The reactions of acetylcholine with receptor and esterase and bioelectric signal generation

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The interactions of the neuroactivator acetylcholine (AcCh) and the two proteins acetylcholine receptor (AcChR) and acetylcholinesterase (AcChE, EC 3.1.1.7) are the basis of the electric signal generation in many nerve and muscle cells. The most elemental electric phenomenon resulting from the AcCh action is the spontaneous miniature end plate current (mepc) of synapses, carried by Na^+-inflow and K^+-outflow.

Among the basic conclusions which may be derived from electrophysiological data for the AcCh gating system are:

(i) The closure phase (decay part) of a mepc is apparently independent of AcCh; it is rate-limited by the return of the AcChR-channel to the closed conformation. This requires that AcCh, once dissociated from the AcChR, is much faster removed than it can return. The effective association of AcCh with the esterase (and hydrolysis) should therefore be faster than with the receptor, or in terms of the effective association rate constants: $k_1(E,eff) > k_1(R,eff)$.

(ii) In a normal mepc the AcChRs are not saturated. The amount of AcCh that is initially available for AcChR appears to be not fully accessible for esterase decomposition: rather, the approach of AcCh to the receptors seems to be separated by a 'partial diffusion barrier' from a microreaction space where both receptor and esterase fully compete for AcCh. The basal structures of the synaptic gap are a candidate for local spatial anisotropies.

The interpretation of the electrophysiological data is supported by the results of recent relaxation kinetic studies with isolated receptors and esterases from electric fish.

AcChE active sites bind cationic ligands extremely rapidly; the bimolecular rate constants at zero ionic strength are about $10^{10}\ M^{-1}s^{-1}$. The analysis of the unusually strong ionic strength dependencies of association rate constants and of catalytic parameters reveals that at least six to seven monovalent anionic groups are effective in trapping cationic substrates like AcCh. It thus appears that the dominantly anionic surface structure of this enzyme is a powerful electrostatic sink for an extremely rapid binding of the AcCh cation.

The smallest AcChR protein which can be isolated under gentle experimental conditions minimizing chemical modifications is the so-called H(heavy)-form (500,000 d). It is a dimer of two probably not identical L(light)-forms (250,000 d) linked to each other by disulfid bonds. As outlined previously the two types of L-forms are candidates for two separate channels for Na^+-inflow and K^+-outflow, both cooperatively controlled by the binding of at least two AcCh. AcChR, functionally stable only in lipid environment, binds large amounts of Ca^{2+}, associated with high (μM) and low (mM) affinity binding sites. The competition of Ca^{2+} with other cations has been used to

estimate some thermodynamic and kinetic constants of the AcCh and Ca^{2+} binding. The association rate constant for the AcCh binding with a life-time of $1/k_{-1}(R)$ = 7ms is $k_1(R)$ = 2.4×10^7 $M^{-1}s^{-1}$, a value which is of the same order of magnitude as that derived from electrophysiological current relaxations.

To the extent to which data on isolated proteins can be used to extrapolate to the cellular level, it is very tempting to compare $k_1(R) = 2.4 \times 10^7$ $M^{-1}s^{-1}$ with $k_1(E) > 2 \times 10^8$ $M^{-1}s^{-1}$, probably 10^9 $M^{-1}s^{-1}$. The number values are in line with the inequality $k_1(E,eff) > k_1(R,eff)$ derivable from electrophysiological data. Thus, the results of the isolated proteins would support and physically rationalize the conclusions from cellular data.

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