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Invited review

Agonist activation of a nicotinic acetylcholine receptor

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ABSTRACT

How does an agonist activate a receptor? In this article I consider the activation process in muscle nicotinic acetylcholine receptors (AChRs), a prototype for understanding the energetics of binding and gating in other ligand-gated ion channels. Just as movements that generate gating currents activate voltage-gated ion channels, movements at binding sites that generate an increase in affinity for the agonist activate ligand-gated ion channels. The main topics are: i) the schemes and intermediate states of AChR activation, ii) the energy changes of each of the steps, iii) the sources of the energies, iv) the three kinds of AChR agonist binding site and v) the correlations between binding and gating energies. The binding process is summarized as sketches of different conformations of an agonist site. The results suggest that agonists lower the free energy of the active conformation of the protein in stages by establishing favorable, local interactions at each binding site, independently.

This article is part of the Special Issue entitled 'The Nicotinic Acetylcholine Receptor: From Molecular Biology to Cognition'.

1. Introduction

An agonist diffuses to a resting receptor and lands on a small target site. The protein changes shape and generates a cellular response. Here I discuss evidence and ideas regarding agonist activation of muscle nicotinic acetylcholine receptors (AChRs), with a focus on energy changes occur in the activation process. (For a review of structural changes see Cecchini and Changeux, this volume). Because AChRs are ion channels I will call the resting state C (for closed-channel) and the active state O (for open-channel). These symbols distinguish only structure and dynamics rather than function; an AChR can have the O shape even if something blocks the pore.

The basic view of receptor activation by an agonist (A) derives from the Henri–Michaelis–Menten kinetic scheme for enzyme function and was encoded into a simple chemical equation for AChRs in 1957 (Del Castillo and Katz, 1957):

$$A + C \leftrightarrow AC \leftrightarrow AO \qquad \text{Scheme 1}$$

Although all nicotinic AChRs have at least two agonist sites, here we imagine a receptor with just one. In Scheme 1 the first step is called 'binding', which is the formation of a ligand–protein complex and the second step is called 'gating', which is the resting ↔ active isomerization of the system. Just as substrate molecule S makes a stable ES complex before catalysis, agonist molecule A makes a stable AC complex separately from the global conformational change. The intermediate ES/AC state is short-lived and was not detected directly until ~30 years after its proposal, in an enzyme in 1943 (Chance, 1943) and in AChRs in 1985 (Colquhoun and Sakmann, 1985). Below I will add states to Scheme 1, even if some have not been detected directly.

In addition to C and O, AChRs adopt stable desensitized (D) states in which the agonist is bound with high affinity but the ion channel is shut. In single-channel recordings of muscle AChRs, sojourns in D states are distinguished from those in C by virtue of their longer lifetimes (Fig. 1A) (Sakmann et al., 1980; Elenes and Auerbach, 2002; Auerbach and Akk, 1998; Katz and Thesleff, 1957). In muscle AChRs desensitization is complex and proceeds mainly from O states, but recovery can also be directly to C. The schemes and models considered here pertain only to binding and gating, with desensitization omitted.

2. Energy from the agonist

Scheme 1 (extended to two sites, for muscle AChRs) describes most of what happens in physiological conditions. However, on rare occasions wild-type (WT) receptors in muscle cells undergo spontaneous C ↔ O conversions in the absence of agonists (Jackson, 1986) and agonists dissociate from the AO conformation (Grosman and Auerbach, 2001). Although these events are infrequent, they demand a cyclic activation scheme that has, in addition

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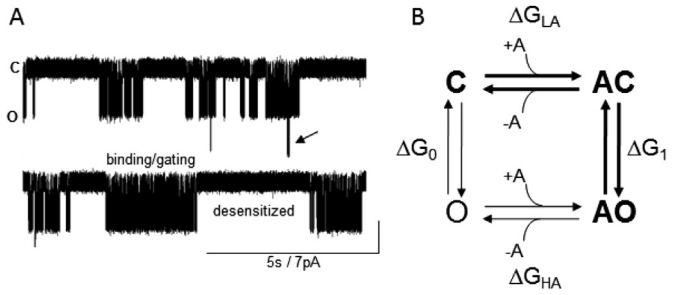

Fig. 1. Currents and states. A. Single-channel currents from muscle AChRs. Clusters of openings arise from binding and gating events (Scheme 1), and silent periods between clusters are sojourns in desensitized states. Arrow, 2 AChRs open at the same time. B. Cyclic model for activation of a receptor having 1 agonist site. Scheme 1 is bold. ΔG, free energy difference between states; subscripts are LA (low affinity), HA (high affinity), *n* (number of bound agonists). The free energy difference between C and AO is the same regardless of the connecting route: $\Delta G_{LA} + \Delta G_1 = \Delta G_0 + \Delta G_{HA}$. The free energy from the affinity change is $\Delta G_{B1} = \Delta G_{HA} - \Delta G_{LA}$, so $\Delta G_1 = \Delta G_0 + \Delta G_{B1}$. Agonists increase the probability that a receptor is active because the O state has the higher affinity.

to Scheme 1, the activation pathway C ↔ O ↔ AO (Fig. 1B). This thermodynamic cycle is sometimes called MWC, after those who first applied it to an allosteric protein (Monod et al., 1965; Karlin, 1967). In WT muscle AChRs the anti-clockwise activation path can be ignored when considering a concentration–response curve or a synaptic current. However, measuring the equilibrium and rate constants for this route is essential for understanding receptor activation mechanisms.

A ligand binds to the C state with a relatively low affinity (LA): ~150 μM for ACh and adult-type mouse AChRs (Chakrapani et al., 2004). Importantly, for ligands that are agonists, the binding site affinity increases when the protein switches from C to O. A diffusing ligand delivers little more force than from a bump of a water molecule, so it cannot 'kick' the receptor into action. Rather, an agonist molecule floats onto its binding site as a small, side-chain-sized, reversible structural perturbation that increases the probability of a global C-to-O isomerization that occurs by thermal energy alone.

The key point is that an agonist at a binding site increases the receptor's probability of being active (P_O) simply because the O conformation of the binding site has a higher affinity for the ligand compared to the C conformation. When AC changes *spontaneously* to AO, favorable (negative) energy is generated from new, local interactions between the protein, the agonist molecule and water. These serve to increase the relative stability of the active form of the receptor (a ground state effect). The affinity change of a ligand-gated ion channel (LGIC) is analogous to the gating current of a voltage-gated ion channel (VGIC). Just as the movement of an S4 following depolarization stabilizes the Open ground state of a VGIC because of a more favorable disposition of charged groups, the movement of loops at an agonist binding site following the arrival of a ligand stabilizes the Open ground state of a LGIC by virtue of the higher agonist affinity.

The AC ↔ AO isomerization involves many different rearrangements throughout the protein that almost certainly do not occur at the exact same instant. The AChR binding sites appear to switch from low-to-high affinity early in the global transition, before the rearrangement of the conductance-regulating gate region (Grosman et al., 2000; Purohit et al., 2013). Accordingly, an extra state (bold) can be inserted into the gating step of Scheme 1, to represent an AChR that has undergone the affinity switch but has not yet opened its pore:

$$A + C \leftrightarrow AC \leftrightarrow \mathbf{AC''} \leftrightarrow AO'' \qquad \text{Scheme 2}$$

The ″ superscript indicates a high-affinity (HA) binding site, so **AC**″ represents a receptor with a HA site but an overall C shape. This state (along with others) has been detected indirectly as part of the gating transition state (Grosman et al., 2000; Auerbach, 2005). In addition, brief closures in frog AChR and glycine receptor single-channel currents have been interpreted as reflecting an intermediate gating state in which the binding site has undergone a rearrangement and the gate region is shut (Lape et al., 2008; Auerbach, 1993). The short-lived, **AC**″ state in Scheme 2 may have been detected directly.

It is possible to estimate the free energy arising from the LA ↔ HA affinity change for the neurotransmitter, ACh. The starting point is to estimate the energy change with ACh at the binding sites, which is relatively easy. The free energy change in a chemical reaction is proportional to the log of the equilibrium constant. In the units kcal/mol, $\Delta G_n^{ACh} = -0.59 \ln E_n^{ACh}$ (at 23 °C), where E_n^{ACh} is the full, C ↔ O gating equilibrium constant with *n* bound ACh molecules. In adult-type mouse muscle AChRs expressed in HEK cells and at −100 mV, $E_2^{ACh} \approx 25$, so $\Delta G_2^{ACh} \approx -1.9$ kcal/mol (hereafter just kcal). (The methods we use for estimating rate and equilibrium constants from single channel electrophysiology are described elsewhere (Jadey et al., 2011)).

ΔG_2^{ACh} is the result of adding favorable free energy from the affinity change at 2 agonist sites to the unfavorable free energy of the intrinsic, C ↔ O isomerization (when only water occupies the binding pockets). Just as you need to know your ending *and* beginning bank balance to learn the deposit, you must know the O vs C energy difference in both in the presence *and* absence of agonists to know how much of the total free energy came from the affinity change.

In adult-type AChRs (at −100 mV) the unliganded gating equilibrium constant is $E_0 \approx 7.4 \times 10^{-7}$, or $\Delta G_0^{WT} \approx +8.3$ kcal (Nayak et al., 2012). From the cycle, $\Delta G_2 = \Delta G_0 + \Delta G_{B2}^{ACh}$ (Eq. 1; Fig. 1), so we simply subtract ΔG_0^{WT} from ΔG_2^{ACh} to learn that in this receptor, the affinity change for 2 neurotransmitter molecules contributes $\Delta G_{B2}^{ACh} = -10.2$ kcal towards increasing P_O. The large, uphill energy gap between unliganded C and O becomes downhill because of the energy deposit arising from new, favorable interactions at two agonist sites with ACh molecules. Similar measurements with fetal-type AChRs, in which a γ subunit replaces ε, show that $\Delta G_{B2}^{ACh} = -12.2$ kcal (Nayak and Auerbach, 2013).

The next step is to determine how this total energy is divided between the two agonist sites. The muscle AChR's agonist sites lie at different subunit interfaces, αδ and either αε in adult or αγ in fetal (Fig. 2). The net binding energy from the affinity change at each agonist site is $\Delta G_{B1} = \Delta G_{HA} - \Delta G_{LA}$ (Fig. 1). This energy can be measured independently for each type of site by studying AChRs in which one site has been knocked out by a mutation (Jha and Auerbach, 2010; Gupta et al., 2013). The result is that in adult AChRs the αδ and αε sites are approximately equivalent energetically for ACh, each providing $\Delta G_{B1}^{ACh} \approx -5.1$ kcal. In fetal AChRs, the αδ site still provides ~−5.1 kcal, but the αγ site provides −7.2 kcal (Nayak et al.). Notice that the αδ site provides the same free energy regardless of whether there is an ε or a γ subunit present, even though these differ by ~250 amino acids.

The average ΔG_{B1} has been estimated for a number of different agonists in adult-type AChRs (Fig. 3) (Jadey et al., 2011; Bruhova et al., 2013; Jadey et al., 2013). These energies range from −5.1 kcal for ACh to −0.9 kcal for betaine, with the other 'physiological' ligands nicotine and choline falling in between. ΔG_{B1} is a quantitative index of how 'partial' an agonist is. The ~−2 kcal extra free energy from αγ allows fetal AChRs to produce a greater cell responds to low [ACh] and also to choline, an ACh precursor, breakdown product and stable component of serum. It may be that this differential sensitivity to choline is a fundamental reason for the γ to ε subunit swap that is necessary for the proper maturation and operation of the neuromuscular synapse. The structural bases for different ΔG_{B1} values are not known, but it could be that a small