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CHEMICAL REPRESENTATION OF ION FLUX GATING IN EXCITABLE BIOMEMBRANES[1]

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ABSTRACT

A review on molecularly specific gating schemes for ion fluxes in excitable biomembranes is presented. Basic electrophysiological data are discussed in terms of recent relaxation kinetic results on isolated acetylcholine receptor-lipid complexes and on acetylcholinesterase from electric fish. A key conclusion of this assay is that rapid bioelectrical signals based on transient permeability changes in axonal and synaptic parts of excitable biomembranes appear to be specialized cases of a more general chemically dissipative control principle involving activator-receptor interactions and metastability for the activated-conducting state.

KEY WORDS

Acetylcholine; gating; receptor; esterase; metastable states.

INTRODUCTION

Excitable membranes are the carriers of the electrical signals in living organisms. Nerve impulse and other membrane potential changes result from transient membrane permeability changes to ions, in many cases selectively to Na^+, K^+, Ca^{2+}, or Cl^- ions. A major objective of modern biophysical chemistry is the study of the, yet unknown, molecular mechanisms underlying the permeability changes (see, e.g., Neumann and Bernhardt, 1977).

A detailed molecular interpretation of electrical-chemical gating of ion transport requires chemical information on the components of the control system. At present there are only a few transport gating systems where intensive biochemical and physical-chemical investigations have led to a fairly advanced molecular picture for the ion

[1] Dedicated to David Nachmansohn on the occasion of his 81st birthday, 1980.

flux control. As far as regulatory mechanisms for the generation of bioelectric signals are concerned, the most thoroughly studied system is the acetylcholine-regulated transport mediated by acetylcholine receptor (AcChR) and acetylcholinesterase (AcChE). It is, however, very probable that this gating system represents the paradigm for ion flux control in biomembranes, including Na^+ channel gating in axonal parts of excitable membranes (Neumann and Bernhardt, 1977; Dorogi and Neumann, 1980 and this volume). In the following account, a review on molecularly specific gating schemes is given; the review is incomplete and is a subjective selection in the context of work done in the author's laboratory, clearly recognizing the conscious and subconscious impact of the ideas and results of other workers to whom only inadequate tribute can be paid in this article.

ION FLUX GATING CONCEPT

In the Harvey lecture Nachmansohn (1953) presented evidence that suggested a cyclic processing of acetylcholine in both synaptic and nonsynaptic, i.e. axonal or conducting, parts of excitable membranes. Nachmansohn proposed that a proper stimulus releases acetylcholine (AcCh) from a storage site; the chemical activator then binds with a, at that time postulated, receptor protein (AcChR) and induces a conformational change: this structural change opens a pathway (channel) for ions. The enzyme acetylcholinesterase (AcChE) rapidly hydrolyzes acetylcholine and thereby terminates the receptor-mediated permeability increase. A slightly shortened form of the original 'Nachmansohn-Cycle' is depicted in Fig. 1 (Nachmansohn, 1955, 1959, 1975a).

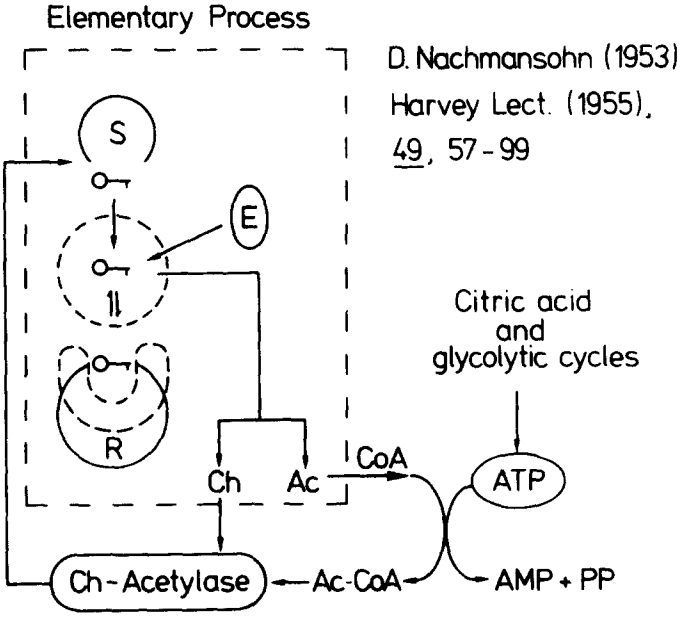


Fig. 1. Cyclic scheme for the acetylcholine-mediated gating of rapid ion flux in excitable biomembranes Nachmansohn (1953, 1955)

A first, formally explicit, chemical representation of the reaction of AcCh, A, with postsynaptic receptors, R, in terms of an induced structural change is due to del Castillo and Katz (1957):

$$A + R \rightleftharpoons AR \rightleftharpoons AR'$$

where AR is the rapidly formed initial, inert complex converting to the depolarizing form AR'.

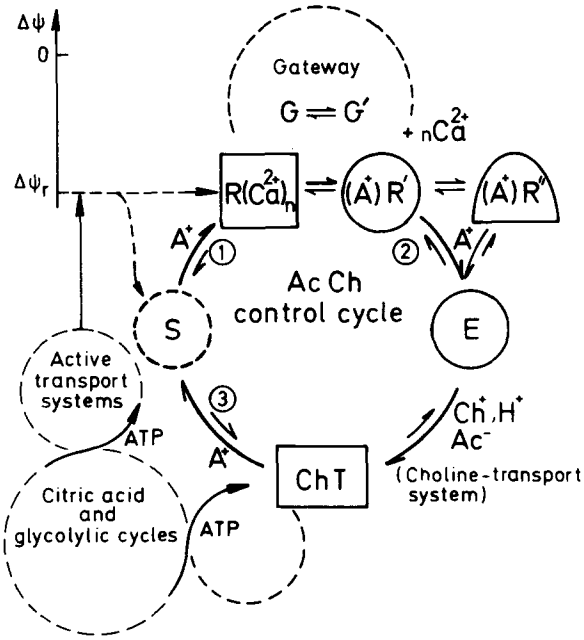


Fig. 2. Acetylcholine(AcCh) cycle of the dissipative chemical control of stationary membrane potentials, $\Delta\psi$, and transient potential changes (and ionic crossmembrane currents). The AcCh control cycle for the (rapidly operating) gateway $G(Ca^{2+})$ binding and closed) and G'(the open permeative configuration) consists of the AcCh receptor system (R), the AcCh esterase (E), the choline-O-acetyl-transferase (ChT), and a (hypothetical) AcCh storage (S) system. The continuous flux of AcCh through a R-E-ChT-S control "subunit" is maintained by ChT (coupled to the choline (Ch^+) uptake system), and by the practically irreversible hydrolytic removal of AcCh ion, A^+, from the reaction space 2 by E.
The opening-closing process $G \rightleftharpoons G'_{2+}$ is controlled by the overall receptor reaction $R(Ca^{2+})_n + A^+ \rightleftharpoons (A^+)R' + nCa^{2+}$, with $n \approx 2$-3. R is the low, and R' the high, conductivity configuration of the receptor. R" accounts for pharmacological desensitization. In the resting steady state, the membrane potential ($\Delta\psi$) reflects dynamic balance between the active transport (including AcCh-synthesis systems) and the flux of AcCh through the control cycles surrounding the gateway. Fluctuations in membrane potentials and ionic currents are amplified by fluctuations in the local AcCh concentrations. The AcCh control cycle is probably coupled to the electric field of the membrane by the receptor system. The encircled numbers refer to different microreaction spaces for the processing of AcCh. This picture summarizes the present knowledge and is a modified version of a former scheme (Neumann & Nachmansohn, 1975).

In programmatic assays (Neumann, Nachmansohn and Katchalsky, 1973; Neumann, 1974; Neumann and Nachmansohn, 1975b, 1975a) the essential aspects of the acetylcholine-specific gating concept were summarized in terms of overall reactions: (1) release of A from a (postulated) storage site S via an electric field induced conformational change $S \rightarrow S'$, (2) induction by A of a structural change in AcChR to a conducting configuration, releasing Ca^{2+}-ions, (3) hydrolysis of A dissociated from the AR'-complex by AcChE, E, (4) return to the R-conformation under re-uptake of Ca^{2+}, closing the channel.

(1) $\quad AS \rightleftharpoons S' + A$

(2) $\quad A + RCa \rightleftharpoons AR' + Ca^{2+}$

(3) $\quad AR' + E \rightleftharpoons E + (Ch, Ac, H^{+}) + R'$

(4) $\quad R' + Ca^{2+} \rightleftharpoons RCa$

Equations (2) and (4) model Ca^{2+}-effects as specifically proposed by Nachmansohn (1968).

The present version of the acetylcholine cycle (Neumann and Nachmansohn, 1975a,b) is depicted in Fig. 2. In this representation the cyclic nature and the essentially sequential processing of AcCh is particularly apparent.

Although the actual permeability and thus conductivity, change in the membrane is mediated by AcChR the electric signals clearly indicate the essential role of the enzyme AcChE for the AcCh-mediated ion transport. It is recalled that, kinetically, the gating concept implies that the time course of the permeability change indicated for instance by electrophysiological voltage-clamp experiments is determined by the kinetics of the chemical-structural reactions of the gating proteins.

ELEMENTAL BIOELECTRICAL SIGNAL

The probably most elemental epiphenomenon resulting from AcCh action is the spontaneous miniature end-plate current, mepc (see, e.g. Gage, 1976). As seen in Fig. 3 where such an mepc is redrawn, a rapid growth phase is followed by a slower decay phase. Gage and McBurney (1975) explicitly state that this decay is exponential from the peak, no rounding is observed. The growth phase probably reflects the AcCh-induced conformational change; the decay phase is determined by AcChE activity, but not in a rate-limited manner.

The voltage dependence of the decay time constant as well as the average life-time t_o of an open channel is not affected by the presence of esterase inhibitors; time constants of mepc and of end-plate currents are the same and equal to t_o (see, e.g. Gage, 1976; Stevens, 1976). It thus appears that the closure phase of the AcCh-activated channels is practically independent of AcCh; it is rate-limited by the return of AcChR to the closed conformation (Stevens, 1976). This observation requires, however, that AcCh is much faster removed than it can return to AcChR; effective association to AcChE should therefore be faster than to AcChR or in terms of association rate constants:

$$k_1(\text{eff},R) < k_1(\text{eff}, E). \qquad (5)$$

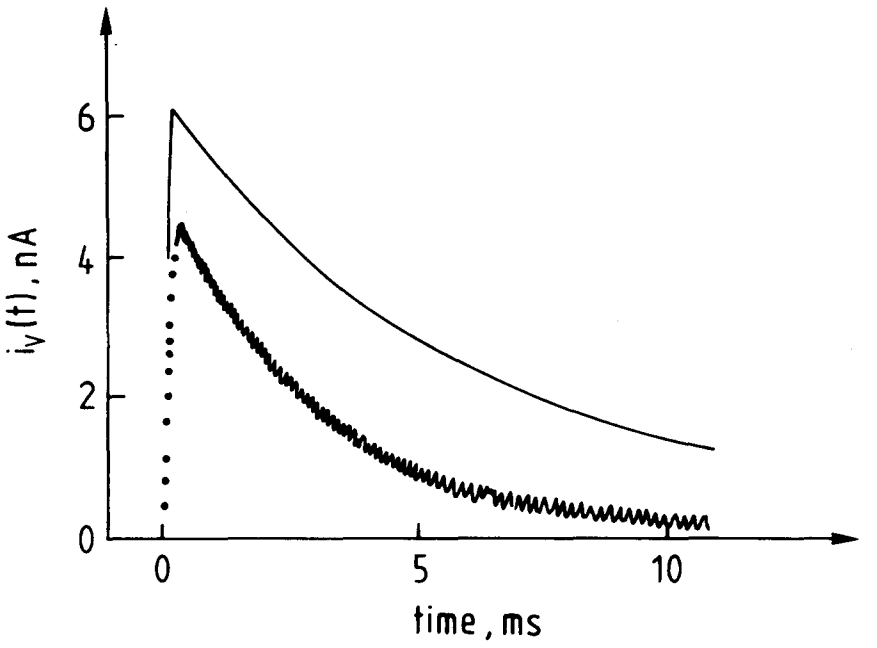


Fig. 3. Miniature end plate current, mepc, in toad neuromuscular junction (redrawn from Fig. 8 of Gage and McBurney, 1975; and Fig. 2 of Gage, 1976); clamp potential - 70 mV, 293K in standard Ringer solution. Upper trace recorded after 30 min exposure to 1mg/l neostigmine in Ringer solution, a condition assumed to completely inhibit esterase activity: the slow decay reflects slow diffusion of acetylcholine in the junction (and probalby also further receptor desensitization).

Since practically complete inhibition of AcChE with neostigmine causes an increase of the peak amplitude of mepc by a factor of 1.4 and a prolongation of the decay phase by a factor of about 2 (see Fig. 3 and Gage and McBurney, 1975), the receptors in a normal mepc appear not to be saturated.

In order to model AcChE inhibitor effects on mepc consistent with the inequality (5), the elementary conductance increase must involve at least two AcCh molecules (Neumann, Rosenberry and Chang, 1978; Rosenberry, 1979). Using several assumptions on receptor density in the synaptic cleft and applying the reactions

$$2A + R_2 \rightleftharpoons ARR' + A \rightleftharpoons (AR')_2 \qquad (6)$$

$$A + E \rightleftharpoons AE \rightleftharpoons E + P \qquad (7)$$

in a competitive model, Rosenberry (1979) successfully simulated mepc; for equation (6) see also Sheridan and Lester (1977). However,

the sharp peak of mepc was not reproduced and it can not be modelled by any competitive model, even if the time course of the AcCh concentration, $[A]$, is a delta function during the growth phase of mepc. Thus the particular shape of mepc indicates an essentially sequential processing of AcCh: the main part of neurally evoked AcCh binds first with AcChR in a microreaction space 1. Because of the inequality (5) it appears that initially in the growth phase of a mepc the AcCh concentration close to activatable receptors, $[A]_1$, is larger than the AcCh concentration $[A]_2$ in a second microreaction space where AcChE fully competes with AcChR for activator. To fulfil the initial mepc condition

$$[A_1] > [A]_2 \tag{8}$$

a partial diffusion barrier between the local reaction spaces 1 and 2 appears to be necessary.

These conclusions from purely electrophysiological data, supported by physical-chemical data on the isolated proteins, have been summarized in a flow scheme (Neumann and Bernhardt, 1977; Neumann, Rosenberry and Chang, 1978). The present form of the AcChR-gating cycle for neurally triggered AcCh is shown in Fig. 4.

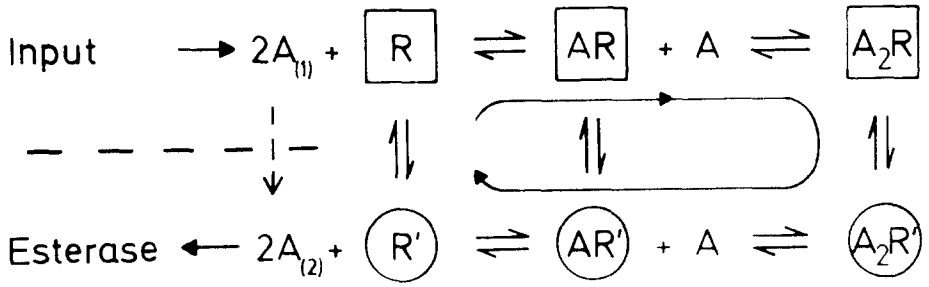


Fig. 4. Flow scheme (AcChR-gating cycle) for neurally triggered acetylcholine (input), which reacts essentially sequentially with receptor and esterase. The curved arrow indicates the flow of acetylcholine A from a reaction space 1 through the closed [R] and open (R') receptor states to a separate reaction space 2 where the esterase has full competitive access to acetylcholine $A_{(2)}$. The dashed line represents a partial diffusion barrier such that initially in a mepc the condition $[A]_1 > [A]_2$ holds.

It is readily realized that, once AcCh is more rapidly removed, i.e. $[A]_2 \cong 0$, closure of a channel can occur solely along intramolecular pathways, probably mainly via the $R' \rightleftarrows R$ step and less via the

$A_2R \rightleftharpoons A_2R'$ step; both pathways are consistent with first order decay of a mepc. The flow scheme contains an extented version of a simple cyclic activation scheme discussed by Colquhoun (1975):

$$\begin{array}{ccc} A + R & \rightleftharpoons & AR \\ \downarrow\uparrow & & \downarrow\uparrow \\ A + R' & \rightleftharpoons & AR' \end{array} \qquad (9)$$

where $[R'] << [R]$ in the absence of activator.

When AcCh is of non-neural origin, for instance applied artificially using a micropipette, AcChRs are multiply activated (Katz and Miledi, 1973) and the decay phase of a mepc is prolonged. The inequality (8) may no longer hold and the opening-closure kinetics is mainly determined by the kinetics of the $A_2R \rightleftharpoons A_2R'$ step, as suggested by experiments (see Neher and Sakmann, 1975; Stevens, 1976; Sheridan and Lester, 1977; Stevens, 1980).

Desensitization. Prolonged exposure to AcCh indicated additional reaction pathways for the closure of AcCh-activated channels. Longer bath application of activators causes inactivation or pharmacological desensitization of AcChRs, according to a cyclic scheme first proposed by Katz and Thesleff (1957).

$$\begin{array}{ccc} A + R & \rightleftharpoons & AR' \\ \downarrow\uparrow & & \downarrow\uparrow \\ A + R'' & \rightleftharpoons & AR'' \end{array} \qquad (10)$$

where R" is one of probably more desensitized receptor states (see also Rang and Ritter, 1970).

A direct consequence of the electro physiological data describable with scheme (10) is that, even in the absence of AcCh, a certain fraction of AcChR exists a priori in the inactivated conformation R", characterized by a higher affinity to AcCh than the R-state. It is therefore suggestive that the 'non-depolarizing' receptors (Katz and Thesleff, 1957) and the 'diffusion barrier' (De Motta and del Castillo, 1977) are just receptors in the desensitized R"-form. Furthermore, AcCh-induced inactivation following the activation phase suggests that the activated, permeable conformation of AcChR is metastable; the inactivated states AR" are thermodynamically the most stable states in the presence of activator.

Ca^{2+}-ions. The permeability changes induced by AcCh in many biomembranes are cation selective. Corresponding to the high concentrations of external Na^+ ions and internal K^+-ions the alkali metal ions predominantly contribute to the ion flows. Since Ca^{2+} selectively inhibits the Na^+-flow there appears competition for sites and thus transient, short-lived binding of Na^+ and Ca^{2+} ions in the Na^+ pathway (Takeuchi, A. and Takeuchi, N., 1972; see also Gage and van Helden, 1979).

The detailed role of Ca^{2+} ions in activation and inactivation is not yet molecularly understood. In frog muscle increased external Ca^{2+} concentration slightly decreases mepc decay time constants and life times of open channels (see Table 3 of Magleby and Weinstock, 1980). Thus Ca^{2+}-ions appear to facilitate the closure reaction and to stabilize the R-conformation of AcChR. In addition, increased Ca^{2+} concentration appears to accelerate the activator-induced desensitization (see, e.g. Nastuk and Parsons, 1970; Manthey, 1972; Fiekers, Spannbauer, Scubon-Mulieri, and Parsons, 1980).

In summary, AcCh-induced rapid activation eventually releasing Ca^{2+}-ions followed by slower inactivation processes accompanied by uptake of Ca^{2+} ions appear to be a fundamental characteristics of functionally intact AcChRs.

GATING MACROMOLECULES

Acetylcholine receptor

The smallest, functionally intact acetylcholine binding protein which can be decoupled under gentle experimental conditions from Torpedo fish, minimizing chemical modifications, is the H-form of receptor rich membrane fragments. According to Chang and Bock (1977), this H-form of a molecular weight of roughly 400 000d (Torp.cal.) is a dimer of two probably not identical, monomers: the L- and the L'-form which are linked by intersubunit disulfide bond (see also Suarez-Isla and Hucho, 1977; Hamilton, McLaughlin, and Karlin, 1977, 1979). Provided that endogenous lipids remain attached to the isolated AcChR receptor-lipid complex, the H-form exhibits essentially the same positive-cooperative acetylcholine binding isotherms as biomembrane fragments (Chang and Bock, 1979).

Ca^{2+} and $AcCh^{+}$ binding. The isolated AcChR in detergent solution probably largely in the L-forms was found to bind large amounts of Ca^{2+} ions associated with high (μM) and low (mM) affinity sites (Eldefrawi, M.E., Eldefrawi, A.T., Penfield, O'Brien and van Campen, 1975; Chang and Neumann, 1976; Rübsamen, Hess, Eldefrawi, 1976); there is competition of Ca^{2+} binding with other cations (see also Raftery, Vandlen, Reed, and Lee, 1976). This competition has been used to estimate some thermodynamic and kinetic constants of acetylcholine binding to AcChR. It was found that upon binding of one AcCh-ion about 2 to 3 Ca^{2+}-ions are released; subsequent binding of α-bungarotoxin causes reuptake of Ca^{2+} (Chang and Neumann, 1976; see also Rübsamen, Hess, Eldefrawi (1976). Relaxation kinetic studies have provided estimates for the (effective) rate constants of acetylcholine and of Ca^{2+} ion binding; see Table I. As seen $k_1 = 2.4(\pm 0.5) \times 10^7\ M^{-1}s^{-1}$ for AcCh suggests that the measured association rate constant is probably an effective, complex rate parameter involving several, more rapid elementary steps. See Fig. 5. The life time of the effective association is $(k_{-1})^{-1}=7$ ms. The kinetic constants for acetylcholine so far characterize a receptor preparation with overall dissociation equilibrium constant $\bar{K}_A = 10^{-6}$ M (at 296 K, pH 8.5 and 0.1 M NaCl). This value is close to the acetylcholine concentration which causes the electrical half-response. On the other hand, K_A for crude extracts, membrane fragments and recent receptor preparations where chemical modifications could be largely reduced (Chang and Bock, 1979), is between 10^{-8} and 10^{-9} M, most likely representing the acetylcholine affinity to the inactivated

receptor. Moreover, the rate constants for the bimolecular overall reaction (Table I) compare well with data from electric current relaxations (Sheridan and Lester, 1977): $k_{on} \simeq 10^7\ M^{-1}\ s^{-1}$ and $k_{off} \simeq 10^2$ to $10^3\ s^{-1}$ depending on membrane potential. The electrophysiological data further indicate that at least two acetylcholine ions must bind in order to open a single permeation site. This may be related to the fact that the H-form of the isolated receptor protein has two binding sites for acetylcholine.

TABLE I Interaction parameters for the isolated acetylcholine receptor from Torpedo californica and acetylcholine and Ca ions in terms of a direct competition of both ligands, for the main reaction path $A + R \rightleftharpoons AR \rightleftharpoons AR^*$ (Neumann and Chang, 1976; Neumann, Rosenberry, and Chang, 1978)

$A + R \rightleftharpoons AR$	$AR \rightleftharpoons AR^*$	$Ca + R \rightleftharpoons CaR$
$k_1 = 2.4(\pm 0.5) \times 10^7\ M^{-1}\ s^{-1}$	$k_2 = 43.5\ s^{-1}$	$k_o = 10^8\ M^{-1}\ s^{-1}$
$k_{-1} = 140\ s^{-1}$	$k_{-2} = 6.5\ s^{-1}$	$k_{-o} = 10^5\ s^{-1}$
$K_1 = 0.6 \times 10^{-5}\ M$	$K_2 = 6.7$	$K_o = 10^{-3}\ M$

Solvent is 0.1 M NaCl, 0.1% Brij, 1 mM Ca^{2+}, 0.05 M Tris HCl, pH 8.5 at 296 K. The overall equilibrium constant for acetylcholine is given by $\bar{K}_A = K_1(1 + K_o^{-1} c_{Ca})(1 + K_2)^{-1} = 10^{-6}$ M, where $K_1 = k_{-1}/k_1$, $K_2 = k_2/k_{-2}$ and for the Ca^{2+}-binding $K_o = k_{-o}/k_o$.

Among the various points of comparisons, in particular the similarity between $k_{on} \simeq 10^7\ M^{-1}\ s^{-1}$ from current relaxations and $k_1(\text{eff}) = 2.5(0.5) \times 10^7\ M^{-1}\ s^{-1}$ from studies on isolated receptors suggests that the rate of coupled Ca^{2+} release (upon binding of acetylcholine) not only reflects the overall rate of effective acetylcholine binding to isolated receptor, but is also characteristic for the rate-limiting step in the conductivity increase of the membrane. Thus, stoichiometry of acetylcholine binding, equilibrium and rate constants suggest the low affinity receptor with $K_A \simeq 10^{-6}$M as a candidate for the in vivo metastable, conducting receptor conformation, which by chemical modification (sulfhydryl-disulfide redox reactions) during isolation may be stabilized in detergent solution.

In contrast to the relatively low value for acetylcholine, the measured association rate constant for the binding of the dicationic inhibitor bis (3-aminopyridinium)-1,10-decane (DAP) to isolated Torp.marm. AcChR is $k_{ass} = 1.2 \times 10^8\ M^{-1}\ s^{-1}$ in 0.1 M ionic strength, pH 7.0 and 293 K. The ionic strength dependence of the association rate of k_{ass} (DAP) suggests an effective charge of $-3(\pm 1)$ on the binding site of the protein (Chang and Neumann, 1980, in press). This value is somewhat less negative than that indicated for the esterase. But it seems that in both proteins of the permeability control system there are larger electrostatic contributions to the

rate with which cationic ligands like acetylcholine are bound.

The estimates for bimolecular rate constants k_1(eff) of AcChR-ligand binding appear to depend strongly on the type of ligand used: for decamethonium 2 x 10^8 $M^{-1}s^{-1}$ and for carbamylcholine and acetylcholine 10^7 $M^{-1}s^{-1}$ (Sheridan and Lester, 1977), for suberyldicholine 0.98 x 10^7 $M^{-1}s^{-1}$ (Barrantes, 1978), for NBD-5-acylcholine $\geq 10^8$ M^{-1} s^{-1} (Jürss, Prinz and Maelicke, 1979).

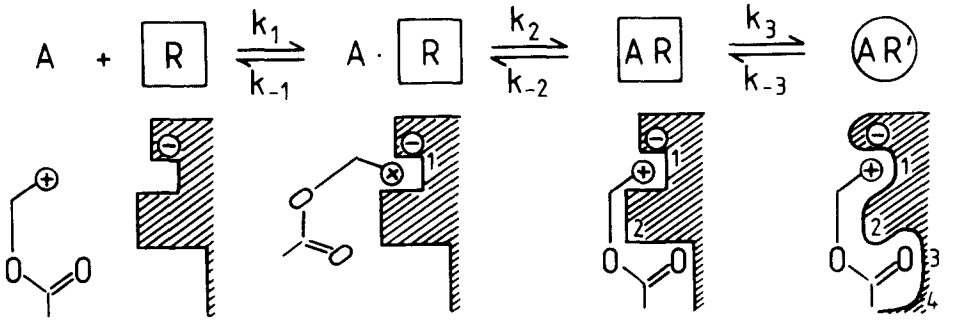


Fig. 5. Chemical Model for the induced conformational change in AcChR: step 1, encounter ion pairing with $K_1=k_{-1}/k_1 \cong$ 0.1 M, $k_1 \geq 10^8$ M^{-1} s^{-1} and $k_{-1} \leq 10^7$ s^{-1}; step 2, contact with a second site in the life-time of the ion-pairing; step 3, conformational change to the permeable state induced by steps 1 and 2. The state AR' may involve a 'distorted' AcCh.

AcChR-lipid complexes. Studies with the isolated AcChR-lipid complexes from Torp. cal. confirm that the Ca^{2+}-binding isotherm is essentially two-phasic with equilibrium constants in the µM and mM range (Dorogi, Chang, Moss, and Neumann, 1980), suggestive for intracellular and extracellular Ca^{2+} sites in AcChR. If this complex is incorporated in lipid vesicles and then transferred via a surface monolayer into planar lipid bilayer, 'specific' conductivity changes inclusively inactivations are evoked upon addition of carbamylcholine Inhibitors like d-tubocurarine and α-bungarotoxin block the membrane (Spillecke, Schindler and Neumann, 1980, in prep.). It thus appears that the H-form of AcChR (Torp. cal.) with its subunits α(40,000d), ß(48,000d), γ(59,000d) and δ(67,000d) contains not only the AcCh binding subunits (Karlin, Weill, McNamee, Valderama, 1976) but also the subunits forming, after structural changes, the ion transporting channel.

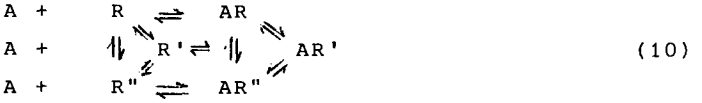
Ca^{2+} ions may be involved to preserve stability of the protein-lipid complex and may also bind to the anionic groups of the channel subunits. A large number of anionic ,probably carboxylate,groups are suggested by the large Ca^{2+}-ion binding capacity of AcChR. Provided that the density of the anionic charges exceeds a certain value, divalent ions like Ca^{2+} are preferentially bound. If AcCh induces a structural change which increases the average distance between the charged groups, this polyelectrolyte-preference for Ca^{2+} ions would be lost and ion exchange with, say, Na^{+}-ions could occur (Neumann, Nachmansohn, and Katchalsky, 1973). The 'indicator' of 'effective binding' of AcCh to AcChR would therefore be allosteric release of bound Ca^{2+}. However, prolonged exposure to receptor activators leads to definitely allosteric uptake of Ca^{2+}-ions; see also Sugiyama and Changeux (1975).

Transient release of Ca^{2+}-ions followed by uptake of Ca^{2+} upon addition of activators to AcChR (Spillecke, Chang, Neumann, 1980, in prep.) suggest at least one metastable state in vitro, parallel to the electrophysiologically indicated in vivo, metastability for the conducting channel configuration, and parallel to the metastability suggested for the permeable state in sealed biomembrane vesicles (Bernhardt and Neumann, 1978, and Neumann, 1979).

Asymmetry in AcChR. Bulger and Hess (1973) found that two types of binding sites in membrane-bound AcChR are apparent; this non-equivalence is induced by α-bungaratoxin and leads to interconversion of the sites (Bulger, Fu, Hindy, Siberstein, and Hess, 1977), which in unbound state must not necessarily be of different type. Binding studies with DAP and α-bungarotoxin have also been interpreted in terms of two classes of binding sites (Raftery, Vandlen, Reed, and Lee, 1976). In the same line are data of Damle, McLaughlin, and Karlin (1978) and of Delegeane and McNamee (1980). Structurally, there appears a distinct asymmetry in the results of digital image processing of electron micrographs of *Torp. marm.* membrane fragments (Zingsheim, Neugebauer, Barrantes, and Frank, 1980).

These data suggestive for intrinsic asymmetry of AcChR structure and function invites speculation on a possible functional role. If the 'monomers' of the dimeric H-form have asymmetric subunit compositions, say an L-form with $\alpha_2\beta\,\delta$, and an L'-form with $\alpha_2\gamma\,\delta$ stoichiometry, a molecular weight of about 200,000 d for each 'monomer' and of about 400 000 d for the asymmetric H-form would result, a value not inconsistent with recent estimates (Raftery, Vandlen, Reed, and Lee 1976; Reynolds and Karlin, 1978), where, however, the 43 000 d chain appears to be included (see, e.g. Wennogle and Changeux, 1980). Functionally, the L and L'-forms are candidates for separate channels for Na^{+} and K^{+}, both, however, controlled by the binding of at least two acetylcholine ions.

These characteristic features of the dimeric AcChR gating protein are only covered by more complicated reaction schemes. It thus appears that chemical representations like that of Changeux and coworkers (1976):



(10)

here presented in our symbols, covers correctly single site binding of ligands to closed (R), conducting (R') and desensitized (R") states as well as the corresponding cyclic structural transitions.

In the light of the previous arguments of a dimeric channel structure it appears however more apropriate to formally express the experimental complexity in terms of the AcChR dimer, see Fig. 6; the scheme is analogous to a general scheme developed by Eigen (1967) for a tetrameric subunit system.

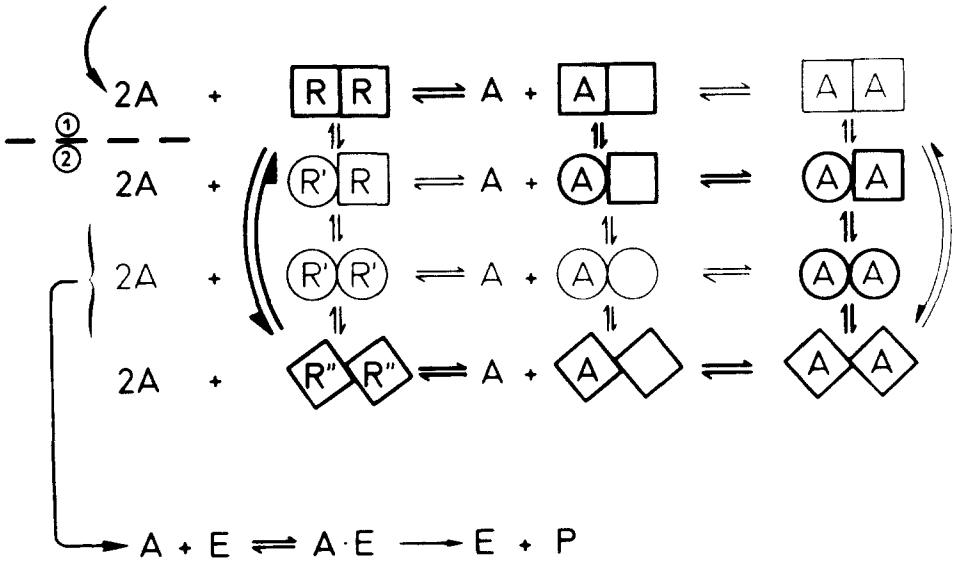


Fig. 6. Chemical representation of acetylcholine (AcCh)-induced activation and inactivation of acetylcholine receptors (AcChR) in terms of a basically sequential processing of AcCh from a microreaction space 1 to a microreaction space 2 where acetylcholinesterase (AcChE) has fully competitive access to AcCh. The receptor states, in terms of activator binding sites, correspond to R (closed), R'R' (open) and R" (desensitized). The main reaction pathways are drawn in thick symbols. For neurally evoked AcCh the changes along the desensitization pathways $R_2 \rightleftharpoons R"_2$ and $(AR')_2 \rightleftharpoons (AR")_2$ appear to be uninvolved.

ACETYLCHOLINESTERASE

The essential role of AcChE in bioelectrogenesis is manifested in an extremely high turnover number $k_{cat} = 1.6 \times 10^4\ s^{-1}$ at 0.1 M NaCl, pH 8 and 298 K. The isoelectric point of the eel protein is pI=4.5; thus, under normal conditions of pH 7 the protein is anionic. In order to explore ionic-electrostatic contributions to substrate binding, fluorescent non-substrates can be used, which bind specifically to the catalytic sites and are cations like acetylcholine, but are not hydrolyzed; particularly suited is the compound N-methylacridinium. A key result of relaxation kinetic studies is that the overall relaxation of AcChE and N-methylacridinium is bimolecularly controlled (Rosenberry and Neumann, 1978) as modelled in Fig. 7. The bimolecular rate constants between 10^{10} and $10^9\ M^{-1}s^{-1}$ are unusually high for enzyme ligand interactions. In addition, the association rate constants k_{12} are very strongly dependent on the ionic strength, I_c, of the solution (Nolte, Rosenberry, Neumann, 1980). Virtually the same strong I_c-dependence has been observed for a catalytic parameter proportional to k_{12} of acetylthiocholine k_{cat}/K_{app}, a substrate whose structure and kinetic properties are very similar to those of acetylcholine.

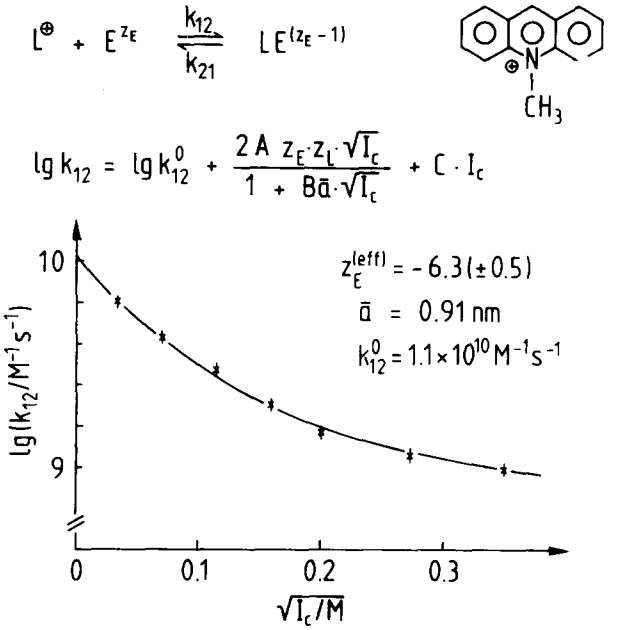


Fig. 7. Ionic strengh (I_c) dependence of the association rate constant k_{12} of the equilibrium of electric eel AcChE and N-methylacridinium, analyzed in terms of the semiempirical Brønsted-Debye-Hückel equation giving $k_{12}(I_c \to 0) = k_{12}^o$, the effective point charge equivalent z_E of an enzyme active site and the approach distance $\bar{a}$.

The I_c-dependencies have been analyzed in terms of a Brønsted-Debye-Hückel relationship as given in Fig. 7. The extended Debye-Hückel equation for the activity coefficient product involved in this relationship, contains the bulk dielectric constant ε of H_2O (at 298K, ε=79) and the charge numbers Z_i of the interacting ionic species; it has been found to describe not only the experimental data but also the rigorous Monte-Carlo results of electrolytes even with higher charge number (Pitzer, 1977). We have chosen a Brønsted-Debye-Hückel relationship to evaluate the effective charge number Z_E as the point charge equivalent of the enzyme active site. The analysis results in $Z_E = -6.3(\pm 0.5)$ when the term $C \cdot I_c$ is not considered. In a recent study of the I_c-dependence of k_{12} and of the corresponding equilibrium the empirical value C=0.5 M^{-1} led to $Z_E = -7$ as a meaningful estimate (Nolte and Neumann, 1980, in prep.) Thus at least seven monovalent anionic groups contribute to the comparatively large values k_{12} for both the non-substrate N-methylacridinium and acetylthiocholine ($k^o_{12} = 0.42 \times 10^{10}$ $M^{-1}s^{-1}$), suggesting that an enzyme surface area larger than the ligand binding site itself is effective in trapping a ligand in encounter complexes. This larger surface area might include peripheral anionic sites from which ligand would move to the active site by surface diffusion. The high effective charge number supports this concept. The charged groups contributing to $Z_E = -7$ would be expected to be dispersed over an enzyme surface greater than the immediate catalytic site. In summary, we may conclude that the high bimolecular association rate constants and the unusually strong ionic strength dependence of kinetic and thermodynamic parameters have its physical origin in a dominantly anionic surface structure of this enzyme. Physiologically, the polyionic enzyme acetylcholinesterase appears to be a powerful electrostatic sink for trapping and decomposing the acetylcholine cation.

To the extent to which data on isolated proteins can be used to extrapolate to the cellular level, it is very tempting to compare the effective association rate constant of the receptor-acetylcholine interaction $k_1(eff) = 2.4(\pm 0.5) \times 10^7$ $M^{-1}s^{-1}$ with that of the enzyme $k_1(eff) = k_{12} \geq 2 \times 10^8$ $M^{-1}s^{-1}$, probably 10^9 $M^{-1}s^{-1}$. It is readily recognized that the inequality (5) suggested by electrophysiological data is parallel to $k_1(eff,R) < k_1(eff, E)$ found for the isolated proteins. The physical reason for the lower k-value of AcChR may reside in the preequilibria preceding the step $(AR)_2 \rightleftharpoons (AR')_2$; see Fig. 5 and 6.

AXONAL GATING

The existence of basic similarities between synaptic and axonal parts of excitable membranes has been frequently discussed see, e.g. , Nachmansohn and Neumann, 1975, Neumann and Bernhardt, 1977). In particular, the conducting conformation of the Na^{2+}-ion channel is clearly a metastable, short-lived state as appears to be the case with the permeable AcChR conformation (Neumann, 1973, 1979). In a recent study it was found that kinetic models which can successfully simulate the ion-permeability features of axonal Na^+ channels, suggest the presence of bimolecular reaction steps in the activation of channels (Dorogi and Neumann, 1980 and this volume). The implied chemical formalism is highly suggestive of an activator-controlled gating system with strong similarities to the acetylcholine regu-

lated ion transport systems; see Fig. 8 and Eqs. (1)-(4). Conformational changes which underlie the ion conductance changes are suggested to possess a greater sensitivity to the membrane field in axonal than at synaptic parts of excitable membranes. This allows

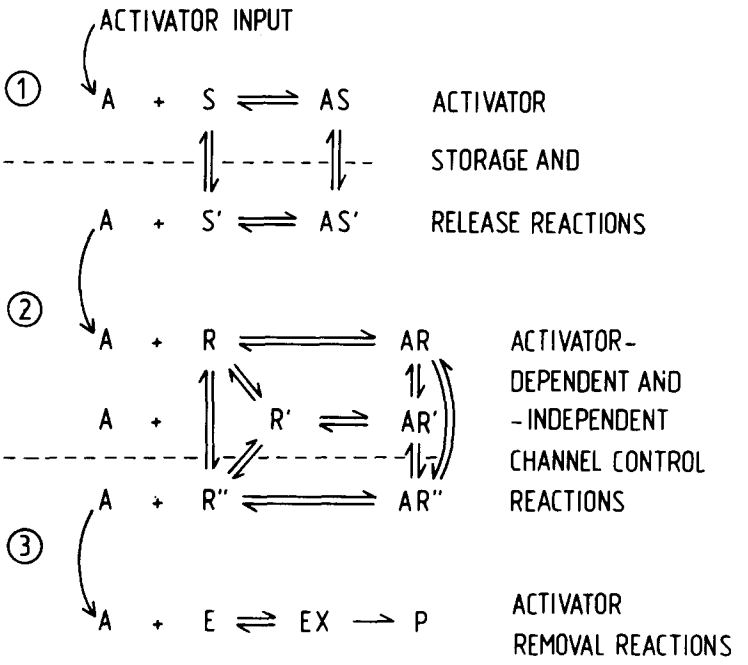


Fig. 8. Overall chemical gating model for the axonal Na^+ channel, showing an essentially sequential processing of activator through three reaction spaces during a maintained d polarization. R is the activatable-closed state, R' and AR' are activated-conducting states, and R" and AR" correspond to inactivated-closed states. During a normal action potential the reactions in the micro-reaction space 3 appear uninvolved, providing minimum dissipation for the activator (Dorogi and Neumann, 1980)

axonal permeability changes to be regulated energetically more conservatively. Axonal K^+ channels with delayed activation kinetics would serve to reverse the increase in membrane permeability to Na^+-ions with a minimum of chemical dissipation (Dorogi and Neumann, 1980).

In summary, an AcCh-mediated or fundamentally similar, chemical system is proposed as a plausible candidate for the regulation of axonal permeability changes leading to the action potential. Thus rapid bioelectrical signals based on transient permeability changes in

axonal and synaptic parts of excitable biomembranes appear to be specialized cases of a more general chemically dissipative control principle involving activator-receptor interactions and structural metastability for the activated, ion conducting state.

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