi_D^{(i)} + \Delta\psi_r$. Thus.

$$\Delta\psi' = (\Delta\psi_N)_{I_m=0} + \Delta\psi_{DU}. \quad (7)$$

Compared to $\Delta\psi_{DU}$, the "Nernst" contributions to $\Delta\psi'$ appear relatively large. Inserting Eqs. (4) and (7) in Eq. (6), we obtain for the average electric field in the resting stationary state:

$$(\bar{E})_{I_m=0} = -\frac{1}{d} \left(\Delta\psi_{DU} + \frac{RT}{F} \sum_i \frac{t_i}{Z_i} \ln \frac{a_i^{(o)}}{a_i^{(i)}} \right) \quad (8)$$

Among the various ions known to contribute to $\Delta\psi$ (*in vivo*) are the metal ions Ca^{2+} , Na^+ , K^+ , protons, and Cl^- ions. In the resting state the value of the stationary membrane potential, $\Delta\psi_r$, is different for different cells and tissues. Distribution and density of fixed charges, ion gradients and the extent to which they contribute to $\Delta\psi_r$ differ for different excitable membranes.

Variations of the natural ionic environment lead also to alterations in the Donnan and interdiffusion terms. Since ions are an integral part of the membrane structure, for instance as counterions of fixed charges, any change in ion type and salt concentration but also variations in temperature and pressure may cause changes in the lipid phase [16] and in the conformation of membrane proteins. As a consequence of such structural changes, membrane "fluidity" and permeability properties may be considerably altered. It is known that, for instance, an increase in the external Ca^{2+} ion concentration increases the electrical resistance of excitable membranes [10].

4. Transient Changes of Membrane Properties

A central problem in bioelectricity is the question: what is the mechanism of the various transient changes of membrane parameters associated with nerve activity? Before discussing a specific physico-chemical model for the control of electrical activity, it appears necessary to recall a few fundamental electrophysiological and chemical observations on excitable membranes.

4.1 Transient Changes in $\Delta\psi$

The various transient expressions of nerve activity, such as depolarizations and hyperpolarizations of the resting stationary potential level, reflect changes of dielectric-capacitive nature, inter-diffusional ion redistributions and "activations" or "inactivations" of different gradients or changes of the extent with which these gradients contribute to the measured parameters. At constant pressure and temperature, the ion gradient contributions to $\Delta\psi$ may be modelled as variations of the t_i parameters.

(It should be realized that, in general, the actually measured parameters such as potential differences, ionic conductivity or membrane resistance may contain larger contributions from cell membranes — of glia or Schwann cells — between the excitable membrane and the measuring electrodes.)

a) Threshold Behavior. Transient changes in $\Delta\psi$ may be caused by various physical and chemical perturbations originating from adjacent membrane regions, from adjacent excitable cells, or from external stimuli. Phenomenologically we differentiate sub- and suprathreshold responses of the excitable membrane to stimulation. Subthreshold changes are, for instance, potential changes which attenuate with time and distance from the site of perturbation; this phenomenon has been called electrotonic spread. If, however, the intensity of depolarizing stimulation exceeds a certain threshold value, a potential change is triggered that does not attenuate, but propagates as such along the entire excitable membrane. This suprathreshold response is called *regenerative* and has been termed *action potential* or *nerve impulse*.

b) Stimulus Characteristics. In order to evoke an action potential, the intensity of the stimulation has to reach the threshold with a certain minimum velocity [10]. If the external stimulus for instance is a current of gradually increasing intensity, I , the minimum slope condition for the action potential may be written

$$dI/dt \geq (dI/dt)^{\min}. \quad (9)$$

The observation of a minimum slope condition for the generation of action potentials is of crucial importance for any mechanistic approach to excitability.

On the other hand, when rectangular current pulses are applied, there is an (absolute) minimum intensity, I_{th} , called rheobase, and a minimum time interval, Δt , of current application. It is found that the product of suprathreshold intensity and minimum duration is approximately constant. Thus, for $I \geq I_{th}$,

$$I \cdot \Delta t \cong \text{constant.} \quad (10)$$

Equation (10) describes the well known strength-duration curve. Whereas the strength-duration product does not depend on temperature, the temperature coefficient of the rheobase (dI_{th}/dT related to a temperature increase of 10°C) Q_{10} is generally about 2 (see e.g., [10]).

It is recalled that the value of I_{th} is history dependent. The threshold changes as a function of previous stimulus intensity and duration. Hyperpolarizing and depolarizing subthreshold prepulses also change the threshold intensity of the stimulating current. See Section 3d).

It appears that, in general, the induction of the action potential requires the *reduction* of the intrinsic membrane potential $\Delta\psi_r$ below a certain threshold value, $\Delta\psi_{th}$. This potential decrease $\Delta(\Delta\psi) = \Delta\psi_r - \Delta\psi_{th}$ is usually about 15–20 mV and has to occur in the *form of an impulse*, $\int \Lambda(\psi) dt$, in which a minimum condition between membrane potential and time must be fulfilled:

$$\frac{d\Delta\psi}{dt} \geq \left(\frac{d\Delta\psi}{dt} \right)^{\min}. \quad (11)$$

The condition for the initiation of an action potential may be written

$$\Delta\psi_r - \int \left(\frac{d\Delta\psi}{dt} \right)^{\min} dt = \Delta\psi_{th}$$

or in the more general form:

$$\Delta\psi_r - \frac{1}{\Delta t} \int \Lambda(\Delta\psi) dt \leq \Delta\psi_{th}. \quad (12)$$

The potential change $\Lambda(\Delta\psi)$ is equivalent to a change in the intrinsic field, E , across the membrane of the thickness d . Thus $\Delta E = -\Lambda(\Delta\psi)/d$, and with Ohm's law we have $d \cdot \Delta E = -R_m \cdot I$, where R_m is the membrane resistance.

Including Eq. (4) we may write

$$\begin{aligned} \int d(\Delta\psi) dt &= -d \int \Delta E dt = \int R_m \cdot I dt \\ &= \frac{RT}{Z_i F} \int \ln \frac{a_i^{(o)}}{a_i^{(i)}} dt. \end{aligned} \quad (13)$$

With Eq. (13) we see how the intrinsic membrane potential may be changed: by an (external) field (voltage or current) pulse or by an ion pulse involving those ions that determine the membrane potential [cf. Eq. (4)]. Such an ion pulse may be produced if the concentration of K^+ ions on the outside of excitable membranes is sufficiently increased within a sufficiently short time interval (impulse condition). Similarly, a pH change can cause action potentials [17].

As seen in Eq. (4), the membrane potential is dependent on temperature. Therefore, temperature (and also pressure) changes alter the stationary membrane potential in excitable membranes. Moreover, action potentials can be evoked by thermal and mechanical shocks (see, *e.g.*, [18]).

It is furthermore remarked that under natural conditions electrical depolarization produces an action potential only if the excitable membrane was kept above a certain (negative) level of polarization; in squid giant axons the minimum membrane potential is about -30 to -40 mV. This directionality of membrane polarization and the requirement of field reduction for regenerative excitation are a further manifestation of the intrinsic anisotropy of excitable membranes.

c) Propagation of Local Activity. In many excitable cells, $\Delta\psi_r$ is about -60 mV to -90 mV, where the potential of the cell interior is negative. Assuming an average membrane thickness, $d = 100 \text{ \AA}$, we calculate with Eq. (2) an average field intensity of approximately 60 – 90 kV/cm (cf., however, [19].) The field vector in the resting stationary state is directed from the outside to the inside across the membrane of the excitable cell. (It is this electric field which partially compensates the chemical potential gradient of the K^+ ions.) Any perturbation which is able to change locally the intrinsic membrane potential, *i.e.* the membrane field, will also affect adjacent parts of the membrane or even adjacent cells. If the field change remains below threshold or does not fulfil the minimum slope condition, there will be only subthreshold attenuation of this

field change. A nerve impulse in its rising phase may, however, reduce the membrane field in adjacent parts to such an extent and within the required minimum time interval, that suprathreshold responses are triggered. It is recalled that electric fields represent long-range forces (decaying with distance).

d) Refractory Phases. Another important observation suggestive for the nature of bioelectricity is the refractory phenomenon. If a nerve fibre is stimulated a second time shortly after a first impulse was induced, there is either an impulse that propagates much slower or only a subthreshold change. Immediately after stimulation a fibre is *absolutely refractory*, no further impulse can be evoked. This period is followed by a *relatively refractory phase* during which the threshold potential is more positive and only a slowly propagated impulse can be induced. The various forms of history dependent behavior are also called *accomodation* or *adaption* of the fibre to preceding manipulations.

4.2 Time Constants of $\Delta\psi$ Changes

If rectangular current pulses of fixed duration but of variable amplitude and amplitude directions are locally applied to an excitable membrane, a series of local responses are obtained. Figure 1 shows schematically the time course of these local responses upon hyper- and depolarizing stimulations: subthreshold potential changes and action potential [20].

It appears that the threshold level reflects a kind of instability. When the stimulus is switched off, fluctuations either result in the action potential (path $c \rightarrow c'$) or in a return to the resting level (path $c \rightarrow c''$). If, for phase c , the stimulus of the same intensity would last longer, an action potential would develop immediately from the hump visible in the late phase of stimulation.

In view of a rather continuous transition from a subthreshold response to the action potential, the notion of a threshold in the context of an all-or-none law for the action potential becomes less rigorous.

It has been found that a large section of the time course of sub- and suprathreshold responses can be described with linear differential equations of first order. Furthermore, the time constants τ_m associated with these sections are very similar for subthreshold

potential changes as well as for the initial rising phase (also called *latency*; see Fig. 1) of the action potential.

The actual values for τ_m are different for different excitable membranes and are dependent on temperature. In squid giant axons the value of τ_m at about 20° C is about 1 msec, the corre-

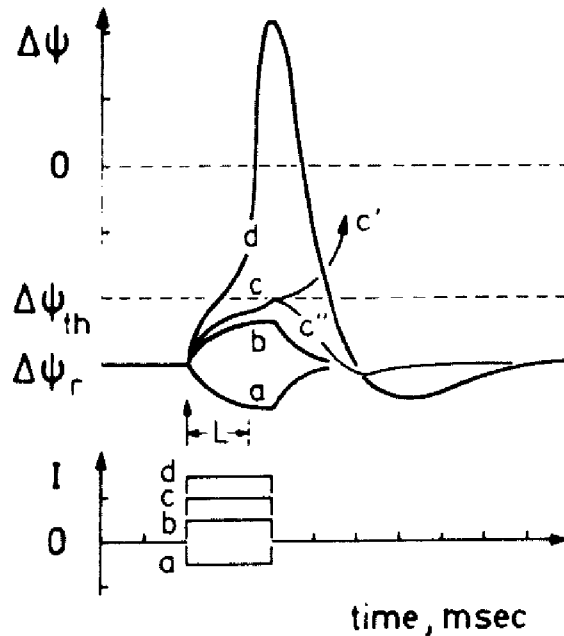


Fig. 1. Sub- and suprathreshold responses of the membrane potential, $\Delta\psi$, to stimulating rectangular current pulses of fixed duration but variable intensity, I . *a* inward current pulse causing hyperpolarization, *b* subthreshold outward current ($I < I_{th}$) causing depolarization, *c* threshold outward current (I_{th}) causing either an action potential (path c') or return to resting stationary potential $\Delta\psi_r$ (path c''), *d* suprathreshold outward pulse ($I > I_{th}$) causing action potential. L , latency phase of the action potential; $\Delta\psi_{th}$, threshold potential

sponding value for lobster giant axons is about 3 msec. The temperature coefficient ($d\tau_m^{-1}/dT$ related to a temperature increase of 10° C) Q_{10} is about 2 [10].

As outlined by COLE, a time constant in the order of milliseconds can hardly be modelled by simple electrodiffusion: an ion redistribution time of 1 msec requires the assumption of an extremely low ion mobility of 10^{-8} cm sec⁻¹/Vcm⁻¹. However, even in "sticky" ion exchange membranes, ionic mobilities are not below 10^{-6} cm sec⁻¹/V cm⁻¹. Furthermore, the temperature coefficients of simple electrodiffusion are only about 1.2–1.5.

Thus, magnitude and temperature coefficient of τ_m suggest that electrodiffusion is not rate-limiting for a large part of the ion redistributions following perturbations of the membrane field.

This conclusion is supported by various other observations. The time course of the action potential can be formally associated with a series of time constants, all of which have temperature coefficients of about 2–3 (see, *e.g.*, [10]). The various phases of the action potential are prolonged with decreasing temperature. In the framework of the classical Hodgkin-Husley phenomenology, prolonged action potentials should correspond to larger ion movements. However, it has been recently found that in contrast to this prediction, the amount of ions actually transported during excitation decreases with decreasing temperature [21].

It thus appears that ion movements (caused by perturbations of stationary membrane states) are largely rate-limited by membrane processes. Such processes may comprise phase changes of lipid domains or conformational changes of membrane proteins. Changes of these types are usually cooperative, and temperature dependencies are particularly pronounced within the cooperative transition ranges.

Prolonged potential changes and smaller ion transport at decreased temperatures may be readily modelled, if at lower temperature configurational rearrangements of membrane components involve smaller fractions of the membrane than at higher temperature.

There is a formal resemblance between the time constant τ of a phase change or a chemical equilibrium and the RC term of an electrical circuit with resistance R and capacity C [22]. If a membrane process is associated with the thermodynamic affinity A and a rate $J_r = d\xi/dt$, where ξ is the fractional advancement of this process, we may formally define a reaction resistance $R_r = (\partial A/\partial J_r)$ and an average reaction capacity $\bar{C}_r = -(\partial A/\partial \xi)^{-1}$. The time constant is then given by $\tau = R_r \cdot \bar{C}_r$.

The time course of electrical parameters may thus be formally modelled in terms of reaction time constants for membrane processes of the type discussed above. For the chemical contribution of the observed subthreshold change in the membrane potential

$$\Delta\psi = I_m R_m (1 - \exp[-t/\tau_m]) \quad (14)$$

caused by the rectangular current pulse of the intensity I_m across the membrane resistance R_m , we have $\tau_m = R_m C_m$.

As already mentioned, the time constants for subthreshold changes and for the first rising phase of the action potential are almost the same. Furthermore, the so-called "Na⁺-ion activation-inactivation curves (in voltage clamp experiments) for both sub- and suprathreshold conditions are similar in shape, although much different in amplitude" (PLOWSEY [23]). These data strongly suggest that sub- and suprathreshold responses are essentially based on the same control mechanism; it is then most likely the molecular organization of the control system that accounts for the various types of response.

4.3 Proteins Involved in Excitation

Evidence is accumulating that proteins and protein reactions are involved in transient changes of electrical parameters during excitation. As briefly mentioned, the action of proteases finally leads to inexcitability. Many membrane parameters such as ionic permeabilities are pH-dependent. In many examples, this dependency is associated with a pK value of about 5.5 suggestive for the participation of carboxylate groups of proteins.

Sulfhydryl reagents and oxidizing agents interfere with the excitation mechanism, *e.g.* prolonging the duration and finally blocking the action potential [24]. The membrane of squid giant axons stain for SH groups provided the fibres had been stimulated [25]. These findings strongly suggest the participation of protein specific redox reactions in the excitation process.

Of particular interest appear the effects of ultraviolet radiation on Ranvier nodes. The spectral radiation sensitivity of the rheobase and of the fast transient inward current (normally due to Na⁺ ions) in voltage clamp is very similar to the ultraviolet absorbance spectrum for proteins [26, 27]. The results of Fox [27] also indicate that the fast transient inward component of the action current is associated with only a small fraction of the node membrane.

The conclusions on locally limited excitation sites are supported by the results obtained with certain nerve poisons. Extremely low concentrations of tetrodotoxin reduce and finally abolish the fast inward component of voltage clamp currents (for review see [28]).

The block action of this toxin is pH dependent and is associated with a pK value of about 5.3.

It thus appears that the ionic gateways responsible for the transient inward current involve protein organizations comprising only a small membrane fraction.

4.4 Impedance and Heat Changes

a) Impedance Change. The impedance change accompanying the action potential is one of the basic observations in electrophysiological studies on excitability [10]. It is generally assumed that the impedance change reflects changes in ionic conductivities (ionic permeabilities). Figure 2 shows schematically that the conductance first rises steeply and in a second phase decays gradually towards the stationary level. In contrast to the pronounced resistance change, the membrane capacity apparently does not change during excitation [29]. This result, too, suggests that only a small fraction of the membrane is involved in the rather drastic permeability changes during excitation.

b) Heat Exchange Cycle. The action potential is accompanied by relatively large heat changes [30]. These heat changes may be

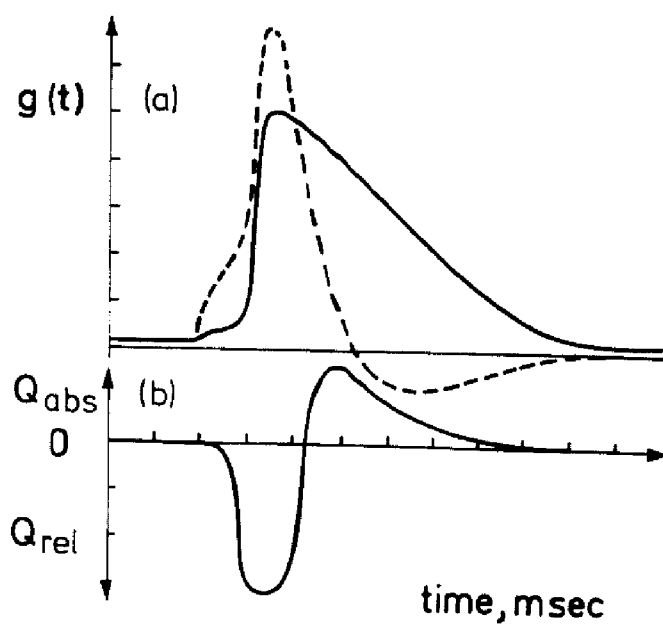


Fig. 2a and b. Schematic representation of (a) conductivity change, $g(t)$, accompanying the action potential (dashed line) and (b) heat exchange cycle. Q_{rel} , heat released during rising phase and Q_{abs} , heat absorbed during falling phase of the action potential

thermodynamically modelled in terms of a cyclic variation of membrane states. The complex chain of molecular events during a spike may be simplified by the sequence of state changes $A \rightarrow B \rightarrow A$, where A represents the resting stationary state and B symbolizes the transiently excited state of higher ionic permeability.

The heat changes occur under practically isothermal-isobaric conditions. If we now associate the Gibbs free energy change, ΔG , with the (overall) excitation process $A \rightleftharpoons B$, we may write

$$\Delta G = \Delta H - T \Delta S, \quad (15)$$

where ΔH is the reaction enthalpy (as heat exchangeable with the environment), and ΔS the reaction entropy.

More recently, it has been confirmed that the rising phase of the action potential is accompanied by heat release, Q_{rel} , while during the falling phase the heat Q_{abs} is reabsorbed [31]; see Fig. 2. In our simple A - B model, the first phase is associated with

$$\Delta G_{A \rightarrow B} = \Delta H_{A \rightarrow B} - T \Delta S_{A \rightarrow B}$$

and the second one with

$$\Delta G_{B \rightarrow A} = \Delta H_{B \rightarrow A} - T \Delta S_{B \rightarrow A}.$$

In general, for a cyclic process (where the original state is restored), $\oint dG = 0$ or

$$\Delta G_{A \rightarrow B} + \Delta G_{B \rightarrow A} = 0.$$

In the hypothetical case of ideality (reversibility), $\Delta H = Q$ and $Q_{\text{rel}} + Q_{\text{abs}} = 0$.

Since no *natural* process occurs ideally (*i.e.* completely reversible), there are always irreversible contributions. This means a part of $\Delta G_{A \rightarrow B}$ and of $\Delta G_{B \rightarrow A}$ will dissipate into heat. We may formally split ΔG in a reversible (exchangeable) contribution ΔG^{rev} and an irreversible contribution ΔG^{irr} [32]. Thus,

$$\begin{aligned} \Delta G_{A \rightarrow B} &= \Delta G_{A \rightarrow B}^{\text{rev}} + \Delta G_{A \rightarrow B}^{\text{irr}} \\ \Delta G_{B \rightarrow A} &= \Delta G_{B \rightarrow A}^{\text{rev}} + \Delta G_{B \rightarrow A}^{\text{irr}}. \end{aligned}$$

By definition $\Delta G^{\text{irr}} = -T \Delta S^{\text{irr}} \leq 0$, since the change in the inner entropy ΔS^{irr} is always larger or equal to zero (see, *e.g.*, [8]).

The measured heats are then given by

$$Q_{\text{rel}} = \Delta H_{A \rightarrow B}^{\text{rev}} + \Delta G_{A \rightarrow B}^{\text{irr}}$$

$$Q_{\text{abs}} = \Delta H_{B \rightarrow A}^{\text{rev}} + \Delta G_{B \rightarrow A}^{\text{irr}}$$

Since in a cycle $\Delta H_{A \rightarrow B}^{\text{rev}} + \Delta H_{B \rightarrow A}^{\text{rev}} = 0$, we find for the "difference" between heat released and heat absorbed,

$$\Delta Q = Q_{\text{rel}} + Q_{\text{abs}} = (\Delta G_{A \rightarrow B}^{\text{irr}} + \Delta G_{B \rightarrow A}^{\text{irr}}) = -T \Delta S^{\text{irr}} \leq 0.$$

Due to irreversible contributions we have for the absolute values $|Q_{\text{rel}}| > |Q_{\text{abs}}|$ (Q_{rel} counting negative!). It is found experimentally that $|Q_{\text{abs}}| \cong 0.9 |Q_{\text{rel}}|$. As stressed by GUGGENHEIM [33], $\Delta G^{\text{irr}} < 0$ or $\Delta S^{\text{irr}} > 0$, *only if phase changes and/or chemical reactions are involved*. Then, for our case we may write

$$\Delta Q = \Delta G^{\text{irr}} = - \sum_j A_j \xi_j < 0,$$

where A is the affinity and ξ is the extent of membrane processes j involved [32].

Since the action potential most likely involves only a small fraction of the excitable membrane, the measured heat changes Q_{rel} and Q_{abs} appear to be very large. Since, on the other hand, the mutual transition $A \rightarrow B \rightarrow A$ "readily" occurs, the value of ΔG ($= \Delta G_{A \rightarrow B} = -\Delta G_{B \rightarrow A}$) cannot be very large. In order to compensate a large ΔH (here $\cong Q$), there must be a large value for ΔS ; see Eq. (15). This means that the *entropy change associated with the membrane permeability change during excitation is also very large*.

It is, in principle, not possible to deduce from heat changes the nature of the processes involved. However, large configurational changes — equivalent to a large overall ΔS — in biological systems frequently arise from conformational changes of macromolecules or macromolecular organizations such as membranes or from chemical reactions. In certain polyelectrolytic systems, such changes even involve metastable states and irreversible transitions of domain structures [32].

The large absolute values of Q and the irreversible contribution ΔQ suggest structural changes and/or chemical reactions to be associated with the action potential. Furthermore, there are obser-

vations indicating the occurrence of metastable states and non-equilibrium transitions in excitable membranes, at least for certain perfusion conditions [6].

Summary of This Section. (1) Due to various similarities, sub- and suprathreshold responses appear to reflect a common basic mechanism (more complex than simple electro-diffusion). (2) Proteins and, in some examples, redox reactions are involved in excitation. (3) Large changes in heat and membrane resistance accompanying the action potential point to configurational rearrangements of membrane components: phase changes, conformational transitions or chemical reactions. (4) Evidence is accumulating suggesting that excitability comprises only a small membrane fraction. (5) Many features of subthreshold responses may be analyzed as membrane relaxations to small perturbations of stationary states (tractable with linear differential equations of first order). Suprathreshold responses are basically nonlinear and may be seen as relaxations to (locally) large perturbations of the excitable membrane.

5. The Cholinergic System and Excitability

Among the oldest known macromolecules associated with excitable membranes are some proteins of the cholinergic system. The cholinergic apparatus comprises acetylcholine (AcCh), the synthesis enzyme choline-*O*-acetyltransferase (ChT), acetylcholinesterase (AcCh-E), acetylcholine-receptor (AcCh-R) and a storage site (*S*) for AcCh. Since details of this system are outlined by NACHMANSOHN in the previous chapter, here only a few aspects essential for our integral model of excitability are discussed.

5.1 Localization of the AcCh System

Chemical analysis has revealed that the concentration of the cholinergic system is very different for various excitable cells. For instance, squid giant axons contain much less AcCh-E (and ChT) than axons of lobster walking legs (see, *e.g.*, [34]); motor nerves are generally richer in cholinergic enzymes than sensory fibres [35].

The search for cholinergic enzymes in nerves was greatly stimulated by the observation that AcCh is released from isolated

axons of various excitable cells, provided inhibitors of AcCh-E such as physostigmine (eserine) are present. This release is appreciably increased upon electric stimulation [36, 37] or when axons are exposed to higher external K^+ ion concentrations [38]. Since larger increases of external K^+ concentration depolarize excitable membranes, reduction of membrane potential appears to be a prerequisite for AcCh liberation from the storage site.

Evidence for the presence of axonal AcCh storage sites and receptors are still mainly indirect. However, direct evidence more and more accumulates for extrajunctional AcCh receptors [39]: binding studies with α -bungarotoxin (a nerve poison with a high affinity to AcCh-R) indicate the presence of receptor-like proteins in axonal membrane fragments [40].

Recently, another protein which also binds AcCh, α -bungarotoxin (and other cholinergic ligands) has been isolated by CHANG from the electric organ of *Electrophorus electricus* [75]. We recall that the receptor protein (*R*) may be operationally defined as the membrane component which upon binding of AcCh and other agonists causes a membrane permeability change and which upon binding of α -bungarotoxin and other antagonists prevents AcCh- (or electrically) induced permeability changes. To account for specificity and efficiency, the receptor protein should bind AcCh with a high binding constant and with a binding stoichiometry of not more than one or a few AcCh molecules per receptor protein. The other protein (called by CHANG AcCh-R II) would not match this operational definition, because it has a high binding capacity for AcCh associated with a relatively low binding constant. This protein would, however, meet the properties required for a membrane storage site for AcCh and may thus be called *S*-protein.

There are still discrepancies as to the presence and localization of the cholinergic system. However, the differences in the interpretations of chemico-analytical data and the results of histochemical light- and electron microscopy investigations gradually begin to resolve; there appears progressive confirmation for the early chemical data of an ubiquitous cholinergic system. For instance, AcCh-E reaction products can be made visible in the excitable membranes of more and more nerves formerly called non-cholinergic (see, *e.g.*, [41]). Catalysis products of AcCh-E are demonstrated in pre- and postsynaptic parts of excitable mem-

branes (see, *e.g.*, [41] and [42]). In a recent study, stain for the α -bungarotoxin receptor complex is visible also in presynaptic parts of axonal membranes (see Fig. 1 in [39]). These findings suggest the presence of the cholinergic system in both junctional membranes and thus render morphological support for the results of previous studies on the pre- and postsynaptic actions of AcCh and inhibitors and activators of the cholinergic system [43, 44].

5.2 The Barrier Problem

Histochemical, biophysical and biochemical and, particularly, pharmacological studies on nerve tissue face the great difficulty of an enormous morphological and chemical complexity. It is now recognized that due to various structural features not all types of nerve tissue are equally suited for certain investigations.

The excitable membranes are generally not easily accessible. The great majority of nerve membranes is covered with protective tissue layers of myelin, of Schwann- or glia cells. These protective layers insulating the excitable membrane frequently comprise structural and chemical barriers that impede the access of test compounds to the excitable membrane. In particular, the lipid-rich myelin sheaths are impervious to many quaternary ammonium compounds such as AcCh and *d*-tubocurarine (curare).

In some examples such as the frog neuro-muscular junction, externally applied AcCh or the receptor inhibitor curare have relatively easy access to the synaptic gap, whereas the excitable membranes of the motor nerve and the muscle fibre appear to be largely inaccessible. On the other hand, the cholinergic system of neuromuscular junctions of lobsters are protected against external action of these compounds, whereas the axons of the walking legs of lobster react to AcCh and curare (*cf.*, *e.g.*, [45]).

Penetration barriers also comprise absorption of test compounds within the protective layers. Furthermore, chemical barriers in the form of hydrolytic enzymes frequently cause decomposition of test compounds before they can reach the nerve membrane. For instance, phosphoryl phosphatases in the Schwann cell layer of squid giant axons cause hydrolysis of organophosphates such as the AcCh-esterase inhibitor diisopropylfluorophosphate (DFP), and impulse conduction is blocked only at very high concentrations of DFP [46].

A very serious source of error in concentration estimates and in interpretations of pharmacological data resides in procedures that involve homogenization of lipid-rich nerve tissue (see, *e.g.*, [47]). For instance, homogenization liberates traces of inhibitors (previously applied) which despite intensive washing still adhered to the tissue. Even when the excitable membrane was not reached by the inhibitor, membrane components react with the inhibitor during homogenization [48]. Thus, block of enzyme activity observed after homogenization is not necessarily an indicator for block during electrical activity (cf. *e.g.*, [20], p. 90).

As demonstrated in radiotracer studies, failure to interfere with bioelectricity is often concomitant with the failure of test compounds to reach the excitable membrane. Compounds like AcCh or *d*-tubocurarine (curare) act on squid giant axons only after (enzymatic) reduction of structural barriers [49]. Diffusion barriers even after partial reduction are often the reason for longer incubation times and higher concentration of test compounds as compared to less protected membrane sites.

In this context it should be mentioned that the enzyme choline-*O*-acetyltransferase, sometimes considered to be a more specific indicator of the cholinergic system, is frequently difficult to identify in tissue and is *in vitro* extremely unstable [50].

In the light of barrier and homogenization problems, it appears obvious that any statements on the absence of the cholinergic system or on the failure of blocking compounds to interfere with excitability are *only useful*, if they are based on evidence that the test compound had actually reached the nerve membrane.

5.3 Electrogenic Aspects of the AcCh System

Particularly suggestive for the bioelectric function of the cholinergic system in axons are electrical changes resulting from eserine and curare application to Ranvier nodes, where permeability barriers are less pronounced [51, 52]. Similar to the responses of certain neuromuscular junctions, eserine prolongs potential changes also at nodes; curare first reduces the amplitude of the nodal action potential and then also decreases the intensity of subthreshold potential changes in a similar way as known for frog junctions.

In all nerves the generation of action potentials is readily blocked by (easily permeating) local anesthetics such as procaine or tetracaine. Due to structural and certain functional resemblance to AcCh which is particularly pronounced for tetracaine (see Fig. 3),

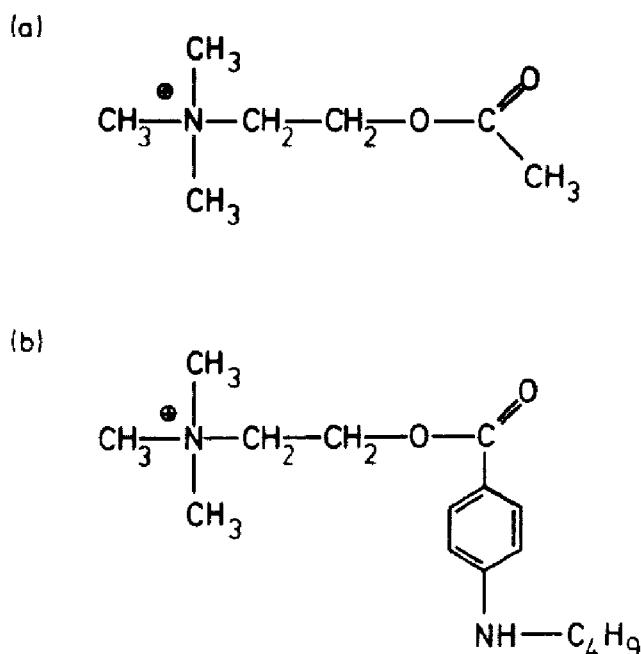


Fig. 3a and b. Chemical structure of the acetylcholine ion (a) and of the tetracaine ion (b). Note that the structural difference is restricted to the acid residue: (a) CH_3 and (b) the amino-benzoic acid residue rendering tetracaine lipid-permeable

these compounds may be considered as analogs of AcCh. In a recent study it is convincingly demonstrated how (by chemical substitution at the ester group) AcCh is successively transformed from a receptor activator (reaching junctional parts only) to the receptor inhibitor tetracaine reaching readily junctional and axonal parts of excitable membranes [53]. Local anesthetics are also readily absorbed in lipid bilayer domains of biomembranes (see for review [54]).

External application of AcCh without esterase inhibitors faces not only diffusion barriers, but esterase activity increases the local proton concentration [45]: the resulting changes in pH may contribute to changes in membrane potential.

Only very few nerve preparations appear to be suited to demonstrate a direct electrogenic action of externally applied AcCh. Dif-

fusion barriers and differences in local concentrations of the cholinergic system may be the reason that the impulse condition for the generation of action potentials cannot be fulfilled everywhere (see Section 4.1b). Some neuroblastoma cells produce subthreshold potential changes and action potentials upon electrical stimulation as well as upon AcCh application [55-57, see also p. 391 ff.].

There are thus, without any doubt, many pharmacological and chemical similarities between synaptic and axonal parts of excitable membranes *as far as the cholinergic system is concerned*. On the other hand, there are various differences. But it seems that these differences can be accounted for by structural and chemical factors.

As to this problem, two extreme positions of interpretation may be distinguished. On the one side more emphasis is put to the differences between axonal and junctional membranes. An extreme view considers the responses of axons to AcCh and structural analogs as a pharmacological curiosity [58, 59]: and, in general, the cholinergic nature of excitable membrane is not recognized and acknowledged. However, the presence of the cholinergic system in axons and the various similarities to synaptic behavior suggest the same basic mechanism for the cholinergic system in axonal and synaptic parts of excitable membranes.

Since until now there has been *no direct experimental evidence for AcCh to cross the synaptic gap*, the action of AcCh may be alternatively assumed to be restricted to the interior of the excitable membrane of junctions and axons. This assumption is based on the fact that no trace of AcCh is detectable outside the nerve unless AcCh-esterase inhibitors are present. According to this alternative hypothesis, *intramembraneous* AcCh combines with the receptor and causes permeability changes mediated by conformational changes of the AcCh receptor.

This is the basic postulate of the chemical theory of bioelectricity (NACHMANSOHN, this issue), attributing the primary events of all forms of excitability in biological organisms to the cholinergic system: in axonal conduction, for subthreshold changes (electronic spread) in axons and pre- and postsynaptic parts of excitable membranes.

In the framework of the chemical model the various types of responses, excitatory and inhibitory synaptic properties are associated with structural and chemical modifications of the *same*

basic mechanism involving the cholinergic system. Participation of neuroeffectors like the catecholamines or γ -aminobutyric acid and other additional reactions within the synapse possibly give rise to the various forms of depolarizing and hyperpolarizing potential changes in postsynaptic parts of excitable membranes. The question of coupling between pre- and postsynaptic events during signal transmission cannot be answered for the time being. It is, however, suggestive to incorporate in transmission models the transient increase of the K^+ ion concentration in the synaptic gap after a presynaptic impulse.

5.4 Control Function of AcCh

It is recalled that transient potential changes such as the action potential result from permeability changes caused by proper stimulation. However, a (normally proper) stimulus does not cause an action potential if (among others) certain inhibitory analogs of the cholinergic agents such as, *e.g.*, tetracaine are present. Tetracaine also reduces the amplitudes of subthreshold potential changes; in the presence of local anesthetics, for instance procaine, mechanical compression does not evoke action potentials [18]. It thus appears that the (electrical and mechanical) stimulus does *not directly* effect sub- and suprathreshold permeability changes, suggesting preceding events involving the cholinergic system.

If the AcCh-esterase is inhibited or the amount of this enzyme is reduced by protease action, subthreshold potential changes and (postsynaptic) current flows as well as the action potential are prolonged (see, *e.g.*, [51, 52, 60]). Thus AcCh-E activity appears to play an essential role in terminating the transient permeability changes. The extremely high turnover number of this enzyme (about 1.4×10^4 AcCh molecules per sec, *i.e.*, a turnover time of 70 μ sec) is compatible with a rapid removal of AcCh [61].

In summary, the various studies using activators and inhibitors of the cholinergic system indicate that both initiation and termination of the permeability changes during nerve activity are (active) processes associated with AcCh. It seems, however, possible to decouple the cholinergic control system from the ionic permeation sites or gateways. Reduction of the external Ca^{2+} ion concentration appears to cause such a decoupling: the result is an increase in

potential fluctuations or even random, *i.e.* uncontrolled firing of action potentials (see, *e.g.*, [10]).

6. The Integral Model

In the previous sections, some basic electrophysiological observations and biochemical data are discussed that any adequate model for bioelectricity has to integrate. It is stressed that among the features excitability models have to reproduce are the threshold behavior, the various similarities of sub- and suprathreshold responses, stimulus characteristics and the various forms of conditioning and history-dependent behavior.

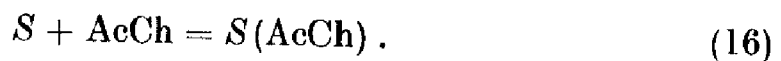
In the present account we explore some previously introduced concepts for the control of electrical membrane properties by the cholinergic system [4]. Among these fundamental concepts are (i) the notion of a basic excitation unit (BEU); (ii) the assumption of an AcCh storage site particularly sensitive to the electric field of the excitable membrane; (iii) the idea of a continuous sequential translocation of AcCh through the cholinergic proteins (AcCh-cycle). Finally, we proceed towards a formulation of various excitation parameters in terms of nonequilibrium thermodynamics.

6.1 Key Processes

In order to account for the various interdependencies between electrical and chemical parameters, it is necessary to distinguish between a minimum number of single reactions associated with excitation.

A possible formulation of some of these processes in terms of chemical reactions has been previously given [4]. The reaction scheme is briefly summarized.

1) *Supply of AcCh to the membrane storage site (S), following synthesis (formally from the hydrolysis products choline and acetate)*

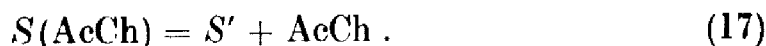


For the uptake reaction two assumptions are made: (i) the degree of AcCh association with the binding configuration *S* increases with increasing membrane potential (cell interior negative), (ii) the uptake rate is limited by the conformational transition from

state S to $S(\text{AcCh})$, and is slow as compared to the following translocation steps.

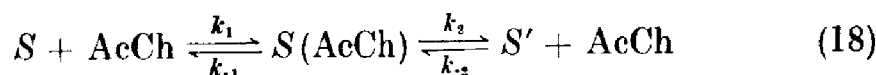
Vesicular storage of AcCh as indicated by WHITTAKER and coworkers [62 see also p. 515 ff.] is considered as additional storage for membrane sites of high AcCh turnover, for instance at synapses.

2) *Release of AcCh* from the storage from $S(\text{AcCh})$, for instance by depolarizing stimulation



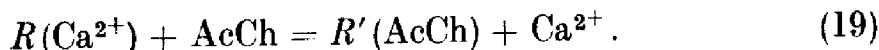
Whereas $S(\text{AcCh})$ is stabilized at large (negative) membrane fields, S' is more stable at small intensities of the membrane fields. The field-dependent conformational changes of S are assumed to gate the path of AcCh to the AcCh-receptors.

The assumptions for the dynamic behaviour of the storage translocation sequence



may be summarized as follows: the rate constant, k_2 , for the release step is larger than the rate constant, k_1 , for the uptake, and also $k_2 \gg k_{-2}$. (See also Section 6.5.)

3) *Translocation of released AcCh* to the AcCh-receptor (R) and association with the Ca^{2+} -binding conformation $R(\text{Ca}^{2+})$. This association is assumed to induce a conformational change to R' that, in turn, releases Ca^{2+} ions



4) *Release of Ca^{2+} ions* is assumed to change structure and organization of gateway components, G . The structural change from a closed configuration, G , to an open state, G' , increases the permeability for passive ion fluxes.

5) *AcCh Hydrolysis*. Translocation of AcCh from $R'(\text{AcCh})$ to the AcCh-esterase, E , involving a conformational transition from E to E'



The hydrolysis reaction causes the termination of the permeability change by re-uptake of Ca^{2+} ions,



concomitant with the relaxation of the gateway to the closed configuration, G .

Thus, the reactions (20) and (21) "close" a reaction cycle which is formally "opened" with reactions (16) and (18).

Since under physiological conditions (i.e. without esterase inhibitor) no trace of AcCh is detectable outside the excitable membrane (axonal and synaptic parts), the sequence of events modelled in the above reaction scheme is suggested to occur in a specifically organized structure of the cholinergic proteins; a structure that is intimately associated with the excitable membrane.

6.2 Basic Excitation Unit

Before proceeding towards a model for the organization of the cholinergic system, it is instructive to consider the following well known electrophysiological observations.

In a great variety of excitable cells the threshold potential change to trigger the action potential is about 20 mV. This voltage change corresponds to an energy input per charge or charged group within the membrane field of only about one kT unit of thermal energy (k Boltzmann constant; T absolute temperature) at body temperature. If only one charge or charged group would be involved, thermal motion should be able to initiate the impulse. Since random "firing" is very seldom, we have to conclude that several ions and ionic groups have to "cooperate" in a concerted way in order to cause a suprathreshold permeability change.

Furthermore, there are various electrophysiological data which suggest at least two types of gateways for ion permeation in excitable membranes (for summary see [10]): a rapidly operating ion passage normally gating passive flow of Na^+ ions (into the cell interior) and permeation sites that normally limit passive K^+ ion flow.

There are various indications such as the direction of potential change and of current flow, suggesting that the rising phase of the action potential has predominantly contributions from the "rapid gateway"; the falling phase of the overall permeability change involves larger contributions of the K^+ ion gateways (see also [63]). There is certainly coupling between the two gateway types: electrically through field changes and possibly also through Ca^{2+} ions

transiently liberated from the "rapid gateways". As explicitly indicated in Eqs. (19) and (21), Ca^{2+} ion movement precedes and follows the gateway transitions. Recent electrophysiological studies on neuroblastoma cells confirm the essential role of Ca^{2+} ions in sub-threshold potential changes and in the gating phase of the action potential [54].

At the present stage of our model development we associate the direct cholinergic control of permeability changes only to the rapidly operating gateway, G . As seen in Fig. 2, the rising phase of the conductance change caused by the permeability increase is rather steep. This observation, too, supports a cooperative model for the mechanism of the action potential.

The experimentally indicated functional cooperativity, together with the (experimentally suggested) locally limited excitation sites, suggest a structural anchorage in a cooperatively stabilized membrane domain.

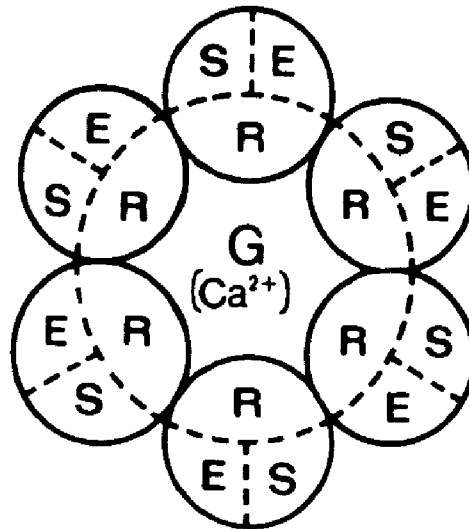
In order to account for the various boundary conditions discussed above we have introduced the *notion of a basic excitation unit* (BEU). Such a unit is suggested to consist of a gateway G that is surrounded by the cholinergic control system. The control elements are interlocked complexes of storage (S), receptor (R) and esterase (E), and are called *SRE*-assemblies. These assemblies may be organized in different ways and, for various membrane types, the BEUs may comprise different numbers of *SRE* assemblies. As an example, the BEU schematically represented in Fig. 4 contains 6 *SRE* units controlling the permeation site, G .

The core of the BEU is a region of dynamically coupled membrane components with fixed charges and counter ions such as Ca^{2+} ions. Figure 4 shows that the receptors of the *SRE* assemblies form a ring-like array. We assume that this structure is cooperatively stabilized and, through Ca^{2+} ions, intimately associated with the gateway components. In this way the Ca^{2+} -dependent conformational dynamics of the receptors is coupled to the transition behavior of the gateway.

The receptor ring of a BEU is surrounded by the "ring" of the storage sites and (spatially separated) by the "ring" of the AcCh esterases. The interfaces between the different rings define local reaction spaces through which AcCh is exchanged and translocated.

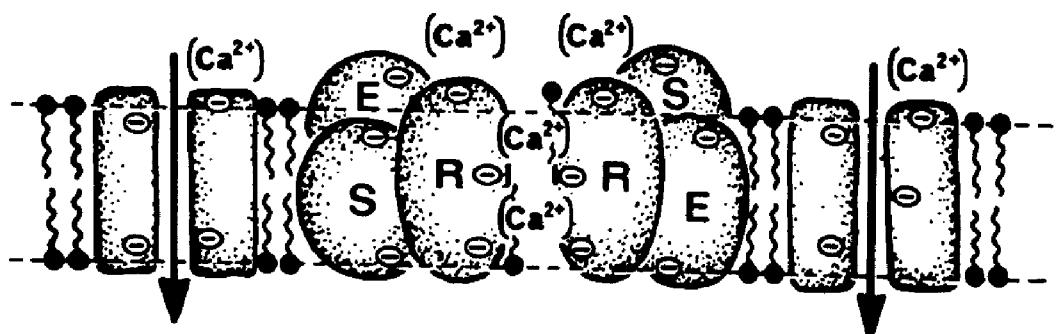
The BEUs are assumed to be distributed over the entire excitable membrane, axonal, pre- and postsynaptic and dendritic parts; the BEU density may vary for different membrane parts. The high density of cholinergic proteins found in some examples may be due to clustering of BEUs.

(a)



(b)

(OUTSIDE)



(INSIDE)

Fig. 4a and b. Scheme of the AcCh-controlled gateway, *G*. (a) Basic excitation unit (BEU) containing in this example 6 *SRE*-assemblies, viewed perpendicular to the membrane surface. *S*, AcCh storage site; *R*, AcCh receptor protein; *E*, AcCh-esterase. (b) Cross section through a BEU flanked by two units which model ion passages for K^+ ions; the arrows represent the local electrical field vectors due to partial permselectivity to K^+ ions in the resting stationary state. The minus signs, \ominus , symbolize negatively charged groups of membrane components

Different membrane types may not only vary in the number of *SRE* assemblies per BEU but also in the type and organization of the gateway components, thus assuring permselectivities for various ion types, particularly in synaptic parts of excitable membranes. It is only the cholinergic control system, the *SRE*-element which is assumed to be the same for all types of rapidly controlled gateways for passive ion flows.

a) Action Potential. In the framework of the integral model, the induction of an action potential is based on cooperativity between several *SRE* assemblies per BEU. In order to initiate an action potential a certain critical number of receptors, \bar{m}^c per BEU, has on average to be activated within a certain critical time interval, Δt^c , (impulse condition). During this time interval at least, say 4 out of 6 *SRE* assemblies have to process AcCh in a concerted manner. Under physiological conditions only a small fraction of BEUs is required to generate and propagate the nerve impulse.

b) Subthreshold Responses. Subthreshold changes of the membrane are seen to involve only a few single *SRE* assemblies of a BEU. On the average not more than one or two *SRE* elements per BEU are assumed to contribute to the measured responses (within time intervals of the duration of Δt^c).

The (small) permeability change caused by Ca^{2+} release from the receptor thus results from only a small part of the interface between receptor and gateway components of a BEU: the ion exchanges $\text{AcCh}^+/\text{Ca}^{2+}$ and Na^+ are locally limited.

In the framework of this model, spatially and temporally attenuating electrical activity such as subthreshold axonal, post-synaptic, and dendritic potentials are the sum of spatially and temporally additive contributions resulting from the local subthreshold activity of many BEUs.

Although the permeability changes accompanying local activity are very small (as compared to those causing the action potentials), the summation over many contributions may result in large overall conductivity changes. Such changes may even occur (to a perhaps smaller extent) when the core of the gateway is blocked. It is suggested that compounds like tetrodotoxin and saxitoxin interact with the gateway core only, thus essentially not impeding subthreshold changes at the interface between receptor ring and gateway.

Influx of Ca^{2+} ions particularly through pre- and postsynaptic membranes may affect various intracellular processes leading, e.g., to release of hormones, catecholamines, etc.

6.3 Translocation Flux of AcCh

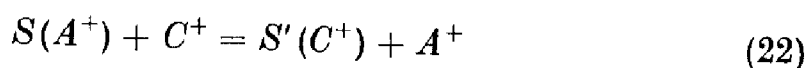
As discussed before, the excitable membrane as a part of a living cell is a nonequilibrium system characterized by complex chemodiffusional flow coupling. Although modern theoretical biology tends to regard living organisms only as quasi-stationary, with oscillations around a steady average, our integral model for the subthreshold behavior of excitable membranes is restricted to stationarity. We assume that the "living" excitable membrane (even under resting conditions) is in a state of continuous subthreshold activity (maintained either aerobically or anaerobically). However, the nonequilibrium formalism developed later on in this section can also be extended to cover non-linear behavior such as oscillations in membrane parameters.

In the frame of the integral model, continuous subthreshold activity is also reflected in a continuous sequential translocation of AcCh through the cholinergic system. The *SRE* elements comprise reaction spaces with continuous input by synthesis (ChT) and output by the virtually irreversible hydrolysis of AcCh. Input and output of the control system are thus controlled by enzyme catalysis.

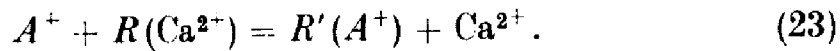
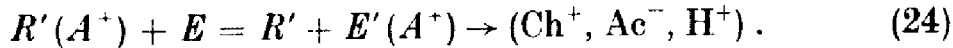
a) Reaction Scheme. Since AcCh is a cation, translocation may most readily occur along negatively fixed charges, may involve concomitant anion transport or cation exchange. The reaction scheme formulated in Section 6.1 gives therefore only a rough picture. Storage, receptor and esterase represent macromolecular subunit complexes with probably several binding sites and the exact stoichiometry of the AcCh reactions is not known.

The conformationally mediated translocation of the AcCh-ion, A^+ , may then be reformulated by the following sequence:

1) Storage Reaction

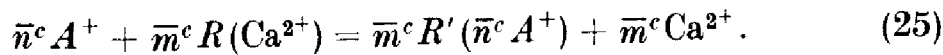


(C^+ symbolizes a cation, $2C^+$ may be replaced by Ca^{2+}).

2) *Receptor Reaction*3) *Hydrolysis Reaction*

As already mentioned, the nucleation of the gateway transition (causing the action potential) requires the association of a critical number of A^+ , \bar{n}^c , with the cooperative number of receptors, \bar{m}^c , in the Ca^{2+} binding form $R(\text{Ca}^{2+})$, within a critical time interval Δt^c . This time interval is determined by the life time of a single receptor-acetylcholine association.

Using formally \bar{n}^c and \bar{m}^c as stoichiometric coefficients the concerted reaction inducing gateway transition may be written:

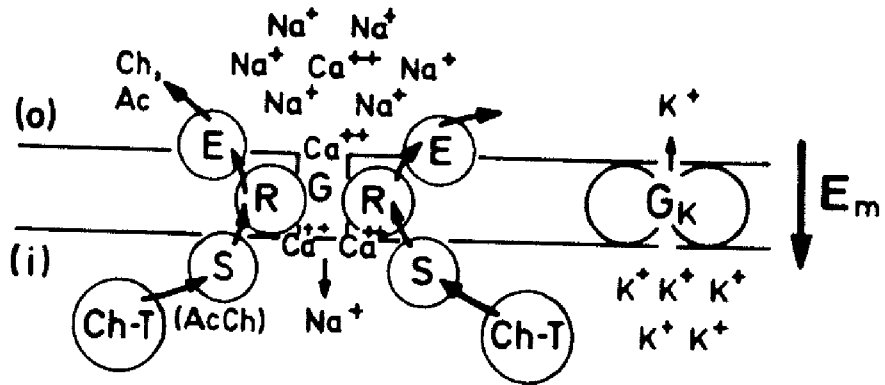


Storage and receptor reactions, Eqs. (22) and (23), represent gating processes preceding gateway opening (“ Na^+ -activation”) and causing the latency phase of the action potential. The hydrolysis process causes closure of the cholinergically controlled gateway (“ Na^+ -inactivation”). In the course of these processes the electric field across the membrane changes, affecting all charged, dipolar, and polarizable components within the field. These field changes particularly influence the storage site and the membrane components controlling the K^+ permeation regions (see [65]). Figure 5 shows a scheme modelling the “resting” stationary state and a transient phase of the excited membrane.

The complexity of the nonlinear flow coupling underlying suprathreshold potential changes may be tractable in terms of the recently developed network thermodynamics covering inhomogeneity of the reaction space and nonlinearity [22]. An attempt at such an approach, which formally includes conformational metastability and hysteretic flow characteristics [66, 67] is in preparation [68].

b) Reaction Fluxes. For the nonequilibrium description of the translocation dynamics we may associate reaction fluxes with the translocation sequence, Eqs. (22–24).

(a) Resting stationary state



(b) Excited state

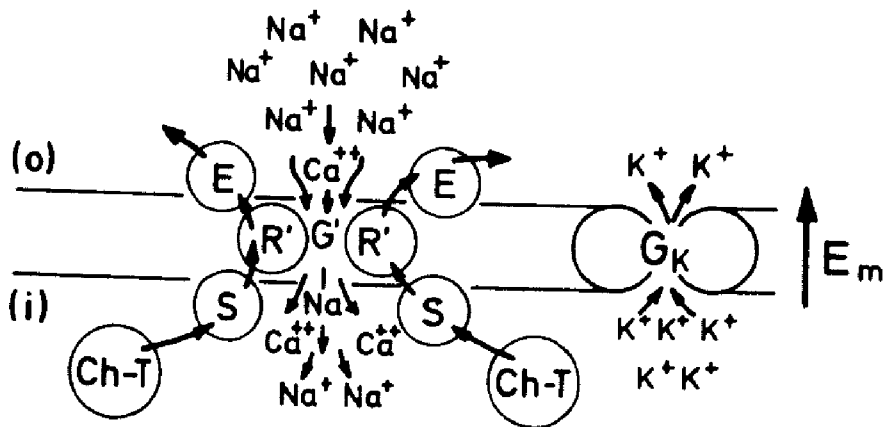


Fig. 5a and b. Schematic representation of a membrane section (a) in the "resting" stationary state and (b) in a transient phase of excitation. In (a), the majority of the acetylcholine receptors is in the Ca^{2+} ion-binding conformation R ; the cholinergically controlled rapidly operating gateway is in the closed state G , and the permeability for Na^+ (and Ca^{2+}) ions is very small as compared to the permeability for K^+ ions through the slow gateway G_K . The electric field vector, E_m , pointing from the outside boundary (o) to the inside boundary (i) of the membrane is largely due to the K^+ ion gradient. In (b), most of the receptors are in the acetylcholine-binding conformation R' , and the rapid gateway is in its open configuration G' (Na^+ -activation phase). The change in the electric field (directed outward during the peak phase of the action potential) accompanying the transient Na^+ (and Ca^{2+}) influx causes a transient (slower) increase in the permeability of G_K , thus inducing a (delayed) transient efflux of K^+ ions. Hydrolysis of acetylcholine (AcCh) leads to relaxation of R' and G' to R and G , restoring the resting stationary state. Translocation of AcCh, occasionally in the resting stationary state and in a cooperatively increased manner after suprathreshold stimulation, through a storage site (S) of relatively large capacity, receptor and AcCh-esterase is indicated by the curved arrows. The hydrolysis products choline (Ch) and acetate (Ac) are transported through the membrane where intracellular choline-*O*-acetyltransferase (ChT) may resynthesize AcCh (with increased rate in the refractory phase)

1) The release flux is defined by

$$J(S) = \frac{d[\bar{n}_r]}{dt} \quad (26)$$

where \bar{n}_r is the average number of A^+ released into the reaction space between storage- and receptor ring.

2) The receptor flux including association of A^+ and conformation change of R is given by

$$J(R) = \frac{d[\bar{n}]}{dt} \quad (27)$$

where \bar{n} is the average number of A^+ associated with R .

3) The esterase (or decomposition flux) is defined by

$$J(E) = \frac{d[\bar{n}_e]}{dt} \quad (28)$$

where \bar{n}_e is the average number of A^+ processed through AcCh-esterase.

Stationary states of the cholinergic activity are characterized by constant overall flow of AcCh; neither accumulations nor depletions of locally processed AcCh occur outside the limit of fluctuations. Thus, for stationary states,

$$J(S) = J(R) = J(E) = \text{constant}. \quad (29)$$

Statistically occurring small changes in membrane properties such as the so-called *miniature end plate potentials* are interpreted to reflect amplified fluctuations in the subthreshold activity of the cholinergic system.

Oscillatory excitation behavior observed under certain conditions (see, e.g., [10]) may be modelled by periodic accumulation and depletion of AcCh in the reaction spaces of the BEUs.

6.4 Field Dependence of AcCh Storage

In the simplest case, a change of the membrane potential affects the chain of translocation events already at the beginning, i.e. at the storage site. Indeed, the observation of AcCh release by electrical stimulation or in response to K^+ -ion induced depolari-

zation support the assumption of a field-dependent storage site for AcCh.

Denoting by \bar{n}_b the amount of AcCh bound on an average to S , we may define a distribution constant for the stationary state of the storage translocation by $K = \bar{n}_b/\bar{n}_r$. This constant (similar to an equilibrium constant) is a function of temperature T , pressure p , ionic strength, I , and of the electric field, E . A field dependence of K requires that the storage translocation reaction involves ionic, dipolar, or polarizable groups.

The isothermal-isobaric field dependence of K at constant ionic strength may be expressed by the familiar relation:

$$\left(\frac{\partial \ln K}{\partial E}\right)_{p,T,I} = \frac{\Delta M}{RT} \quad (30)$$

where ΔM is the reaction moment; ΔM is (proportional to) the difference in the permanent (or induced) dipole moments of reaction products and reactants. If a polarization process is associated with a finite value of ΔM , K should be proportional to E^2 (for relatively small field intensities up to 100 kV/cm). Furthermore, a small perturbation of the field causes major changes in K only on the level of higher fields (see, *e.g.*, [69]). It is therefore of interest to recall that, under physiological conditions, excitable membranes generate action potentials only above a certain (negative) potential difference.

The suggestion of a field-induced conformational change in a storage protein to release AcCh derives from recent studies on field effects in macromolecular complexes and biomembranes. It has been found that electric impulses in the intensity similar to the depolarization voltage changes for the induction of action potentials are able to cause structural changes in biopolyelectrolytes [70, 71] and permeability changes in vesicular membranes [72]. In order to explain the results, a polarization mechanism has been proposed that is based on the displacement of the counterion atmosphere of polyelectrolytes or of oligo-electrolytic domains in membrane organizations.

If the conformational dynamics of the storage site does indeed involve a polarization mechanism, we may represent the dependence of bound AcCh, \bar{n}_b , on the electric field of the membrane as

shown in Fig. 6. Increasing membrane potential increases the amount of bound AcCh and thus also the number of AcCh ions that, after fast reduction of the membrane potential, are translocatable to the receptor.

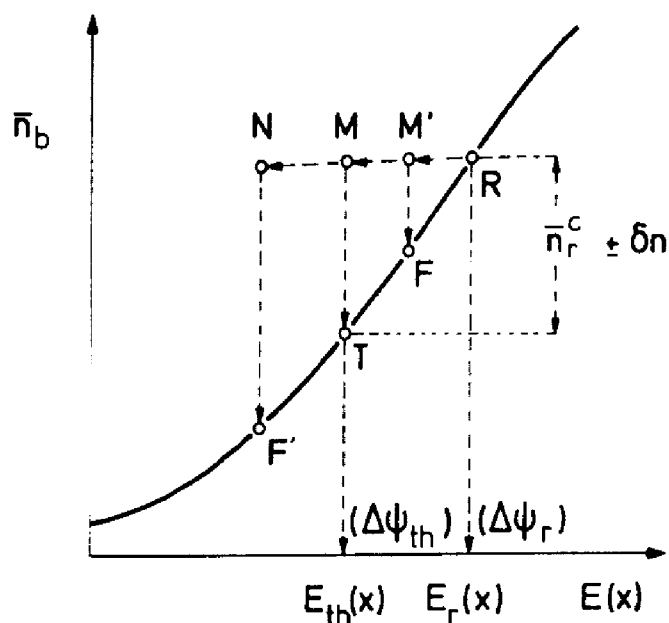


Fig. 6. Model representation of the field-dependent stationary states for AcCh storage. The mean number, \bar{n}_b , of AcCh ions bound to the storage site at the membrane site, x , of the release reaction, as a function of the electric field, $E(x)$, (at constant pressure, temperature and ionic strength). The intervals $M'F$, MT , and NF' correspond to the maximum number of AcCh ions released, n_r , for 3 different depolarization steps: (a) a subthreshold change from the resting state R to F , (b) a threshold step R to T , releasing the threshold or critical number $n_r^c (\pm \delta n, \text{fluctuation})$, and (c) a suprathreshold step R to F' with $n_r > n_r^c$

6.5 Relaxations of AcCh Translocation Fluxes

It is recalled that the receptor reaction [cf. Eqs. (23) and (25)] plays a key role in coupling the control function of AcCh with the permeability change of the gateway. Uptake of AcCh from the storage ring, conformational transition and Ca^{2+} ion release comprise a sequence of three single events. It is therefore assumed that the processing of AcCh through the receptor is slower than the preceding step of AcCh release from the storage. The receptor reaction is thus considered to be rate-limiting. Therefore, any (fast) change in the membrane field will lead to either a transient accumulation or

a depletion of AcCh in the reaction space between *S*-ring and *R*-ring of a BEU.

In the case of a fast depolarization, there is first a transient increase in the storage flux $J(S)$ causing transient accumulation of AcCh in the *S*-*R* reaction space. The accumulation rate $J(A^+)$ is defined by

$$J(A^+) = \frac{d[\bar{n}_a]}{dt} = J(S) - J(R) \quad (31)$$

where the receptor flux is also considered as rate-limiting for the (fast) esterase flux $J(E)$. The number \bar{n}_a of transiently accumulated AcCh is calculated by integration of Eq. (31). Recalling the definitions of the single fluxes, Eqs. (26) and (27), $\bar{n}_a = \bar{n}_r - \bar{n}$; after adjustment of the flux system to stationarity $\bar{n}_a = 0$.

Since flux intensities increase with increasing driving forces (see, e.g., KATCHALSKY [7]), $J(S)$ will increase with increasing perturbation intensity, thus causing an increase in the rate of all following processes.

It is recalled that in the framework of our integral model, the time course of changes in electrical membrane parameters such as the membrane potential is controlled by the cholinergic system and the gateway dynamics.

a) Subthreshold Relaxations. Subthreshold perturbations do not induce the gateway transitions and are considered to cause membrane changes of small extent only. The time constant, τ_m , for subthreshold relaxations of chemical contributions to membrane potential changes [see Eq. (14)] is thus equal to the time constant, τ_R , of the rate limiting receptor flux. For squid giant axons $\tau_R = \tau_m = 1$ msec, at 20° C.

The relaxation of $J(R)$ to a lasting subthreshold perturbation (e.g. current stimulation) is given by

$$\frac{dJ(R)}{dt} = -\frac{1}{\tau_R} (J(R) - J'(R)) \quad (32)$$

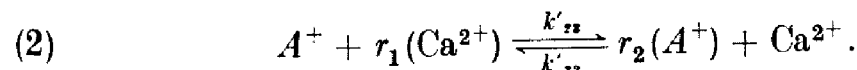
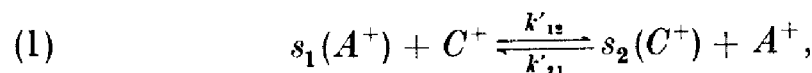
where $J'(R)$ is the stationary value of the new flux. Equivalent to Eq. (32), we have for \bar{n} ,

$$\frac{d[\bar{n}]}{dt} = -\frac{1}{\tau_R} ([\bar{n}] - [\bar{n}]') \quad (33)$$

describing an exponential "annealing" to a new level of AcCh, \bar{n}' , processed through the receptor-ring.

It is evident from the reaction scheme, Eqs. (22)–(33), that the time constant τ_R is the relaxation time of a coupled reaction system. In order to demonstrate the dependencies of τ_R on various system parameters such as the local concentrations of the reaction partners, we may calculate τ_R using a few simplifying assumptions.

We recall that the release step is fast as compared to the receptor reaction. Furthermore, for subthreshold perturbations the changes in the local concentration of the metal ions is certainly small in comparison to the concentration changes of the cholinergic reaction partners (buffer-condition). We denote by s and r the single binding sites of S and R , and use the simplified reaction scheme



Due to the buffer condition, we may approximate

$$k''_{12} \cong k'_{12}[C^+] \quad \text{und} \quad k''_{32} = k'_{32}[\text{Ca}^{2+}].$$

With

$$s_1(A^+) = \bar{n}_b, \quad r_2(A^+) = \bar{n}, \quad [A^+] = [\bar{n}_r] \cong [s_2(C^+)],$$

the two reaction fluxes are:

$$(1) \quad j(s) = -\frac{d[\bar{n}_b]}{dt} = k''_{12}[\bar{n}_b] - k'_{21}[\bar{n}_r]^2,$$

$$(2) \quad j(r) = \frac{d[\bar{n}]}{dt} = k'_{23}[\bar{n}_r][r_1(\text{Ca}^{2+})] - k''_{32}[\bar{n}].$$

Applying normal mode analysis [73], we obtain the relaxation times $\tau(s)$ and $\tau(r)$:

$$1/\tau(s) = 4k'_{21}[\bar{n}_r]' + k'_{12}[C^+]$$

$$1/\tau(r) = k'_{23} \left\{ [\bar{n}_r]' + \frac{[r_1(\text{Ca}^{2+})]' \cdot (k'_{12} + k'_{23}[\bar{n}_r]')}{k'_{12} + 4k'_{21}[\bar{n}_r]'} \right\} + k'_{32}[\text{Ca}^{2+}][\bar{n}]'.$$

It is noted that the rate coefficients k' contain conformational contributions; the concentration terms are primed and represent the stationary values of the new flux conditions.

In case of strongly concerted interactions, we may approximate $\tau(s) = \tau_S$ associated with $J(S)$, and $\tau(r) = \tau_R$ associated with $J(R)$.

b) Parameters of Suprathreshold Changes. It is recalled that the induction of an action potential is associated with three critical parameters:

$$\bar{n}^c, \bar{m}^c, \Delta t^c = \tau_R.$$

For a perturbation, the intensity of which increases gradually with time, the condition $\bar{n} \geq \bar{n}^c$ corresponding $\bar{n}_r \geq \bar{n}_r^c$ (within a BEU) can only be realized, if the minimum slope condition leading to

$$\frac{dJ(S)}{dt} \geq \frac{dJ(S)^{\min}}{dt}$$

and to [the equivalent expression for $J(R)$]

$$\frac{dJ(R)}{dt} \geq \frac{dJ(R)^{\min}}{dt} \quad (34)$$

is fulfilled (see Section 4.1 b; Eqs. (9) and (11)).

For rectangular (step) perturbations the threshold conditions are:

$$J(S) = \frac{[\bar{n}_r]}{\tau_R} \geq \frac{[\bar{n}_r^c]}{\tau_R},$$

$$J(R) = \frac{[\bar{n}]}{\tau_R} \geq \frac{[\bar{n}^c]}{\tau_R}.$$

Since $J(S)$ increases with the intensity of the (step) perturbation, the time intervals Δt ($< \tau_R$) in which \bar{n}^c AcCh ions start to associate with the receptor become smaller with larger stimulus intensities.

We may write this "strength-duration" relationship for supra-threshold perturbations in the form

$$J(S) \cdot \Delta t = [\bar{n}_r^c] \quad \text{and} \quad J(R) \cdot \Delta t = [\bar{n}^c]. \quad (35)$$

[Compare Eq. (10).] The time intervals Δt of the receptor activation correspond to the observed latency phases.

The expressions corresponding to the rheobase (see Section 4.1) are

$$J(S)_{\text{th}} = \frac{[\bar{n}_r^c]}{\tau_R} \quad \text{and} \quad J(R)_{\text{th}} = \frac{[\bar{n}^c]}{\tau_R}. \quad (36)$$

These equations clearly demonstrate the impulse condition [release of $\bar{n}_r \geq \bar{n}_r^c$ within Δt^c , cf. Eq. (12)] for the induction of action potentials.

Since \bar{n}_r^c and \bar{n}^c are numbers describing functional cooperativity, the strength-duration products Eq. (35) do not depend on temperature. The fluctuations $\pm \delta n$ for \bar{n}_r , however, increase with increasing temperature (and may finally lead to thermal triggering of action potentials). The flux equivalents of the rheobase, Eq. (36) are reaction rates which in general are temperature dependent (having a Q_{10} coefficient of about 2).

There are further aspects of electrophysiological observations which the integral model at the present stage of development may (at least qualitatively) reproduce.

If the membrane potential is slowly reduced, subthreshold flux relaxation of the ratio \bar{n}_b/\bar{n}_r may keep \bar{n}_r always smaller than \bar{n}_r^c . Thus, corresponding to experience, slow depolarization does not (or only occasionally) evoke action potentials.

In order to match the condition $\bar{n}_r > \bar{n}_r^c$ starting from the resting potential, the depolarization has in any case to go beyond the threshold potential, where

$$\bar{n}_b(M) - \bar{n}_b(T) = \bar{n}_r^c \quad (\text{see Fig. 6}).$$

For stationary membrane potentials $\Delta\psi < \Delta\psi_{th}$, the maximum number of AcCh-ions that can be released by fast depolarization within Δt^c is smaller than \bar{n}_r^c . Thus, corresponding to experience, below a certain membrane potential, near below $\Delta\psi_{th}$, no nerve impulse can be generated.

c) Refractory Phenomena. After the gateway transition to the open state, the receptors of the BEUs have to return to the Ca^{2+} -binding conformation $R(\text{Ca}^{2+})$ before a second impulse can be evoked [cf. Eq. (25)]. Even if \bar{n}^c AcCh ions were already available, the time interval for the transition of \bar{m}^c receptors is finite and causes the observed absolutely refractory phase.

Hyperpolarizing prepulses shift the stationary concentration of \bar{n}_b to higher values (see Fig. 6). Due to increased "filling degree", the storage site appears to be more sensitive to potential changes (leading, among others, to the so-called "off-responses"). On the other hand, depolarizing prepulses and preceding action potentials

temporarily decrease the actual value of \bar{n}_b , thus requiring increased stimulus intensities for the induction of action potentials.

The assumptions for the kinetic properties of the storage site mentioned in the discussion of Eq. (18) are motivated by the accommodation behavior of excitable membranes. The observation of a relatively refractory phase suggests that the uptake of AcCh into the storage form $S(A^+)$ is slow compared to the release reaction. Therefore, after several impulses there is partial "exhaustion" of the storage site. If during the (slow) refilling phase there is a new stimulation, \bar{n}_b may still be lower than the stationary level. Therefore, the membrane has to be depolarized to a larger extent in order to fulfil the action potential condition $\bar{n}_r \geq \bar{n}_r^c$.

6.6 The AcCh Control Cycle

The cyclic nature of a cholinergic permeability control in excitable membranes by processing AcCh through storage, receptor, esterase and synthetase is already indicated in a reaction scheme developed 20 years ago (see Fig. 11 of [2]). The complexity of mutual coupling between the various cycles directly or indirectly involved in the permeability control of the cholinergic gateway is schematically represented in Fig. 7. In this representation it may be readily seen that manipulations such as external application of AcCh and its inhibitory or activating analogs may interfere at *several sites* of the AcCh cycle. In particular, the analysis of pharmacological and chemical experiments faces the difficulty of this complexity.

In the previous sections it has been shown that basic parameters of electrophysiological phenomenology may be modelled in the framework of a nonequilibrium treatment of the cholinergic reaction system. The various assumptions and their motivations by experimental observations are discussed and the cholinergic reaction cycle is formulated in a chemical reaction scheme.

In conclusion, the integral model at the present level of development appears to cover all essential pharmaco-electrophysiological and biochemical data on excitable membranes. The model is expressed in specific reactions subject to further experimental investigations involving the reaction behavior of isolated membrane components as well as of membrane fragments containing these components in structure and organization.

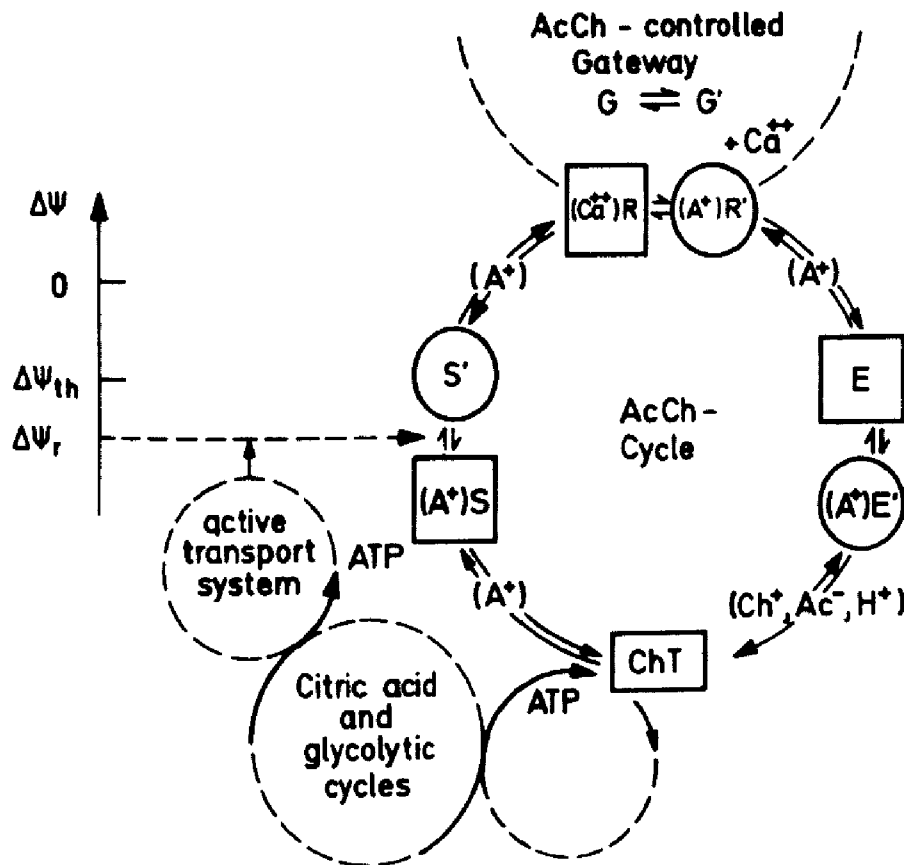


Fig. 7. Acetylcholine (AcCh) cycle, for the cyclic chemical control of stationary membrane potentials $\Delta\psi$ and transient potential changes. The binding capacity of the storage site (S) for AcCh is assumed to be dependent on the membrane potential, $\Delta\psi$, and is thereby coupled to the active transport system (and the citric acid and glycolytic cycles). The control cycle for the gateway G (Ca^{2+} -binding and closed) and G' (open) comprises the SRE assemblies (see Fig. 4) and the choline- O -acetyltransferase (ChT); ChT couples the AcCh synthesis cycle to the translocation pathway of AcCh through the SRE -assemblies. The continuous subthreshold flux of AcCh through such a subunit is maintained by the virtually irreversible hydrolysis of AcCh to choline (Ch^+), acetate (Ac^-) and protons (H^+) and by steady supply flux of AcCh to the storage from the synthesis cycle. In the resting stationary state, the membrane potential ($\Delta\psi$) reflects dynamic balance between active transport (and AcCh synthesis) and the flux of AcCh (through the control cycles surrounding the gateway) and of the various ions asymmetrically distributed across the membrane. Fluctuations in membrane potential (and exchange currents) are presumably amplified by fluctuations in the local AcCh concentrations maintained at a stationary level during the continuous translocation of AcCh through the cycle [74]

Acknowledgements

This study is based on numerous discussions with Prof. DAVID NACHMAN-SOHN whom I thank for the many efforts to reduce my ignorance in the bio-

chemistry of excitable membranes. Thanks are also due to Prof. MANFRED EIGEN for his critical interest and generous support of this work. Finally, I would like to thank the Stiftung Volkswagenwerk for a grant.

Note Added in Proof

Recently it has been found that acetylcholine induces a conformational change in the isolated acetylcholine-receptor protein (from *Electrophorus electricus*). This configurational change controls the binding of calcium ions to the polyelectrolytic macromolecule. The kinetic analysis of this fundamentally important biochemical reaction [see Eq. (19)] results in number values for apparent rate constants and equilibrium parameters of the participating elementary processes, but also reveals the stoichiometry of the interactions between receptor, acetylcholine, and calcium ions. [CHANG, H. W., NEUMANN, E.: Proc. Natl. Acad. Sci. USA, (in press).]

References

1. AGIN, D.: Excitability phenomena in membranes. In: ROSEN, R. (Ed.): Foundations of mathematical biology, pp. 253—277. New York: Academic Press 1972.
2. NACHMANSOHN, D.: Metabolism and function of the nerve cell. In: Harvey Lectures 1953/1954, pp. 57—99. New York: Academic Press 1955.
3. NACHMANSOHN, D.: Proteins in bioelectricity. Acetylcholineesterase and -receptor. In: LOEWENSTEIN, W. R. (Ed.): Handbook of sensory physiology, Vol. 1, pp. 18—102. Berlin-Heidelberg-New York: Springer 1971.
4. NEUMANN, E., NACHMANSOHN, D., KATCHALSKY, A.: An attempt at an integral interpretation of nerve excitability. Proc. nat. Acad. Sci. (Wash.) **70**, 727—731 (1973).
5. HODGKIN, A. L.: The conduction of the nervous impulse, Springfield, Ill.: C. C. Thomas 1964.
6. TASAKI, I.: Nerve excitation. Springfield, Ill.: C. C. Thomas 1968.
7. KATCHALSKY, A.: Membrane thermodynamics. In: QUARTON, G. C., MELNECHUK, T., SCHMITT, F. O. (Eds.): The neurosciences, pp. 326—343. New York: The Rockefeller University Press 1967.
8. PRIGOGINE, I.: Thermodynamics of irreversible processes, 3rd ed. Springfield, Ill.: Thomas Publ. 1968.
9. TASAKI, I., SINGER, I.: Membrane macromolecules and nerve excitability: a physico-chemical interpretation of excitation in squid giant axons. Ann. N. Y. Acad. Sci. **137**, 793—806 (1966).
10. COLE, K. S.: In: TOBIAS, C. A. (Ed.): Membranes, ions, and impulses. Berkeley, Calif.: University of California Press 1968.
11. HODGKIN, A. L., KEYNES, R. D.: The potassium permeability of a giant nerve fibre. J. Physiol. (Lond.) **128**, 61—88 (1955).
12. TASAKI, I., TAKENADA, T.: Ion fluxes and excitability in squid giant axon. In: HOFFMAN, J. F. (Ed.): The cellular functions of membrane transport. Englewood Cliffs, N. J.: Prentice-Hall Inc. 1964.

13. AGIN, D.: Electron neutrality and electrondiffusion in the squid axon. *Proc. nat. Acad. Sci. (Wash.)* **57**, 1232—1238 (1967).
14. SEGAL, J. R.: Surface charge of giant axons of squid and lobster. *Biophys. J.* **8**, 470—489 (1968).
15. ZELMAN, A., SHIH, H. H.: The constant field approximation: numerical evaluation for monovalent ions migrating across a homogeneous membrane. *J. theor. Biol.* **37**, 373—383 (1972).
16. TRÄUBLE, H., EIBL, H.: Electrostatic effects on lipid phase transitions: membrane structure and ionic environment. *Proc. nat. Acad. Sci. (Wash.)* **71**, 214—219 (1974).
17. TASAKI, I., SINGER, I., TAKENAKA, T.: Effects of internal and external ionic environment on excitability of squid giant axon. *J. gen. Physiol.* **48**, 1095—1123 (1965).
18. JULIAN, F. J., GOLDMAN, D. E.: The effects of mechanical stimulation on some electrical properties of axons. *J. gen. Physiol.* **46**, 197—313 (1962).
19. CARNAY, L. D., TASAKI, I.: Ion exchange properties and excitability of the squid giant axon. In: ADELMAN, W. J., JR. (Ed.): *Biophysics and physiology of excitable membranes*, pp. 379—422. New York: Van Nostrand Reinhold Co. 1971.
20. KATZ, B.: *Nerve, muscle, and synapse*. New York: McGraw-Hill 1966.
21. LANDOWNE, D.: Movement of sodium ions associated with the nerve impulse. *Nature (Lond.) New Biol.* **242**, 457—459 (1973).
22. OSTER, G. F., PERELSON, A. S., KATCHALSKY, A.: Network thermodynamics: dynamic modelling of biophysical systems. *Quart. Rev. Biophys.* **6**, 1—134 (1973).
23. PLONSEY, R.: *Bioelectric phenomena*. New York: McGraw-Hill 1969.
24. HUNNEUS-COX, F., FERNANDEZ, H. L., SMITH, B. H.: Effects of redox and sulfhydryl reagents on the bioelectric properties of the giant axon of the squid. *Biophys. J.* **6**, 675—689 (1966).
25. ROBERTSON, J. D.: The ultrastructure of synapses. In: SCHMITT, F. O. (Ed.): *The neurosciences*, Vol. 2, pp. 715—728. New York: The Rockefeller University Press 1970.
26. MURALT, A. V., STÄMPFLI, R.: Die photochemische Wirkung von Ultraviolettlicht auf den erregten Ranvierschen Knoten der einzelnen Nervenfasern. *Helv. physiol. Acta* **11**, 182—193 (1953).
27. FOX, J. M.: Veränderungen der spezifischen Ionenleitfähigkeiten der Nervenmembran durch ultraviolette Strahlung. Dissertation. Homburg-Saarbrücken: 1972.
28. EVANS, M. H.: Tetrodotoxin and saxitoxin in neurobiology. *Int. Rev. Neurobiol.* **15**, 83—166 (1972).
29. COLE, K. S.: Dielectric properties of living membranes. In: SNELL, F. et al. (Eds.): *Physical principles of biological membranes*, pp. 1—15. New York: Gordon and Breach 1970.
30. ABBOT, B. C., HILL, A. V., HOWARTH, J. V.: The positive and negative heat production associated with a single impulse. *Proc. Roy. Soc. B* **148**, 149—187 (1958).

31. HOWARTH, J. V., KEYNES, R. D., RITCHIE, J. M.: The origin of the initial heat associated with a single impulse in mammalian non-myelinated nerve fibres. *J. Physiol. (Lond.)* **194**, 745—793 (1968).
32. NEUMANN, E.: Molecular hysteresis and its cybernetic significance. *Angew. Chem. (int. Edit.)* **12**, 356—369 (1973).
33. GUGGENHEIM, E. A.: *Thermodynamics*. New York: Interscience 1949.
34. BRZIN, M., DETTBARN, W.-D., ROSENBERG, PH., NACHMANSOHN, D.: Cholinesterase activity per unit surface area of conducting membranes. *J. Cell Biol.* **26**, 353—364 (1965).
35. GRUBER, H., ZENKER, W.: Acetylcholinesterase: histochemical differentiation between motor and sensory nerve fibres. *Brain Res.* **51**, 207—214 (1973).
36. CALABRO, W.: Sulla regolazione neuro-umorale cardiaca. *Riv. biol.* **15**, 299—320 (1933).
37. LISSÁK, K.: Liberation of acetylcholine and adrenaline by stimulating isolated nerves. *Amer. J. Physiol.* **127**, 263—271 (1939).
38. DETTBARN, W.-D., ROSENBERG, PH.: Effects of ions on the efflux of acetylcholine from peripheral nerve. *J. gen. Physiol.* **50**, 447—460 (1966).
39. PORTER, C. W., CHIU, T. H., WIECKOWSKI, J., BARNARD, E. A.: Types and locations of cholinergic receptor-like molecules in muscle fibres. *Nature (Lond.) New Biol.* **241**, 3—7 (1973).
40. DENBURG, J. L., ELDEFRAWI, M. E., O'BRIEN, R. D.: Macromolecules from lobster axon membranes that bind cholinergic ligands and local anesthetics. *Proc. nat. Acad. Sci. (Wash.)* **69**, 177—181 (1972).
41. KOELLE, G. B.: Current concepts of synaptic structure and function. *Ann. N. Y. Acad. Sci.* **183**, 5—20 (1971).
42. LEWIS, P. R., SHUTE, C. C. D.: The distribution of cholinesterase in cholinergic neurons demonstrated with the electron microscope. *J. Cell Sci.* **1**, 381—390 (1966).
43. MASLAND, R. L., WIGTON, R. S.: Nerve activity accompanying fasciculation produced by Prostigmine. *J. Neurophysiol.* **3**, 269—275 (1940).
44. RIKER, W. F., JR., WERNER, G., ROBERTS, J., KUPERMAN, A.: The pre-synaptic element in neuromuscular transmission. *Ann. N. Y. Acad. Sci.* **81**, 328—344 (1959).
45. DETTBARN, W.-D.: The acetylcholine system in peripheral nerve. *Ann. N. Y. Acad. Sci.* **144**, 483—503 (1967).
46. HOSKIN, F. C. G., ROSENBERG, PH., BRZIN, M.: Re-examination of the effect of DFP on electrical and cholinesterase activity of squid giant axon. *Proc. nat. Acad. Sci. (Wash.)* **55**, 1231—1235 (1966).
47. NACHMANSOHN, D.: Proteins of excitable membranes. *J. gen. Physiol.* **54**, 187—224 (1969).
48. HOSKIN, F. C. G., KREMZNER, L. T., ROSENBERG, PH.: Effects of some cholinesterase inhibitors on the squid giant axon. *Biochem. Pharmacol.* **18**, 1727—1737 (1969).
49. ROSENBERG, P., HOSKIN, F. C. G.: Demonstration of increased permeability as a factor in the effect of acetylcholine on the electrical activity of venom-treated axons. *J. gen. Physiol.* **46**, 1065—1073 (1963).

50. NACHMANSOHN, D.: Actions on axons and the evidence for the role of acetylcholine in axonal conduction. In: KOELLE, G. B. (Ed.): *Cholinesterases and anticholinesterase agents. Handb. d. exp. Pharmakologie, Erg. XV*, pp. 701—740. Berlin-Heidelberg-New York: Springer 1963.
51. DETTBARN, W.-D.: The effect of curare on conduction in myelinated, isolated nerve fibres of the frog. *Nature (Lond.)* **186**, 891—892 (1960).
52. DETTBARN, W.-D.: New evidence for the role of acetylcholine in conduction. *Biochim. biophys. Acta (Amst.)* **41**, 377—386 (1960).
53. BARTELS, E.: Relationship between acetylcholine and local anesthetics. *Biochim. biophys. Acta (Amst.)* **109**, 194—203 (1965).
54. SEEMAN, P.: The membrane actions of anesthetics and tranquilizers. *Pharmacol. Rev.* **24**, 583—655 (1972).
55. HARRIS, A. J., DENNIS, M. J.: Acetylcholine sensitivity and distribution on mouse neuroblastoma cells. *Science* **167**, 1253—1255 (1970).
56. NELSON, P. G., PEACOCK, J. H., AMANO, T.: Responses of neuroblastoma cells to iontophoretically applied acetylcholine. *J. Cell Physiol.* **77**, 353—362 (1971).
57. HAMPRECHT, B.: Cell cultures as model systems for studying the biochemistry of differentiated functions of nerve cells. *Hoppe-Seylers Z. physiol. Chem.* **355**, 109—110 (1974).
58. ARMETT, C. J., RITCHIE, J. M.: The action of acetylcholine on conduction in mammalian non-myelinated fibres and its prevention by anti-cholinesterase. *J. Physiol. (Lond.)* **152**, 141—158 (1960).
59. RITCHIE, J. M.: The action of acetylcholine and related drugs on mammalian non-myelinated nerve fibres. *Biochem. Pharmacol.* **12**(S), 3 (1963).
60. TAKEUCHI, A., TAKEUCHI, N.: Actions of transmitter substances on the neuromuscular junctions of vertebrates and invertebrates. In: KOTANI, M. (Ed.): *Advan. in Biophys.* **3**, 45—95. Baltimore: University Park Press 1972.
61. NACHMANSOHN, D.: *Chemical and molecular basis of nerve activity.* New York: Academic Press 1959.
62. WHITTAKER, V. P.: The biochemistry of synaptic transmission. *Naturwissenschaften* **60**, 281—289 (1973).
63. NEHER, E., LUX, H. D.: Rapid changes of potassium concentration at the outer surface of exposed single neurons during membrane current flow. *J. gen. Physiol.* **61**, 385—399 (1973).
64. SPECTOR, I., KIMHI, Y., NELSON, P. G.: Tetrodotoxin and cobalt blockage of Neuroblastoma action potentials. *Nature (Lond.) New Biol.* **246**, 124—126 (1973).
65. ADAM, G.: Theory of nerve excitation as a cooperative cation exchange in a two-dimensional lattice. In: SNELL, F., et al. (Eds.): *Physical principles of biological membranes*, pp. 35—64. New York: Gordon and Breach 1970.
66. KATCHALSKY, A., SPANGLER, R.: Dynamics of membrane processes. *Quart. Rev. Biophys.* **1**, 127—175 (1968).
67. BLUMENTHAL, R., CHANGEUX, J.-P., LEFÈVRE, R.: Membrane excitability and dissipative instabilities. *J. Membrane Biol.* **2**, 351—374 (1970).

68. RAWLINGS, P.K., NEUMANN, E.: In preparation.
69. EIGEN, M.: Dynamic aspects of information transfer and reaction control in biomolecular systems. In: QUARTON, G.C., MELNECHUK, T., SCHMITT, F.O. (Eds.): The neurosciences, pp. 130—142. New York: The Rockefeller University Press 1967.
70. NEUMANN, E., KATCHALSKY, A.: Long-lived conformation changes induced by electric impulses in biopolymers. Proc. nat. Acad. Sci. (Wash.) **69**, 993—997 (1972).
71. REVZIN, A., NEUMANN, E.: Conformational changes in rRNA induced by electric impulses. Biophys. Chem. **2**, 144—150 (1974).
72. NEUMANN, E., ROSENHECK, K.: Permeability changes induced by electric impulses in vesicular membranes. J. Membrane Biol. **10**, 279—290 (1972).
73. EIGEN, M., DEMAEYER, L.: Relaxation methods. In: FRIESS, S.L., LEWIS, E.S., WEISSBERGER, A. (Eds.): Technique of organic chemistry, Vol. 8, p. 895. New York: Interscience Publ. Inc. 1963.
74. NEUMANN, E., NACHMANSOHN, D.: In: MANSON, L. (Ed.): Biomembranes, Vol. 7. London. New York: Academic Press 1974.
75. CHANG, HAIWON: Purification and characterization of acetylcholine receptor-I from *Electrophorus electricus*. Proc. nat. Acad. Sci. (Wash.) **71**, 2113—2117 (1974).

Discussion

H. STIEVE (Jülich): This is a very potent model — which reminds me in some respects of ADAM's model — but I was unable to grasp so many details in the short time. Therefore I would like to ask you: Can you derive from your model critical experiments which can verify or disprove essential parts of the model?

E. NEUMANN: ADAM's molecular model for excitation does not specify the protein matrix on which ion exchange is assumed to take place upon depolarization; furthermore, the experimental data on the various inhibitors such as curare or physostigmine that interfere with electrical activity not only of synaptic junctions but also of axons (provided these structural analogs of AcCh can reach the excitable membrane) are not taken into account. Thus the elegant model of ADAM covers only part of experimental observations on axonal excitation.

I recall that our integral model is suggested by experimental facts, direct and partially indirect biochemical and pharmaco-electrophysiological data. These experimental results have been correlated and modelled in a specific reaction scheme for the initiation and termination of the excitation process. If it turns out that the *in vitro* measured thermodynamic and kinetic parameters associated with the reaction scheme are beyond reasonable values (*e.g.* compared to the rate of increase and decrease of the ion flows during excitation), then our model has to be modified. Thus the specific reaction scheme itself suggests critical experiments.