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Activation of endplate nicotinic acetylcholine receptors by agonists

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ABSTRACT

The interaction of a small molecule made in one cell with a large receptor made in another is the signature event of cell signaling. Understanding the structure and energy changes associated with agonist activation is important for engineering drugs, receptors and synapses. The nicotinic acetylcholine receptor (AChR) is a ~300 kD ion channel that binds the neurotransmitter acetylcholine (ACh) and other cholinergic agonists to elicit electrical responses in the central and peripheral nervous systems. This mini-review is in two sections. First, general concepts of skeletal muscle AChR operation are discussed in terms of energy landscapes for conformational change. Second, adult vs. fetal AChRs are compared with regard to interaction energies between ACh and agonist-site side chains, measured by single-channel electrophysiology and molecular dynamics simulations. The five aromatic residues that form the core of each agonist binding site can be divided into two working groups, a triad (led by αY190) that behaves similarly at all sites and a coupled pair (led by γW55) that has a large influence on affinity only in fetal AChRs.

Each endplate AChR has 5 homologous subunits, two of α(1) and one each of β, δ, and either γ (fetal) or ε (adult). These nicotinic AChRs have only 2 functional agonist binding sites located in the extracellular domain, at αδ and either αγ or αε subunit interfaces. The receptor undergoes a reversible, global isomerization between structures called C and O. The C shape does not conduct ions and has a relatively low affinity for ACh, whereas O conducts cations and has a higher affinity. When both agonist sites are empty (filled only with water) the probability of taking on the O conformation (P_O) is low, $<10^{-6}$. When ACh molecules occupy the agonist sites the C → O opening rate constant and C ↔ O gating equilibrium constant increase dramatically. Following a pulse of ACh at the nerve-muscle synapse, the endplate current rises rapidly to reach a peak that corresponds to P_O ~0.96.

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1. Energy landscape

An energy landscape depicts the relationship between structure and function (Fig. 1). The *x*-axis represents the positions of all atoms and bonds, and the *y*-axis relates to the rate and equilibrium constants that together determine the cell response. As drawn, such landscapes are oversimplified because all structural rearrangements are projected onto a single dimension ('reaction progress'), there may be multiple pathways connecting the various structures and, undoubtedly, countless small energy barriers and wells on the surface of each trajectory. Nonetheless, the simple landscape is a useful starting point for understanding receptor operation.

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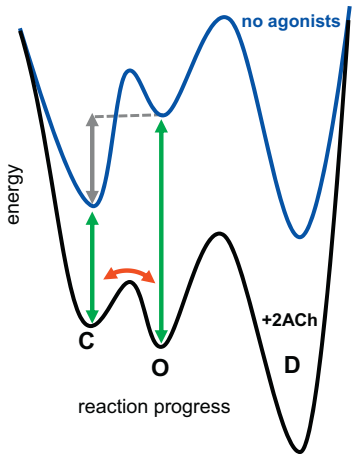

Fig. 1. Energy landscape for adult endplate AChRs.
Without agonists, C(losed)-to-O(pen) gating is uphill (+8.3 kcal/mol; gray arrow). This energy change becomes downhill with 2 ACh molecules bound to the agonist sites, because the affinity of O is higher than that of C (−10.2 kcal/mol; the *difference* between the green arrows). Red arrow, C ↔ O oscillations that give rise to clusters of single-channel openings (see Fig. 2).

In endplate AChRs the main, stable structures (energy wells) are connected in series: C(losed) ↔ O(pen) ↔ D(esensitized). This scheme, too, is oversimplified. AChRs that have desensitized appear to recover directly to C without passing through O, so a cyclic model is likely required [1]. Also, single-channel recordings show that there are multiple C, O and D states connected in a web that may be necessary to unravel in order to fully-account for the macroscopic behaviors of some nicotinic receptors [2]. However, the fundamental energy changes that undergird agonist activation of endplate AChRs can be explored by using the basic scheme shown in Fig. 1.

The lifetime of a stable state is related exponentially to the heights of the barriers that thwart escape from the well. In the absence of agonists, the O state has a lifetime that is about a million times shorter than the others (∼0.1 s vs. ∼5 min): It is a brief layover on the journey between C and D. The intrinsic, unliganded C ↔ O gating equilibrium constant (the allosteric constant) of wild-type (WT) muscle AChRs is small and constitutive openings are rare, so in whole-cell recordings without agonists it appears that the membrane current is zero. However, single-channel recordings of WT AChRs in muscle cells show that in the absence of agonists there are brief openings that occur at a low frequency [3]. These events generate a tiny cellular current that is too small to be noticed in macroscopic recordings.

The low value for the allosteric constant is a consequence of natural selection. More than a thousand mutations of muscle AChRs have been studied at the single-channel level, and most of these do nothing more than increase constitutive activity above the WT level [4,5]. For most amino acids away from the neurotransmitter binding sites, Nature has selected side chains that are locally more stable in C vs. O. For many, the free energy changes in gating occur independently and therefore sum to enforce a small allosteric constant.

Consider a mutation of an amino acid in the transmembrane domain that makes the side chain a bit more stable in O (or less stable in C) compared to the WT, to increase the allosteric constant, but without a noticeable effect on the baseline current. The agonist binding energies (green arrows in Fig. 1) remain unchanged, so this substitution has the same quantitative effect on the stability of diliganded O. Consequently, the gating equilibrium constant in the presence of the neurotransmitter will increase to the same extent as does the allosteric constant. The midpoint of the concentration-response curve will shift to the left and the maximum will P_O increase. For some natural mutations the increase in the allosteric constant changes the pharmacological profile enough to cause disease, even if the baseline current remains undetectably small in whole-cell recordings [6]. For instance, a 40-fold increase in the allosteric constant caused by the congenital myasthenic syndrome (CMS) mutation αN217K will reduce EC_{50} and slow the synaptic current decay even though it has no effect on agonist binding or, apparently, the macroscopic baseline current.

A receptor moves along the landscape under the influence *only* of thermal energy ('spontaneously'). The small value of the allosteric constant in WT AChRs implies that the free energy of the unliganded O structure is higher (more positive; less stable) than that of C, making the adoption of the O shape uncommon under physiological conditions. However, a resting, unliganded receptor is always sampling microscopic structures near C, some of which are on the pathway to O.

An ACh molecule arrives at a neurotransmitter binding site by diffusion, having no more momentum than a few water molecules. The ligand nestles into the core of the agonist site by a process that requires some local rearrangements [7]. In mouse muscle AChRs the overall equilibrium dissociation constant for ACh binding to a resting agonist site (K_d) is ∼175 μM at both αδ and αε (adult) and ∼8 μM at the fetal, αγ site. The low-affinity equilibrium constant (represented by the shorter green arrow in Fig. 1) is a function of both diffusion and the local rearrangements.

The protein continuously samples conformational space randomly, but the energy profile changes when ACh is present. Instead of the exit path from C being steeply uphill, the route(s) connecting C with O becomes more favorable. Without an agonist, the uphill gating profile and thermal energy fluctuations usually result in the system rolling back down to the bottom of the C energy well. With ACh the landscape is less uphill, allowing the system to reach the CO transition state more readily. Agonists enable receptors to take on the active conformation with a higher rate and probability because microstructures along the activation pathway as well as the active, O structure are stabilized.

A bound ligand can be thought of as a reversible 'mutation' of the agonist site. Just as covalent amino acid substitutions can increase the relative stability of O, agonists are (transient) perturbations that tilt the energy landscape downward to favor conformational change. As a consequence, both the opening rate constant and P_O are larger when there is an ACh molecule in an agonist site. Agonists increase receptor activation because they foster the crossing of a pre-existing energy landscape that is sampled spontaneously.

It is possible to estimate the amount of favorable binding energy each ACh molecule provides [8–10]. Another way to describe the above scenario of agonist action is to say that the O state has a higher affinity for ACh than the C state (in Fig. 1, the green arrow connecting the O states is the longer). The affinity *difference*, O vs. C, is the amount of favorable free energy provided by the ligand to tilt the energy and stabilize the O structure. It is difficult to estimate the equilibrium dissociation constant of ACh to O (J_d) in WT AChRs directly, but several approaches yield estimates of ∼30 nM at αδ and αε and 0.05 nM at αγ.

The low/high affinity ratio (K_d/J_d) is called the coupling constant, and its logarithm is proportional to the extra, stabilizing free energy provided by the agonist (the difference between the green arrows). At each adult mouse muscle AChR agonist site this ratio is ∼5700, so the favorable energy from each ACh molecule is (at 23 °C) −0.59ln(5700) = −5.1 kcal/mol (a 1 kcal/mol increment translates to a 5.5-fold change in equilibrium constant). In adult AChRs two ACh molecules stabilize O by ∼−10.2 kcal/mol, which increases the diliganded gating equilibrium constant by a factor of