Defining the determinants of nicotine selectivity

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Nicotine, with its simple structure connecting pyridine and methylpyrrolidine rings, acts with a high degree of selectivity on an extensive variety of nicotinic subtypes of cholinergic receptors (nAChR) in the peripheral and central nervous systems of humans (1). The addictive properties of nicotine, initiated by frequent bolus dosing through inhalation of tobacco smoke, result in chronic cardiovascular and pulmonary diseases that shorten life expectancy. Tobacco use is the most common cause of preventable death in the Western world, with increasing global consequences.

Cloning and identification of the many nAChR subtypes have led to a far more detailed understanding of the sites and activity of these receptors in the peripheral and central nervous systems (2, 3). Detailed electrophysiology measuring single-channel events of individual cloned receptors as well as the elucidation of the structure of an acetylcholine-binding protein (AChBP) from mollusks (4), and more recently, proton-gated channels from various bacterial species (5, 6) have yielded mechanistic and comparative structural information on pentameric ligand-gated ion channels at molecular and atomic levels (Fig. 1). In particular, AChBP provides an accurate structural representation of a single state of the extracellular domain of the receptor, whereas the proton-gated channel is beginning to yield information on channel-gating characteristics.

Apart from elucidating the mechanism and sites of action of nicotine, the characterization of nicotinic receptors is important for understanding signaling pathways within the brain and periphery and for the development of selective pharmacologic agents that may prove therapeutically important in various CNS disorders such as schizophrenia, parkinsonism, and dementias of the Alzheimer type. Moreover, nAChRs are members of a larger family of pentameric ligand-gated ion channels that also encompass the serotonin (5HT₃) receptor and the inhibitory amino acid receptors to glycine and γ -aminobutyric acid in humans (2, 3). Finally, because nicotinic receptors in the periphery are responsible for motor activity in mammalian and certain marine species, a large number of naturally occurring compounds are used in nature for predation or protection from predation (9). Hence, the variety of nicotinic re-

Fig. 1. Radial and topical views of overlay of the acetylcholine-binding protein in blue (7), with the nicotinic receptor from *Torpedo* sp. in gray (8). Both proteins are pentameric assemblies of subunits, where the AChBP is truncated at the end of the extracellular domain (residue ~210), therein lacking the four transmembrane-spanning regions and a large cytoplasmic domain. Both structures are pentameric where the agonist and antagonist bind at the five subunit interfaces at the radial perimeter (modified from ref. 7).

ceptor subtypes, composed of homomeric and heteromeric associations of five subunits, and an array of alkaloids, peptides, macrocyclic imines, and terpenes interacting with nAChRs provide a rich base for structure–activity analyses.

The study by Blum et al. (1) in PNAS combines nonsense suppression techniques to insert unnatural amino acids in the recombinant protein and detailed electrophysiologic measurements to analyze the determinants of specificity for nicotine and its congeners. Previously, the Dougherty

and Lester groups (10–12) teamed up in an extensive series of experiments to establish the overall importance the cation– π interaction in stabilizing quaternary nicotinic ligands in their interfacial binding site. Aromatic side chains at both the principal and complementary subunit faces form a nest or cage outlining the binding surface. The strength of the approach stems from the structural analysis being done in solution, rather than a crystalline phase. Importantly, the energetics of the interaction can be quantitated and correlated with physical parameters intrinsic to both the ligand and receptor. Critical to these considerations for the arrangement of a nest of multiple aromatic side chains around the quaternary ammonium ion is a conserved tryptophan in the principal subunit face (10–12). The backbone carbonyl oxygen of this residue (Trp B) serves as a hydrogen bond acceptor for protonated amines and imines that serve as donors. Although the proton is not visualized in the crystal structure, the distance between the amine nitrogen and carbonyl oxygen is consistent with a hydrogen bond (12–14). Also, difference spectroscopy and the pH dependence of ligand association are consistent with the protonated form being in the bound state (15). Although an aromatic nest can be defined in active center gorge of acetylcholinesterase (16), the hydrogen-bonding interaction seems unique to the nicotinic receptor.

The study of Blum et al. (1) focuses on the stabilization of the pyridine in nicotine, where the unprotonated nitrogen is a hydrogen-bond acceptor for stabilizing this ring. It seems that a network of H₂O molecules is stabilized and connects to solvent in the vestibule. In turn, polar side chains in the receptor and AChBP are involved. Here again, Blum et al. (1) use an ingenious approach, where esters are substituted for amides in the protein backbone, the ester oxygen cannot serve as a hydrogen-bond donor, and the backbone carbonyl oxygen is a far weaker acceptor. The study uses mutant cycle analysis to ascertain the linkage energy using both the ester and nonpyridine (benzene) ring. Their findings illustrate the power of the

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mutation-suppression techniques, because they use protein-backbone modifications and are not simply limited to known amino acid side-chain substitutions to analyze interaction energies obtained from mutant cycle analysis.

Overall, this cornerstone study, when melded with others in the literature, reveals that a variety of sophisticated techniques and systems are required for the proper analysis of ligand interactions with target molecules, and all of these

techniques should weigh in for drug discovery considerations. As the study by Blum et al. (1) points out, AChBP lacks the complement of conformational changes associated with ligand binding and receptor activation. For example, binding measurements with the AChBP show ligand specificities and ligand-binding rates characteristic of the receptor but do not reveal the cooperativity of ligand binding that is seen for multiple-state receptor binding and activation elicited by agonists

(17). Also, solution-based techniques that measure ligand binding and activation examine the receptor in a native environment and potentially are not constrained by crystallization in a solid phase dictated by symmetry-related molecules and unit crystal parameters. Finally, the mutagenesis analysis presented enables one to use physical parameters to correlate with structural changes that influence the energetics of ligand occupation and activation.

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