

AN INTEGRAL PHYSICO-CHEMICAL MODEL

FOR BIOEXCITABILITY

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1. Introduction

Bioelectrical excitability is a universal property of all higher organisms. Although numerous experimental data have been accumulated in the various disciplines working on bioelectricity, the mechanism of nerve excitability and synaptic transmission, however, is still an unsolved problem. Many mechanistic interpretations of nerve behaviour and the various molecular and mathematical models generally cover only a part of the known facts, are thus selective and of only limited value. According to Agin, 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 behavior identical with that observed experimentally. At the present time such a theory does not exist." (Agin, 1972).

The first proposal of a physically specific mechanism for the control of bioelectricity is due to Nachmansohn (Nachmansohn 1955, 1971). The chemical hypothesis of Nachmansohn remained, however, 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 (Neumann et al., 1973).

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. 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 (Hodgkin, 1964; Tasaki, 1968). 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 (Katchalsky, 1967). The intrinsic nonequilibrium nature of membrane excitability is most obviously reflected in the unsymmetric 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 (Prigogine, 1968), including the membrane structure (Katchalsky, 1967).

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 (Tasaki and Singer, 1966).

The membrane components of excitable membranes include ionic constituents: fixed charges (some of them may serve to facilitate locally perm-selective 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 the 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 (Cole, 1968). 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 (Hodgkin and Keynes, 1955; Tasaki and Takenada, 1964). 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 electrochemical behaviour of excitable membranes, within a limited range of physico-chemical state variables (Agin, 1967). 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. Nevertheless, the Nernst approximation for the resting stationary potential is useful for a number of practical cases.

For the condition of stationarity, i.e. at zero net membrane current ($I_m=0$), we may write

$$\Delta\psi_r \approx (\Delta\psi)_{I_m=0} = \frac{RT}{F} \sum_i \frac{t_i}{Z_i} \ln \frac{a_i^{(o)}}{a_i^{(i)}} \quad (1)$$

In Eq. (1), 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); RT is the (molar) thermal energy, F is the Faraday; Z_i is the valency and a_i is the thermodynamic activity of ion i , (o) outside and (i) inside the excitable cell.

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.

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 .

$$\bar{E} = - \frac{1}{d} \Delta\psi' \quad (2)$$

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 (Segal, 1968) there are Donnan potentials $\Delta\psi_D^{(o)}$ and $\Delta\psi_D^{(i)}$ from inside (i) and outside (o) interfaces between membrane and environment. Furthermore, there are interdiffusion potentials $\Delta\psi_U$ arising from differences in ionic mobilities within ion exchange domains of intramembraneous fixed charges (Tasaki, 1968). We may sum up these terms to $\Delta\psi_{DU} = \Delta\psi_D^{(o)} + \Delta\psi_D^{(i)} + \Delta\psi_U$. Thus,

$$\Delta\psi' = \Delta\psi_N + \Delta\psi_{DU} \quad (3)$$

Compared to $\Delta\psi_{DU}$ the "Nernst" contributions to $\Delta\psi$ appear relatively large. Inserting Eqs. (1) and (3) in Eq. (2) we obtain for the resting state:

$$(\bar{E})_{I_m=0} = - \frac{1}{d} \left(\Delta\psi_{DU} + \frac{RT}{F} \sum_i \frac{t_i}{z_i} \ln \frac{a_i^{(o)}}{a_i^{(i)}} \right) \quad (4)$$

Among the various ions known to contribute to $\Delta\psi$ (in vivo) are the metal ions Na^+ , K^+ , Ca^{2+} , 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 Donan 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 (Traeuble and Eibl, 1974) 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 (Cf., e.g. Cole, 1968).

4. Transient Changes of Membrane Potentials

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.

The various transient expressions of nerve activity such as depolarizations and hyperpolarizations of the resting stationary potential level reflect changes of dielectric-capacitive nature, interdiffusional 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; see Eq. (1).

4.1 Threshold behaviour.

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 christened action potential or nerve impulse.

4.2 Stimulus characteristics

In order to evoke an action potential the intensity of the stimulation has to reach the threshold with a certain minimum velocity (Cole, 1968). If the external stimulus for instance is a current the intensity, I , of which gradually increases with time, t , the minimum slope condition for the action potential may be written

$$dI/dt \geq (dI/dt)^{\min} \quad (5)$$

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 stimulus duration. It is found that the product of suprathreshold intensity and minimum duration is approximately constant. Thus, for $I \geq I_{th}$,

$$I \cdot \Delta t = \text{constant}, \quad (6)$$

describing 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., Cole, 1968).

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 3(d).

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 to 20 mV and has to occur in the form of an impulse, $\int \Delta(\Delta\psi) dt$, in which a minimum condition between membrane potential and time must be fulfilled.

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

$$\Delta\psi_r - \int \left(\frac{d \Delta\psi}{dt} \right)_{\min} dt = \Delta\psi_{th} \quad (7)$$

or in the more general form:

$$\Delta\psi_r - \frac{1}{\Delta t} \int \Delta(\Delta\psi) dt \leq \Delta\psi_{th}. \quad (8)$$

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

Including Eq. (1) we may write

$$\int \Delta(\Delta\psi) dt = -d \int \Delta E dt = \int R_m \cdot I dt = \frac{RT}{Z_i F} \int \Delta \ln \frac{a_i^{(0)}}{a_i^{(1)}} dt. \quad (9)$$

With Eqs. (8) and (9) 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. 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 (Tasaki, Singer, and Takenaka, 1965).

As seen in Eq. (1) the membrane potential is dependent on temperature. Therefore temperature (and also pressure) changes alter the membrane potential in excitable membranes. Moreover, action potentials can be evoked by thermal and mechanical shocks (see, e.g., Julian and Goldman, 1962).

It is furthermore remarked that under natural conditions electrical depolarization produces an action potential only if the excitable membrane is 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.

4.3 Propagation of local activity

In many excitable cells $\Delta\psi_x$ 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 to 90 kV/cm. (Cf., however, Carnay and Tasaki, 1971). 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 fulfill 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).

4.4 Refractory phases

Important observations suggestive for the nature of bioelectricity are the refractory phenomena. 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 behaviour are also called accommodation or adaptation of the fibre to preceding manipulations (see, e.g., Tasaki, 1968).

4.5 Time constants

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. Fig. 1 shows schematically the time course of these local responses upon hyper- and depolarizing stimulations: subthreshold potential changes and action potential (Katz, 1966).

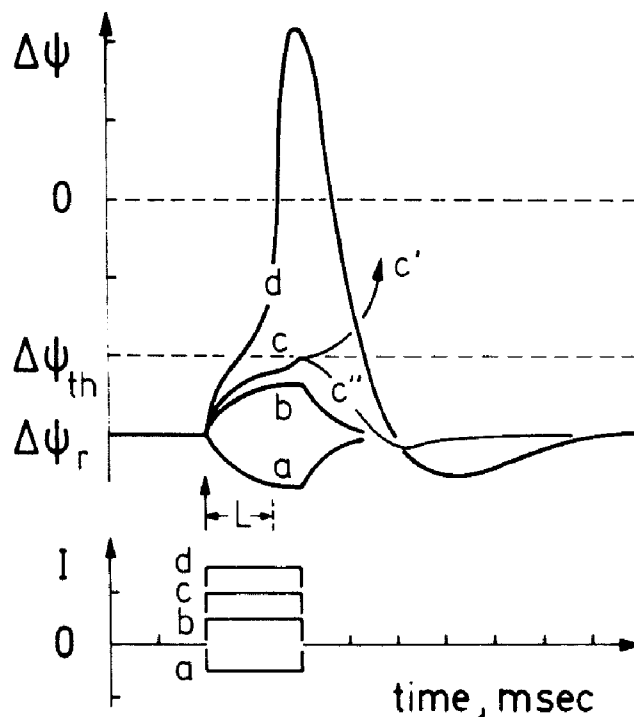


Fig. 1. Sub- and suprathreshold responses of the membrane potential, $\Delta\psi$, to stimulating rectangular current pulses of fixed duration but variable intensity, I . (a) inward current pulse causing hyperpolarization, (b) subthreshold outward current ($I < I_{th}$) causing depolarization, (c) threshold outward current ($I = I_{th}$) 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.

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.

It has been found that large sections of the time course of sub-threshold responses and the first phase of the action potential (also called latency; see Fig. 1) can be described with linear differential equations of first order. Furthermore, the time constants τ_m associated with these sections are nearly equal.

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 corresponding 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 (Cole, 1968).

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 extremely low ion mobilities (about 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 to 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 to 3 (see, e.g., Cole, 1968). The various phases of the action potential are prolonged with decreasing temperature. In the framework of the classical Hodgkin-Huxley phenomenology prolonged action potentials should correspond to larger ion movements. However, it has been found recently that in contrast to this prediction, the amount of ions actually transported during excitation decreases with decreasing temperature (Landowne, 1973).

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 usually are 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 (Oster, Perelson and Katchalsky, 1973). 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

$$\tau = R_R \cdot C_R. \quad (10)$$

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 part of the observed subthreshold change in the membrane potential

$$\Delta\psi = I_m R_m (1 - \exp[-t/\tau_m]) \quad (11)$$

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

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; see Fig. 2) for both sub- and suprathreshold conditions are similar in shape, although much different in amplitude" (Plonsey, 1969). 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.

5. 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

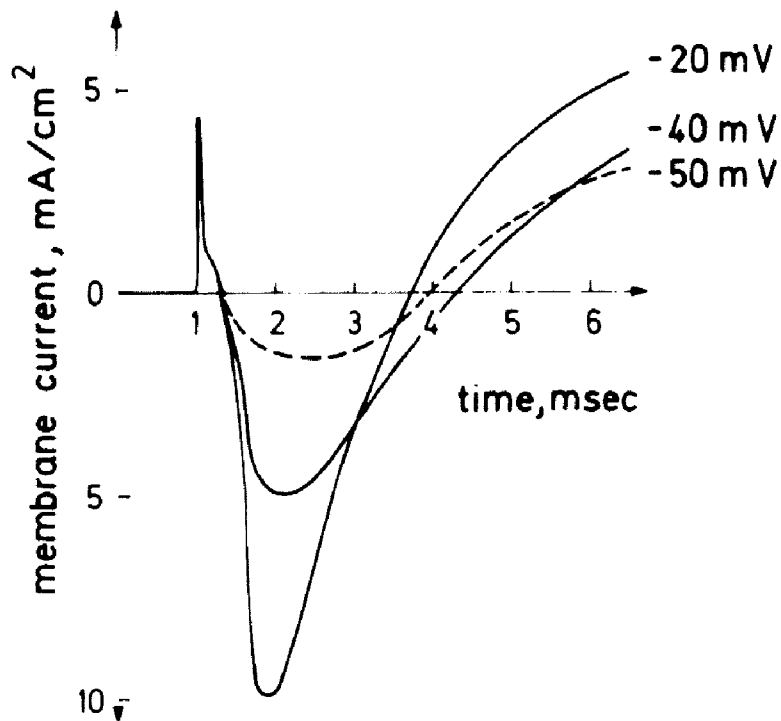


Fig. 2. Time course of membrane currents during voltage clamp experiments of internally perfused giant axons of squid *Loligo pealii*; resting potential $\Delta\psi \approx -65$ mV. Dashed line: response to a clamp of -50 mV, i.e. a depolarization of 15 mV that, under unclamped conditions, does not evoke an action potential. The solid lines represent suprathreshold membrane currents. (Scheme of data by Narahashi et al., 1969).

action potential (Huneus-Cox et al., 1966). The membrane of squid giant axons stain for SH groups provided the fibres had been stimulated (Robertson, 1970). These findings strongly suggest the participation of protein specific red-ox reactions in the excitation process.

Of particular interest appear the effects of ultraviolet radiation on nodes of Ranvier. 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 (von Muralt and Stämpfli 1953; Fox 1972). The results of Fox 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 Evans, 1972). 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.

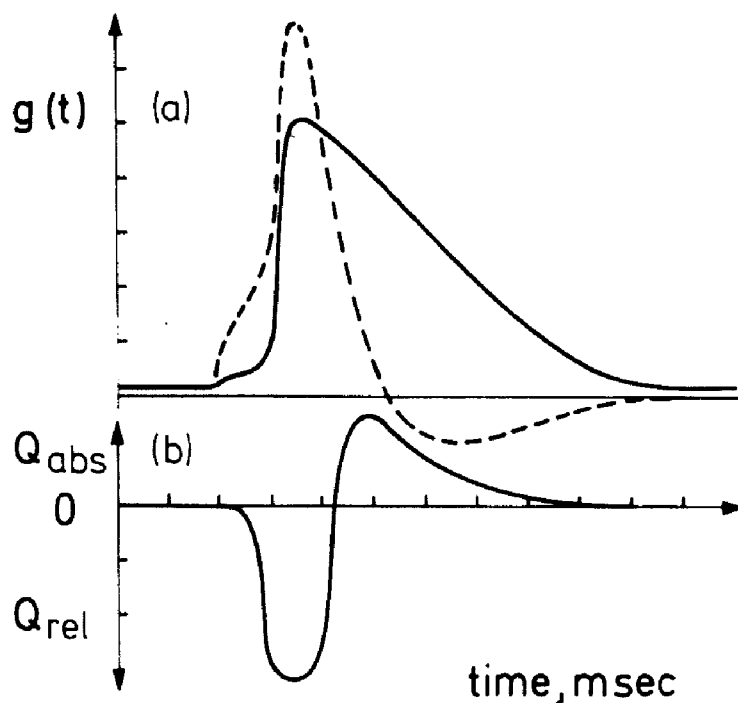


Fig. 3. Schematic representation of (a) conductivity change, $g(t)$, accompanying the action potential (dashed line) and (b) heat exchange cycle. Q_{rel} , heat released during rising phase and Q_{abs} , heat absorbed during falling phase of the action potential.

6. Impedance and Heat Changes

The impedance change accompanying the action potential is one of the basic observations in electrophysiological studies on excitability (Cole, 1968). It is generally assumed that the impedance change reflects changes in ionic conductivities (ionic permeabilities). Fig. 3 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 (Cole, 1970). This result, too, suggests that only a small fraction of the membrane is involved in the rather drastic permeability changes during excitation.

The action potential is accompanied by relatively large heat changes (Abbot, Hill and Howarth, 1958; Howarth, Keynes and Ritchie, 1968); see Fig. 3. These heat changes may be 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

$$\Delta G = \Delta H - T\Delta S, \quad (12)$$

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

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 $\approx Q$), there must be a large value for ΔS ; see Eq. (12). 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 (Neumann, 1973).

The large absolute values of Q (and the irreversible contribution ΔQ , see Neumann, 1974) suggest structural changes and/or chemical reactions to be associated with the action potential. Furthermore, there are observations indicating the occurrence of metastable states and nonequilibrium transitions in excitable membranes at least for certain perfusion conditions (Tasaki, 1968).

7. 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 (Ch-T), acetylcholine-esterase (AcCh-E), acetylcholine-receptor (AcCh-R) and a storage site (S) for AcCh. Since details of this system are outlined elsewhere (Neumann and Nachmansohn, 1974), only a few aspects essential for our integral model of excitability are discussed here.

7.1 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 Ch-T) than axons of lobster walking legs (see e.g. Brzin et al., 1965); motor nerves are generally richer in cholinergic enzymes than sensory fibres (Gruber and Zenker, 1973).

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 (Calabro, 1933; Lissak, 1939) or when axons are exposed to higher external K^+ ion concentrations (Dettbarn and Rosenberg, 1966). 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 is still mainly indirect. However, direct evidence continuously accumulates for extrajunctional AcCh receptors (Porter et al., 1973); 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 (Denburg et al., 1972).

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 are gradually being resolved; 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., Koelle, 1971). Catalysis products of AcCh-E are demonstrated in pre- and postsynaptic parts of excitable membranes (see, e.g., Barnett, 1962; Koelle, 1971; Lewis and Shute, 1966). In a recent study stain for the α -bungarotoxin-receptor complex is visible also in presynaptic parts of axonal membranes (see Fig. 1 in Porter et al. 1973). 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 (Masland and Wigton, 1940; Riker et al., 1959).

7.2 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 are 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 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., Dettbarn, 1967).

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 (Hoskin et al., 1966).

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., Nachmansohn, 1969). 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 (Hoskin et al., 1969). Thus block of enzyme activity observed after homogenization is not necessarily an indicator for block during electrical activity (cf., e.g., Katz, 1966, 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 (Rosenberg and Hoskin, 1963). 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 (Nachmansohn, 1963).

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.

7.3 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 nodes of Ranvier, where permeability barriers are less pronounced (Dettbarn, 1960). Similar to the responses of certain neuro-muscular 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. See Fig. 4.

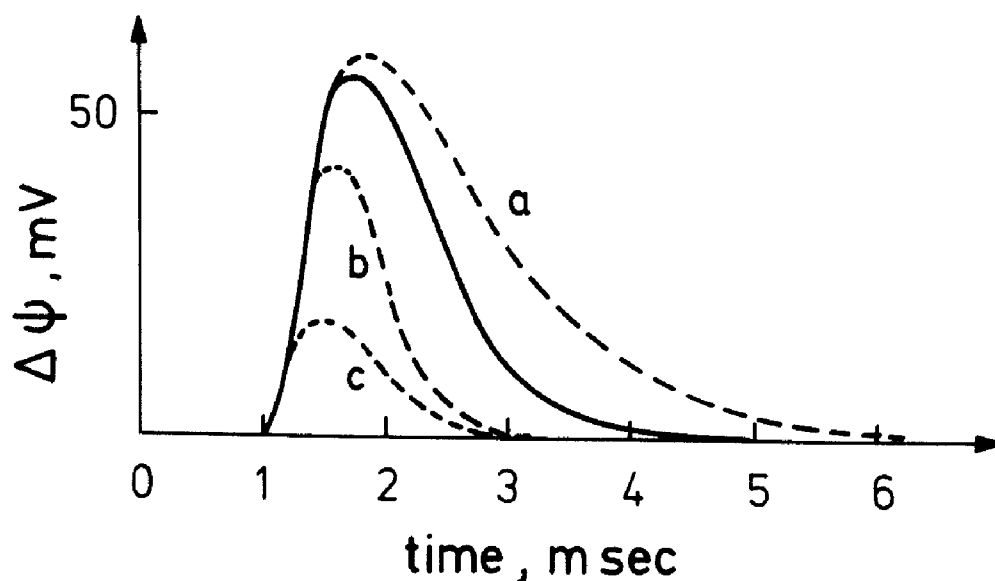


Fig. 4. Action potentials measured at a node of Ranvier of a single fiber of frog Sciatic nerve, $T = 23^{\circ} \text{C}$; solid line: reference; dashed lines: (a) in the presence of $5 \times 10^{-6} \text{ M}$ eserine at pH 7.7 (10^{-3} M eserine blocks the action potential within 30 sec); (b) and (c): gradual decrease of the amplitude with time in the presence of 10^{-3} M curare. The inhibitor solution has been applied externally on top of the Schwann cell layers covering the excitable membrane at the nodes. (Scheme from data by Dettbarn, 1960).

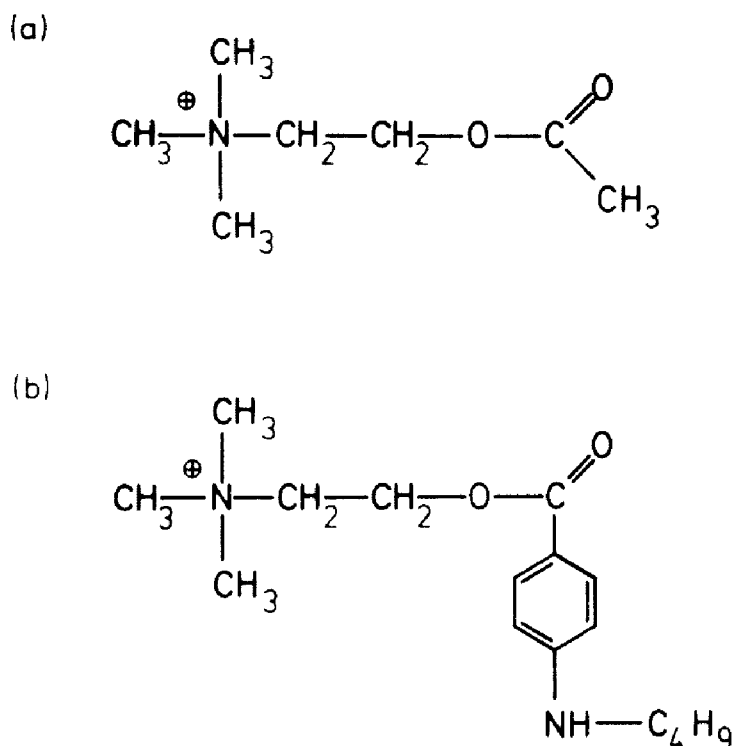


Fig. 5. Chemical structure of the acetylcholine ion (a) and of the tetracaine ion (b). Note that the structural difference is restricted to the acid residue: (a) CH_3 and (b) the amino-benzoic acid residue rendering tetracaine lipid-permeable.

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. 5), these compounds may be considered as analogs of AcCh. In a recent study it was 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 (Bartels, 1965). Local anesthetics are also readily absorbed in lipid bilayer domains of biomembranes (see for review Seeman, 1972).

External application of AcCh without esterase inhibitors faces not only diffusion barriers but esterase activity increases the local proton concentration (Dettbarn, 1967); the resulting changes in pH may contribute to potential changes.

Only very few nerve preparations appear to be suited to demonstrate a direct electrogenic action of externally applied AcCh. Diffusion 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 4.2). Some neuroblastoma cells produce subthreshold potential changes and action potentials upon electrical stimulation as well as upon AcCh application (Harris and Dennis, 1970; Nelson et al., 1971; Hamprecht, 1974).

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 (Ritchie, 1963); 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 behaviour suggest the same basic mechanism for the cholinergic system in axonal and synaptic parts of excitable membranes.

Since there is until now 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, 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 GABA 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.

7.4 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 (Julian and Goldman, 1962). 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., Takeuchi and Takeuchi, 1972; Dettbarn, 1960). 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 (Nachmansohn, 1959).

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 possible, however, 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., Cole, 1968).

8. 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 behaviour, the various similarities of sub- and suprathreshold responses, stimulus

characteristics and the various forms of conditioning and history-dependent behaviour.

In the present account we explore some previously introduced concepts for the control of electrical membrane properties by the cholinergic system (Neumann et al., 1973). Among these fundamental concepts are the notion of a basic excitation unit, the assumption of an AcCh storage site particularly sensitive to the electric field of the excitable membrane, and the idea of a continuous sequential translocation of AcCh through the cholinergic proteins (AcCh-cycle). Finally we proceed toward a formulation of various excitation parameters in terms of nonequilibrium thermodynamics.

8.1 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 (Neumann et al., 1973). 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_1 increases with increasing membrane potential (cell interior negative), (ii) the uptake rate is limited by the conformational transition from state S to S_1 , and is slow as compared to the following translocation steps; see also Sect. 8.5 (c).

Vesicular storage of AcCh as substantiated by Whittaker and co-workers (see, e.g., Whittaker, 1973) is considered as additional storage for membrane sites of high AcCh turnover, for instance at synapses

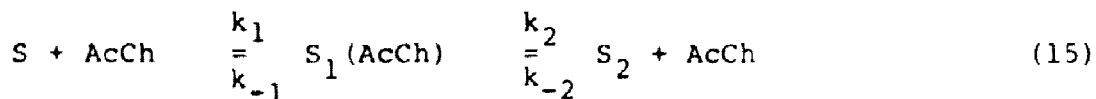
(2) Release of AcCh from the storage form $S_1(\text{AcCh})$, for instance by depolarizing stimulation.



Whereas $S_1(\text{AcCh})$ is stabilized at large (negative) membrane fields, S_2 is more stable at small intensities of the membrane field. The field-dependent conformational changes of S are assumed to gate the

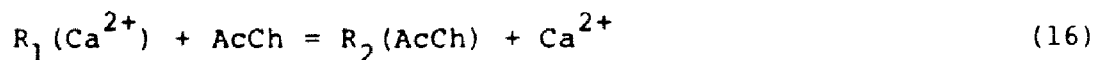
path of AcCh to the AcCh-receptors.

The assumptions for the dynamic behaviour of the storage translocation sequence



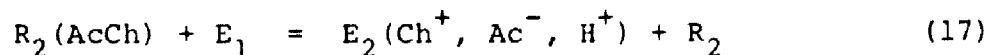
may be summarized as follows: the rate constant, k_2 , for the release step is larger than the rate constant, k_1 , for the uptake, and also $k_2 \gg k_{-2}$. (See also Sect. 8.5)

(3) Translocation of released AcCh to the AcCh-receptor (R) and association with the Ca^{2+} -binding conformation $R_1(\text{Ca}^{2+})$. This association is assumed to induce a conformational change to R_2 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_1 , to an open state, G_2 , increases the permeability for passive ion fluxes.

(5) AcCh hydrolysis. Translocation of AcCh from $R_2(\text{AcCh})$ to the AcCh-esterase, E, involving a conformational transition from E_1 to E_2 .



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



concomitant with the relaxation of the gateway to the closed configuration, G_1 .

Thus, the reactions (17) and (18) "close" a reaction cycle which is formally "opened" with reactions (13) and (15).

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.

8.2 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 Cole, 1968): 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 Neher and Lux, 1973). 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. (16) and (18), 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 subthreshold potential changes and in the gating phase of the action potential (Spector et al., 1973)

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. 3 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. 6 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 counterions such as Ca^{2+} ions. Fig. 6 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 behaviour 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

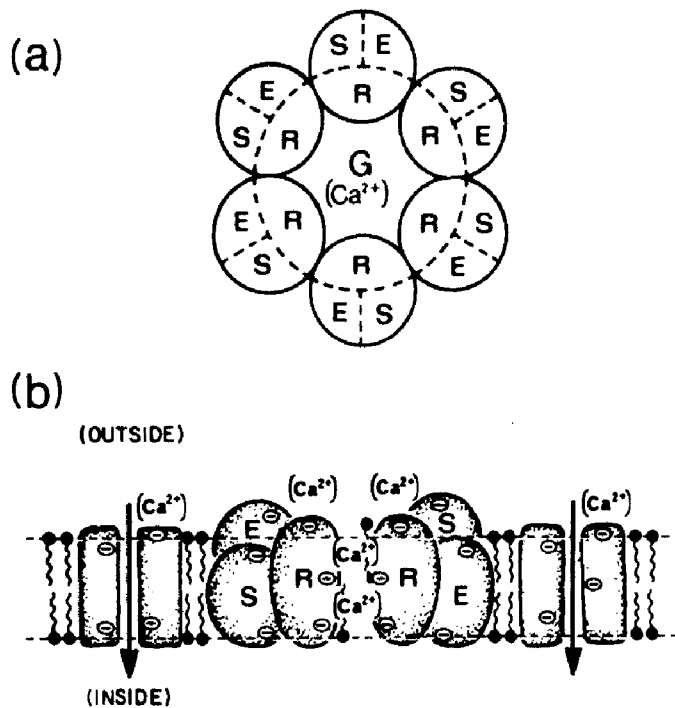


Fig. 6. Scheme of the AcCh-controlled gateway, G. (a) Basic excitation unit (BEU) containing in this example 6 SRE-assemblies, viewed perpendicular to the membrane surface. S, AcCh-storage site; R, AcCh-receptor protein; E, AcCh-esterase. (b) Cross section through a BEU flanked by two units which model 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.

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; 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.

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 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-, postsynaptic-, 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.

8.3 Translocation flux of AcCh

As discussed before, the excitable membrane as a part of a living cell is a nonequilibrium system characterized by complex chemo-diffusional 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 behaviour 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 behaviour such as oscillations in membrane parameters.

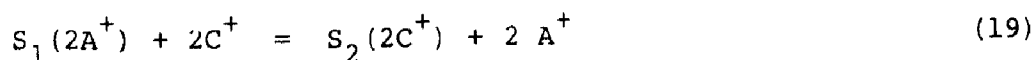
In the frame of the integral model, continuous subthreshold activity is also reflected in a continuous sequential translocation of AcCh through the cholinergic system. The SRE elements comprise reaction spaces with continuous input by synthesis (Ch-T) and output by the virtually irreversible hydrolysis 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 Sect. 8.1 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. As far as local electroneutrality is concerned, it appears in any case more realistic to assume that for one Ca^{2+} -ion released (or bound) there are two AcCh-ions bound (or released).

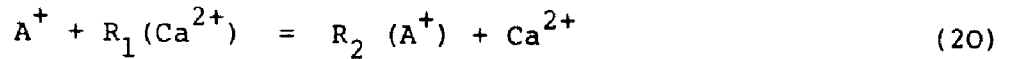
The conformationally mediated translocation of the AcCh-ion, A^+ , may then be reformulated by the following sequence:

(1) Storage reaction

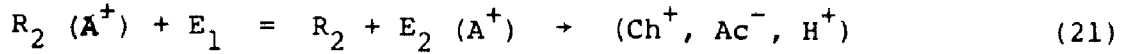


(C⁺ symbolizes a cation, 2 C⁺ may be replaced by Ca²⁺.)

(2) Receptor reaction

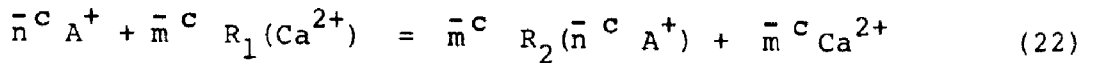


(3) Hydrolysis reaction



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 Ca²⁺ binding form R₁(Ca²⁺), within a critical time interval Δt^c . This time interval is determined by the life time of a single receptor-acetylcholine association.

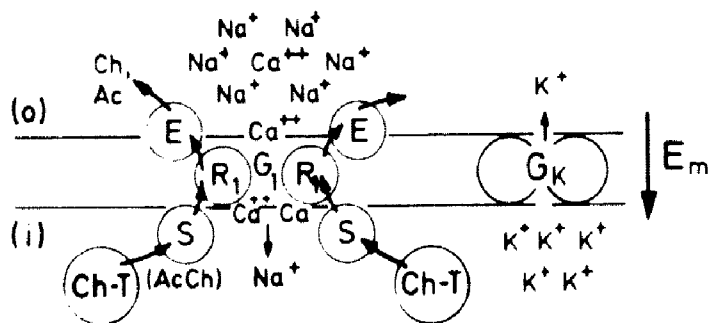
Using formally \bar{n}^c and \bar{m}^c as stoichiometric coefficients the concerted reaction inducing gateway transition may be written:



Storage- and receptor reactions, Eqs. (19) and (20), 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 Adam, 1970). Fig. 7 shows a scheme modelling the "resting" stationary state and a transient phase of the excited membrane.

The complexity of the non-linear flow coupling underlying supra-threshold potential changes may be tractable in terms of the recently developed network thermodynamics covering inhomogeneity of the reaction space and nonlinearity (Oster et al., 1973). An attempt at such an approach, which formally includes conformational metastability and hysteretic flow characteristics (Katchalsky and Spangler, 1968; see also Blumenthal et al., 1970) is in preparation (Rawlings and Neumann, 1974).

Resting stationary state



Excited state

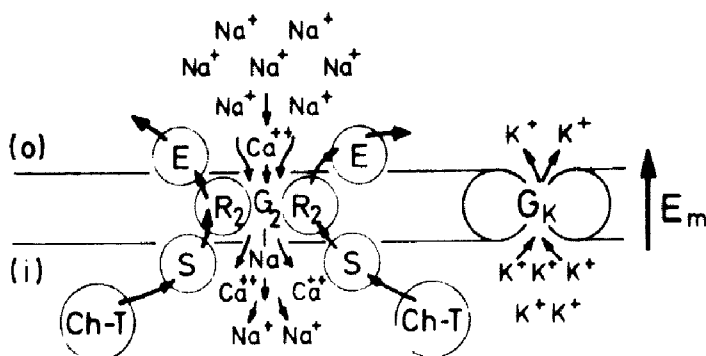


Fig. 7. Schematic representation of a membrane section (a) in the "resting" stationary state and (b) in a transient phase of excitation. In (a), the majority of the acetylcholine-receptors (R) is in the Ca^{2+} ion-binding conformation R_1 ; the cholinergically controlled rapidly operating gateway (G) is in the closed state G_1 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_2 and the rapid gateway is in its open configuration G_2 (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_2 and G_2 to R_1 and G_1 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 (E) is indicated by the curved arrows. The hydrolysis products choline (Ch) and acetate (Ac) are transported through the membrane where intracellular choline-O-acetyltransferase (Ch-T) may resynthesize AcCh (with increased rate in the refractory phase).

(b) Reaction fluxes

For the nonequilibrium description of the translocation dynamics we may associate reaction fluxes with the translocation sequence, Eqs. (19) to (21).

(1) The release flux is defined by

$$J(S) = \frac{d[\bar{n}_r]}{dt} \quad (23)$$

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

$$J(R) = \frac{d[\bar{n}]}{dt} \quad (24)$$

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

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

$$J(E) = \frac{d[\bar{n}_e]}{dt} \quad (25)$$

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,

$$J(S) = J(R) = J(E) = \text{constant.} \quad (26)$$

Statistically occurring small changes in membrane properties such as the so-called miniature-end-plate-potentials (caused by artificially reduced external Ca^{2+} ion concentration at synaptic parts) are interpreted to reflect amplified fluctuations in the subthreshold activity of the cholinergic system.

Oscillatory excitation behaviour observed under certain conditions (see, e.g., Cole, 1968) may be modelled by periodic accumulation and depletion of AcCh in the reaction spaces of the BEUs.

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

Denoting by \bar{n}_b the amount of AcCh bound on average to S_1 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:

$$\left(\frac{\partial \ln K}{\partial E} \right)_{p,T,I} = \frac{\Delta M}{RT} \quad (27)$$

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., Eigen, 1967). 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 (Neumann and Katchalsky, 1972; Revzin and Neumann, 1974) and permeability changes in vesicular membranes (Neumann and Rosenheck, 1972). 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 involves indeed 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. 8. 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, is translocatable to the receptor.

8.5 Relaxations of AcCh translocation fluxes

It is recalled that the receptor reaction (cf. Eqs. (20) and (22)) 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+} 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

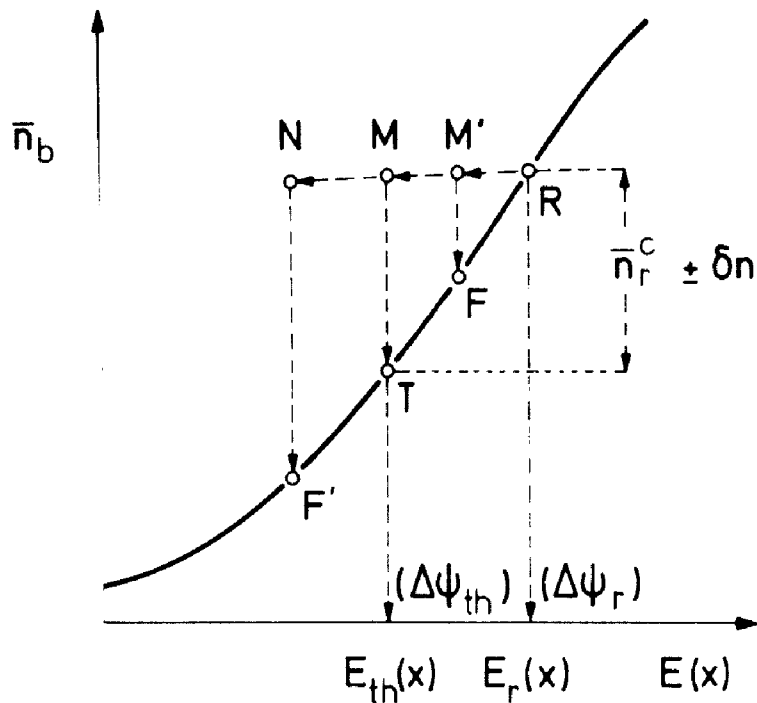


Fig. 8. Model representation of the field-dependent stationary states for AcCh storage. The mean number, \bar{n}_b , of AcCh ions bound to the storage site at the membrane site, x , of the release reaction, as a function of the electric field, $E(x)$, (at constant pressure, temperature and ionic strength). The intervals $M'F$, MT , and NF' correspond to the maximum number of AcCh ions released, \bar{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 \bar{n}_r^C ($\pm \delta n$, fluctuation), and (c) a suprathreshold step R to F' with $\bar{n}_r > \bar{n}_r^C$.

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.

Since flux intensities increase with increasing driving forces (see, e.g., Katchalsky, 1967), $J(S)$ 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 membrane potential changes (see Eq. (11)) 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

$$\frac{d J(R)}{dt} = - \frac{1}{\tau_R} (J(R) - J'(R)) \quad (28)$$

where $J'(R)$ is the stationary value of the new flux. Equivalent to Eq. (28), we have for \bar{n} ,

$$\frac{d[\bar{n}]}{dt} = - \frac{1}{\tau_R} ([\bar{n}] - [\bar{n}]') \quad (29)$$

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. (23) to (25), that the time constant τ_R is the relaxation time of a coupled reaction system.

Applying a few simplifying assumptions and using normal mode analysis (see Eigen and DeMaeyer, 1963), we may readily calculate τ_R as a function of the various reaction parameters (Neumann, 1974).

(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

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

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

$$\frac{d J(R)}{dt} \geq \frac{d J(R)^{\min}}{dt} = \frac{d}{dt} J(R)_{th} \quad (30)$$

is fulfilled.

Eqs. (30) represent the "chemical minimum slope condition" and the threshold receptor flux $J(R)_{th}$ may be considered as the equivalent to the rheobase; see Sect. 4.2.

For the fraction of BEUs necessary to evoke an action potential, the "chemical rheobase" may be specified by

$$J(R)_{th} = \frac{[\bar{n}^c]}{\tau_R} \quad (31)$$

For rectangular (step) perturbations the threshold condition is

$$J(R) \geq \frac{[\bar{n}^c]}{\tau_R} \quad (32)$$

Since $J(R)$ 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 suprathreshold perturbations in the form

$$J(R) \cdot \Delta t = [\bar{n}^c] \quad (33)$$

Compare Eq. (6). The time intervals Δt of the receptor activation correspond to the observed latency phases.

Since \bar{n}_r^c and \bar{n}^c are numbers describing functional cooperativity, the strength-duration product Eq. (33) does 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 chemical rheobase, Eq. (31), is a reaction rate which in general is temperature dependent (normally with 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}^c . Thus corresponding to experience slow depolarization does not (or only occasionally) evoke action potentials.

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

$$\bar{n}_b(M) - \bar{n}_b(T) = \bar{n}^c. \quad (\text{See Fig. 8})$$

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 $R_1(Ca^{2+})$ before a second impulse can be evoked (cf. Eq. (22)). Even if \bar{n}^C AcCh ions would already be 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. 8). 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. (15) are motivated by the accommodation behaviour of excitable membranes. The observation of a relatively refractory phase suggests that the uptake of AcCh into the storage form $S_1(A^+)$ 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 fulfill the action potential condition $\bar{n}_r \geq \bar{n}_r^C$.

8.6 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 Nachmansohn, 1955). 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. 9. In this representation it may be readily seen that manipulations such as external application of AcCh and its inhibitory or activating congeners may interfere at several sites of the AcCh cycle. In particular the analysis of pharmacological and chemical experiments faces the difficulty of this complexity.

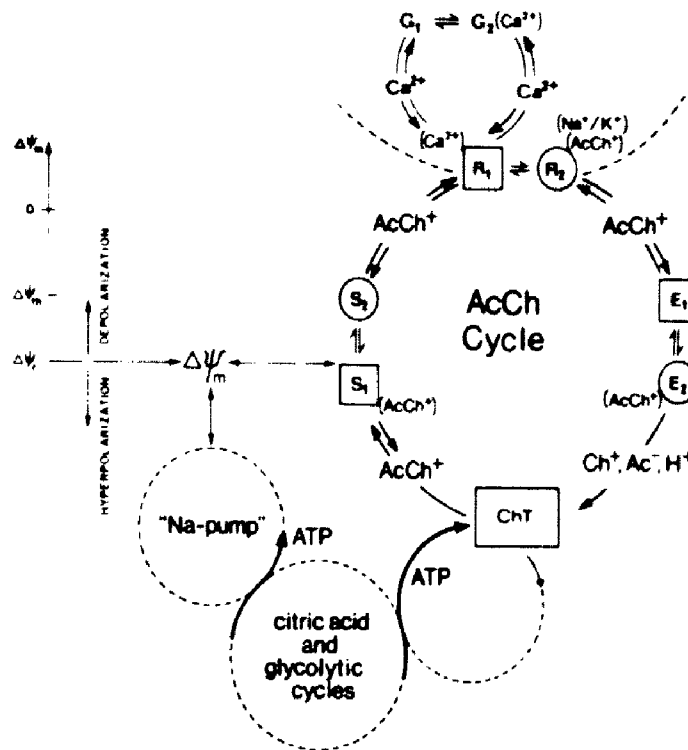


Fig. 9. AcCh-cycle, for the cyclic chemical control of stationary membrane potentials $\Delta\psi$ and transient potential changes. The binding capacity of the storage site for AcCh is assumed to be dependent on the membrane potential, $\Delta\psi_m$, and is thereby coupled to the "Na⁺/K⁺ exchange-pump" (and the citric acid and glycolytic cycles). The control cycle for the gateway G_1 (Ca^{2+} binding and closed) and G_2 (open) comprises the SRE assemblies (see Fig. 4) and the choline-O-acetyltransferase (Ch-T); Ch-T 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 ($\Delta\psi_r$) reflects dynamic balance between active transport (and AcCh synthesis) and passive fluxes of AcCh (through the control cycles surrounding the gateway) and of the various ions unsymmetrically 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 (Neumann and Nachmansohn, 1974).

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.

Summary: The mechanism of nerve excitability is still an unsolved problem. There are various mechanistic interpretations of nerve behaviour, but these approaches cover only a part of the known facts, are thus selective and unsatisfactory.

An attempt at an integral interpretation of basic data well-established by electrophysiological, biochemical and biophysical investigations was inspired by the late Aharon Katchalsky and a first essay had been given (Neumann, Nachmansohn and Katchalsky, 1973). The present account on nerve excitability is a further step towards a specific physico-chemical theory of bioelectricity. In order to account for the various pharmaco-electrophysiological and biochemical observations on excitable membranes, the notion of a basic excitation unit is introduced. This notion is of fundamental importance for modelling details of sub- and suprathreshold responses such as threshold behaviour and strength-duration curve, in terms of kinetic parameters for specific membrane processes.

Our integral model of excitability is based on the original chemical hypothesis for the control of bioelectricity (Nachmansohn, 1959, 1971). This specific approach includes some frequently ignored experimental facts on acetylcholine (AcCh)-processing proteins in excitable membranes. According to the integral model, acetylcholine-ions are continuously processed through the basic excitation units within excitable membranes: axonal, pre- and postsynaptic parts. Excitability, i.e. the generation and propagation of nerve impulses is due to a cooperative increase in the rate of AcCh translocation through the cholinergic control system.

At the present stage of the model, the cholinergic control is restricted to the rapidly operating, normally Na^+ -ion carrying permeation sites. The variations in the electric field of the membrane, caused by the cholinergically controlled rapid gateway, in turn, affects the permeability of the slower, normally K^+ -ion carrying permeation sites in the excitable membrane.

The basic biochemical data suggesting a cyclic cholinergic control (AcCh-cycle) of the ion movements are presented in a concise form and some of the controversial interpretations of biochemical and electrophysiological data on excitability are discussed.

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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. 16) results in number values for apparent rate constants and equilibrium parameters of the participating elementary processes, but also reveals the molecularity of the interactions between receptor, acetylcholine, and Calcium ions. (H.W. Chang and E. Neumann, *Proc. Natl. Acad. Sci. USA*, in press.)