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Potential applications of nicotinic ligands in the laboratory and clinic

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Abstract—The nicotinic acetylcholine receptor (nAChR) is a receptor, ion channel complex composed of five polypeptide subunits. There are many different nAChR subtypes constructed from a variety of different subunit combinations. This structural diversity contributes to the varied roles of nAChRs in the peripheral and central nervous system, and this diversity offers an excellent opportunity for chemists who are producing ligands. Subunit specific ligands could have wide and varied effects in the laboratory as experimental tools and in the clinic as therapeutic agents. Because presynaptic nAChRs have been shown to enhance the release of many neurotransmitters, new nicotinic ligands that potentiate nAChR activity would be very useful. Such ligands could enhance the release of various neurotransmitters during degenerative diseases that cause neurotransmitter systems to decrease their output. For example, boosting the release from cholinergic neurons would help patients with Alzheimer's disease, and boosting the release from dopaminergic neurons would help patients with Parkinson's disease.

Nicotinic acetylcholine receptors (nAChRs) are members of a superfamily of ligand-gated ion channels that includes glycine, $GABA_A$, and $5\text{-}HT_3$ receptors. The nAChR macromolecule is a ligand-receptor, ion-channel complex that is composed of five polypeptide subunits assembled like the staves of a barrel around a central water-filled pore.[1–12] The receptor has three main functional states. In the resting state, the receptor is non-conducting because the ion channel is closed. Binding (usually 2) agonist molecules stabilizes the open state of the receptor for several milliseconds, allowing permeant cations to pass through the membrane via the water-filled pore. In the desensitized state, the ion channel is closed, and the receptor is refractory to agonist. These three basic functional states (closed, open, and desensitized) do not account for the complete kinetic behavior of nAChRs. Particularly desensitization can encompass more than one time constant, having both short and longer lasting substates.[13,14] The overall process is a dynamic one. At each moment the population of nAChRs will distribute among the many possible conformational states depending on the differences in free energy among those states. The binding of agonists or allosteric modulators will shift that distribution. The rate of activation, the amplitude of the ionic current, the rates of desensitization and recovery from desensitization, the pharmacology, and the regulatory controls depend on the subunit composition of the nAChRs. In addition, other factors influence function, including temperature, voltage, post-translational modifications, various ligands, ionic milieu, and cytoskeletal interactions. Therefore, in vivo there is a range of kinetic behaviors available to nAChRs even after considering their particular subunit composition.

The various subunits that compose nAChRs have sequence homology, share a general linear structure, and have similar topologies. Each subunit is one continuous polypeptide chain, with the transmembrane segments connected by alternating intracellular and extracellular loops. The amino and carboxyl termini are located extracellularly. The subunits have four hydrophobic transmembrane domains, which are referred to as M1–M4. The M2 segment from each subunit provides the main lining of the ionic pore, while M1, M3, and M4 serve to separate the pore-lining region from the hydrophobic membrane.[15]

The most well characterized nAChRs are those found at the mammalian neuromuscular junction. Muscle nAChRs have been more extensively studied because

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they are more accessible at peripheral synapses, and they mediate easily measured fast synaptic transmission. In addition, muscle nAChRs share many similarities with nAChRs found in the *Torpedo* electric ray, which provides an extremely rich source of nAChRs. The muscle nAChR is composed of two α1 subunits and one each of β1, δ, and either γ or ε. During development of the neuromuscular junctions, the γ subunit (embryonic-form) is present; but after synaptogenesis is complete, the ε subunit (adult-form) replaces it.

Neuronal nAChRs are much more diverse than muscle nAChRs because many more subunit combinations are possible (Fig. 1). Cloned neuronal subunits that have homology to the muscle α1 subunit are α2–α10, and cloned neuronal non-α subunits are β2–β4 (Fig. 1A). Many neuronal nAChRs are formed by αβ combinations, with the most common being α4β2 (Fig. 1B). Homo-oligomeric nAChRs can be formed only by α7, α8, or α9 subunits (Fig. 1C), but only α7 is widely distributed in the mammalian central nervous system (CNS). There also are more complex nAChR subtypes, where the combinations include more than 1 or 2 different subunits (e.g., Fig. 1D).

All this diversity offers an excellent opportunity for chemists who are producing nicotinic ligands. Subunit specific ligands could have wide and varied effects in the laboratory as experimental tools and in the clinic as therapeutic agents. Newly produced subunit-selective nicotinic ligands would be immediately valuable as research tools in the laboratory. Highly specific ligands would be extremely useful for dissecting the contributions of specific nAChR subtypes during cellular and synaptic physiologic studies. Many such experiments now rely on less than ideal ligands or rely on genetically engineered mice to eliminate the contribution of a particular subunit by preventing its expression. The 'knockout' mice are irreplaceable for many studies, particularly developmental, behavioral, and systems-level investigations. Synaptic studies, however, are more rigorous when specific ligands can be used to obtained a control response, a test response with the ligand, and a recovery response following a wash to remove the ligand. All three of these responses can be determined on one cellular preparation using specific ligands, but using mutant mice requires comparing results from different genotypes across littermates of normal and knockout mice. Therefore, a set of specific nicotinic ligands would be immediately useful for cellular and synaptic functional studies.

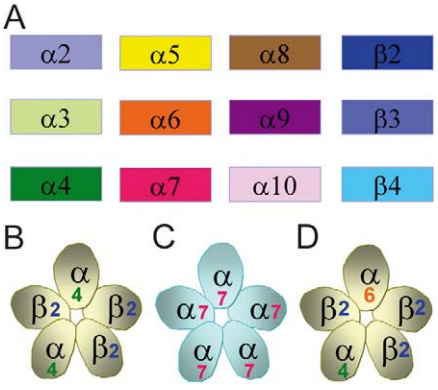

Figure 1. The diversity of neuronal nAChRs arises from the many possible subunit combinations that form the receptors. A. The 12 cloned neuronal nAChR subunits are represented in different colors. B. Many nAChRs are constructed from an α and a β subunit, with the most common being the α4β2 nAChR. C. The α7 subunit forms the most common homo-oligomeric nAChR in the mammalian brain. D. More complex subunit combinations are possible, with more than one α and/or more than one β subunit combining to form the nAChR. This example, α4α6β2, is a common nAChR in the midbrain dopamine areas and in the target fields of those midbrain neurons.

Selective nicotinic ligands also would be extremely important for society because nAChRs have been implicated in devastating diseases, such as various forms of dementia, Parkinson's disease, sleep disorders, Tourette's syndrome, nicotine addiction, schizophrenia, and epilepsy.[3–7,10] Specific nicotinic ligands could be directed toward specific health problems because the nAChR subunits are not uniformly distributed in the brain.[16–19] For example, α4 and α7 are widely distributed, but α4 has a particularly high density in the thalamus while α7 is densest in the hippocampus. A careful analysis of the distributions of the specific subunits would suggest what kinds of neuronal processing would be influenced by particular subunit-specific ligands. The cognitive processing associated with the cerebral cortex would more likely be influenced by ligands to α4, α7, or β2 because they are widely distributed and richly expressed in the cortex. On the other hand, α6 and β4 would not be good candidates because they are not widely distributed or highly expressed in the cortex. Attempts to treat nicotine addiction, however, might well consider α6 as a target. α6 is highly expressed in midbrain dopamine areas and their targets, and those regions have been implicated in addiction to nicotine and other drugs.[3,4,20]

Selective ligands to each of the nAChR subunits would provide an invaluable arsenal against a wide range of neurological and psychiatric diseases. A nicotinic ligand that we believe would be especially valuable is suggested by considering a major role of nAChRs in the brain. The most easily observed and most widely studied nAChR mechanism in the CNS is the enhancement of neurotransmitter release by presynaptic nAChRs;[21,22] (see refs 1–6, 9, 12). When activated, presynaptic nAChRs located on axon terminals initiate a calcium signal (both directly and indirectly) that enhances the release of every major neurotransmitter that has been studied. If ligands could be found to increase the activity of nAChRs, then we should expect enhanced neurotransmitter release. Ligands that enhanced nAChR activity would be analogous to the enhancement of $GABA_A$ receptor/channels by benzodiazepines. Evidence indicates that producing this effect on nAChRs is possible. The mutation of a channel-lining leucine to threonine produces larger and more prolonged nAChR currents.[23–25] Also, allosteric ligands, such as ivermectin, have been shown to enhance nAChRs slightly.[26,27]

The enhancement of neurotransmitter release by increased nAChR activity would be highly beneficial in

degenerative diseases. In fact, enhanced cholinergic activity is the basis for the present treatments of Alzheimer's disease. Although many neurotransmitter systems decline during Alzheimer's disease, the most characterized loss is in the cholinergic system.[28] As the disease advances, cholinergic neurons are progressively lost, and the number of nAChRs declines in the hippocampus and cortex.[10,29] The only approved treatments for mild to moderate Alzheimer's disease are acetylcholinesterase inhibitors. By decreasing the rate of ACh breakdown, the acetylcholinesterase inhibitors increase the concentration and lifetime of ACh that is released from cholinergic terminals. One such treatment, galantamine (Reminyl®), also enhances nAChRs, possibly by an allosteric mechanism.[30,31] Ligands that more potently enhanced nAChRs would have a ready patient base, and could have a rapid positive impact on human health. Ligands that potentiate nAChR also could serve other roles. For example, a nicotinic ligand that preferentially enhanced dopamine release would benefit Parkinson's disease patients, and one that boosted serotonin release might positively affect mood as an anxiolytic. The possible approaches and the potential impact are varied and extensive. It is an exciting time to be a chemist working in the nicotinic-receptor field.

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