NICOTINE MECHANISMS IN ALZHEIMER’S DISEASE

Overview of Nicotinic Receptors and Their Roles in the Central Nervous System

John A. Dani

Alzheimer’s disease is a complex disorder affecting multiple neurotransmitters. In particular, the degenerative progression is associated with loss within the cholinergic systems. It should be anticipated that both muscarinic and nicotinic mechanisms are affected as cholinergic neurons are lost. This review focuses on the basic roles of neuronal nicotinic receptors, some subtypes of which decrease during Alzheimer’s disease. Nicotinic acetylcholine receptors belong to a superfamily of ligand-gated ion channels that play key roles in synaptic transmission throughout the central nervous system. Neuronal nicotinic receptors, however, are not a single entity, but rather there are many different subtypes constructed from a variety of nicotinic subunit combinations. This structural diversity and the presynaptic, axonal, and postsynaptic locations of nicotinic receptors contribute to the varied roles these receptors play in the central nervous system. Presynaptic and preterminal nicotinic receptors enhance neurotransmitter release, and postsynaptic nicotinic receptors mediate a minor fraction of fast excitatory transmission. In addition, some nicotinic receptor subtypes have roles in synaptic plasticity and development. Nicotinic receptors are distributed to influence many neurotransmitter systems at more than one location, and the broad, but sparse, cholinergic innervation throughout the brain ensures that nicotinic acetylcholine receptors are important modulators of neuronal excitability. Biol Psychiatry 2001;49:166–174 © 2001 Society of Biological Psychiatry

Key Words: Acetylcholine, nicotine, Alzheimer’s disease, tobacco, addiction, plasticity

Introduction

Nicotinic acetylcholine receptors (nAChRs) belong to the superfamily of ligand-gated ion channels that includes γ-aminobutyric acid A (GABA A ), glycine, and serotonin 3 (5-HT 3 ) receptors (Albuquerque et al 1997; Dani 2000; Dani and Heinemann 1996; Dani and Mayer 1995; Jones et al 1999; Lena and Changeux 1998; Lindstrom 1997; Lindstrom et al 1996; Luetje et al 1990; McGehee and Role 1995; Role and Berg 1996; Sargent 1993; Wonnacott 1997). Agonists, such as endogenous acetylcholine or exogenous nicotine, stabilize the open conformation of the nAChR channel, which transiently permeates cations before closing back to a resting state or to a desensitized state that is unresponsive to agonists.

Multiple Subunits Produce Nicotinic Receptor Diversity

The structure of the nicotinic receptor–channel complex arises from five polypeptide subunits assembled like staves of a barrel around a central water-filled pore (Cooper et al 1991). Although various subunit combinations can produce many different nAChR subtypes, the nicotinic receptor/channel family can be separated into three general functional classes that are consistent with their evolutionary development and their pharmacologic and physiologic properties: muscle subunits (α1, β1, δ, ε, γ), which are not discussed here; standard neuronal subunits (α2–α6 and β2–β4) that form nAChRs in αβ combinations; and subunits (α7–α9) capable of forming homomeric nAChRs that are inhibited by α-bungarotoxin (Colquhoun and Patrick 1997; Le Novère and Changeux 1995; McGehee and Role 1995). In the third classification, only the α7 subunit (not α8 or α9) is widely distributed in the mammalian central nervous system (CNS). There is evidence that subunits from the separate classes may combine to form nAChRs, possibly making these groupings less than perfectly distinct (Girod et al 1999; Yu and Role 1998; Zoli et al 1998).

Heterologous expression systems have been used to determine possible combinations of nAChR subunits capable of forming active ion channels (McGehee and Role 1995; Patrick et al 1993). Those studies indicated groupings of nAChRs that are easily formed in the Xenopus oocyte expression system: α7 homomeric receptors, heterodimeric αβ nAChRs formed by a combination of α subunits (α2, α3, or α4) and β subunits (either β2 or β4), and complex receptors that include more than one type of α or β subunit (e.g., α4β2β4). This third group of complex combinations also includes nAChRs containing subunits such as α5 and β3 that do not form channels when they are expressed alone or in combination with any other single α or β subunit (e.g., α4α5β2) (Conroy and Berg 1995; Conroy et al 1992; Ramirez-Latorre et al
Expression of nAChRs in the CNS

Most nAChRs in the mammalian brain contain either α4β2 or α7 (Charpantier et al 1998; Cimino et al 1992; Clarke et al 1985; Schoepfer et al 1990; Séguela et al 1993; Wada et al 1989, 1990). Although many of the nAChRs contain α4 and β2 subunits, agonist and antagonist profiles differ for cells derived from different nuclei. For example, both medial habenula and locus coeruleus neurons express functional nAChRs with a pharmacologic profile that is consistent with α3 and β4 subunits in those regions (Mulle et al 1991). Studies indicate that there are few β2 subunits contributing to medial habenular nAChRs (Quick et al 1999). Another example is that α7-containing nAChRs mediate the predominant nicotinic current in hippocampal neurons, but other nAChR responses may be attributable to α4β2-containing, α3β4-containing, and other nAChRs (Albuquerque et al 1997; Alkondon and Albuquerque 1993; Zarei et al 1999; Zoli et al 1998). These electrophysiology results are consistent with in situ hybridization studies that indicated high expression of α7 and β2 throughout the rat hippocampus and weaker expression of α3, α4, α5, and β4 (Séguela et al 1993; Wada et al 1989, 1990; Zoli et al 1998).

Radiolabeled nicotinic ligands and in situ hybridization for nAChR messenger RNA indicate a wide, nonuniform distribution of various subunits, and nuclei often contain subgroups of nAChR subunits (Charpantier et al 1998; Cimino et al 1992; Clarke et al 1985; Lena et al 1999; Le Novère et al 1996; Schoepfer et al 1990; Séguela et al 1993; Silver et al 1998; Wada et al 1989, 1990). Although one class of nAChRs often predominates within a region, more than one class or subclass of nAChRs is often present (e.g., Pidoplichko et al 1997). In addition, individual neurons often express multiple classes of nAChRs, and even related neighboring neurons can express nicotinic responses that are significantly different (Dani et al 2000).

Basic Functions of Nicotinic Receptors

Upon binding ACh, the nAChR ion channel is stabilized in the open conformation for several milliseconds. Then the open pore of the receptor/channel closes to a resting state or closes to a desensitized state that is unresponsive to ACh or other agonists for many milliseconds or more. While open, nAChRs conduct cations, which can cause a local depolarization of the membrane and produce an intracellular ionic signal.

Although sodium and potassium carry most of the nAChR current, calcium can also make a significant contribution (Castro and Albuquerque 1995; Decker and Dani 1990; Dani and Mayer 1995; Séguela et al 1993; Vernino et al 1992, 1994). Calcium entry through nAChRs can be biologically important and is different from calcium influx mediated by voltage-gated calcium channels or by the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors. Both voltage-gated calcium channels and NMDA receptors require membrane depolarization to pass current freely. At hyperpolarized (negative) potentials, voltage-gated calcium channels generally do not open and NMDA receptors are blocked by extracellular magnesium ions. Nicotinic nAChRs do open and pass current freely at negative potentials that provide a strong voltage force driving cations into the cell. Thus, calcium currents mediated by nAChRs can have a different voltage dependence than is seen for other calcium-permeable ion channels. Also, incoming calcium has a different spatial distribution that depends on the location of nAChRs on the cell surface.

The most basic conformational states of nAChRs are the closed state at rest, the open state, and the desensitized state. The rate at which the nicotinic receptor proceeds through the various conformational states and the
selectivity with which it conducts cations in the open state depend on many factors, including the subunit composition. Therefore, the extensive nAChR diversity has the potential to produce many different responses to endogenous or exogenous agonists. The speed of activation, the intensity of the membrane depolarization, the size of the ionic signal, the rates of desensitization and recovery from desensitization, the pharmacology, and the regulatory controls of the ACh response will all depend on the subunit composition of nAChRs as well as other local factors. To add further complexity, the three basic conformational states (rest, open, and desensitized) do not account for the actual kinetic properties of nicotinic receptors. Rather, there are multiple conformations involved in the gating. Desensitization, in particular, can encompass many time constants (Dani et al 2000; Fenster et al 1999b). Thus, there may be short- and long-lived states of desensitization. Long exposures to low concentrations of agonist will favor deeper levels of desensitization, and this situation is often the case for smokers, who maintain low concentrations of nicotine throughout the day (Benowitz et al 1989; Benwell et al 1995; Clarke 1990, 1991; Dani and Heinemann 1996; Ochoa et al 1990; Reitstetter et al 1999; Russell 1989; Wonnacott 1990).

In progressing through this section, our view of the nAChR has grown from an on-off switch regulating cationic conductance to a sophisticated allosteric macromolecule (Lena and Changeux 1993, 1998). Figure 1 represents sites on a nAChR that can alter function. Competitive antagonists and partial agonists can occupy the agonist binding sites. Open channel blockers, such as phencyclidine, can bind within the pore, and there are multiple sites for other noncompetitive inhibitors and modulators. In addition, nAChRs are regulated by several
other factors, including peptide transmitters, various protein kinases, the cytoskeleton, and calcium. Although calcium modulation can act intracellularly (as is usually the case), nAChRs can also be allosterically modulated by extracellular calcium, leading to dramatic changes in the channel opening probability (Adams and Nutter 1992; Amador and Dani 1995; Mulle et al 1992; Vernino et al 1992). This modulation occurs over the physiologic concentration range of external calcium. Therefore, high levels of neuronal activity that can diminish extracellular calcium (Heinemann et al 1990; Wiest et al 2000) could cause a negative feedback that lowers the opening probability of nAChRs.

Calcium modulation is well documented, but there is great potential for nAChR modulation by other allosteric effectors. This potential offers points of entry for therapeutic drugs, such as those that can enhance nAChR activity to assist Alzheimer’s disease (AD) patients. Presently, cholinergic activity can be enhanced in patients with AD by antiacetylcholinesterase drugs that prolong the time that ACh is present before being broken down. There is evidence, however, that some antiacetylcholinesterase drugs (such as physostigmine and galantamine) can allosterically potentiate nAChRs (Maelicke and Albuquerque 2000). Drugs that could act specifically to enhance the activity of the required nAChR subtype, while also prolonging the action of ACh, could be vital in combating the debilitating effects of AD.

In addition to loss of nicotinic mechanisms, as seen in AD (Nordberg 1994; Paterson and Nordberg 2000; Perry et al 1995), there are other physiologic forms of regulation that may become disrupted during pathologic conditions (Dani, in press; Dani and Heinemann 1996; Lena and Changeux 1998; Lindstrom 1997; Perry et al 1995; Steinlein et al 1995). For example, during chronic tobacco use, low concentrations of nicotine cause certain neuronal nAChRs to increase in number and to accumulate in deep states of desensitization (Fenster et al 1999c; Peng et al 1994, 1997). Chronic desensitization may uncouple regulatory mechanisms important for proper nAChR functioning and promote incorrect recycling of receptors, thereby leading to an increase in the number of receptors. Inappropriate changes in the expression of nAChRs may be important in the process of tobacco addiction.

Competing Processes of nAChR Activation and Desensitization

At a cholinergic synapse, approximately 1 mmol/L ACh is rapidly released into the cleft, immediately activating the nicotinic receptors. In a few milliseconds, the ACh is hydrolyzed by acetylcholinesterase and/or diffuses away. The delivery and removal of ACh is very rapid, and therefore desensitization is usually not thought to be important even though the desensitization process is complex. As neuronal nicotinic receptors are diverse and neuronal synapses are anatomically and compositionally varied, the role of desensitization in the brain is not well understood and remains a difficult problem. Repeatedly exposing a synapse to about 1 mmol/L ACh might normally produce little desensitization. If the synaptic stimulation is extremely high, however, even though the rates of recovery from desensitization are fast they may not allow complete recovery (Dilger and Liu 1992; Fenster et al 1999b; Franke et al 1992). Furthermore, the breakdown of ACh releases choline, which could reach locally high concentrations. Higher concentrations of choline could desensitize α7-containing nicotinic receptors (Alkondon et al 1997b; Papke et al 1996).

The physiologic role of desensitization may become especially important when considering the desensitizing levels of nicotine that bathe the brains of smokers (Clarke 1991; Dani and Heinemann 1996; Ochoa et al 1990) or when considering the effects of drugs that inhibit acetylcholinesterase (e.g., to treat patients with AD). Under those two conditions, some nAChRs are likely to desensitize, but not in a uniform manner. At extremely active cholinergic synapses, nAChRs are more susceptible to the desensitizing influence of the endogenous agonist (ACh). When high rates of cholinergic activity are occurring in conjunction with antiacetylcholinesterase or long exposures to nicotine, then the synaptic nAChRs are more susceptible to desensitization. Evidence indicates that longer exposures to agonist allow slower rates of desensitization to come into play, such that some nicotinic receptors can enter longer lasting desensitization (Lester and Dani 1994; Reitstetter et al 1999).

If desensitization comes into play (normally, pathologically, or owing to drugs), then the rate of synaptic firing will be an important determinant of how much current enters the synapse via nicotinic receptors per unit time. The highest synaptic firing rates would not necessarily produce the most effective nicotinic signal because the nAChR would desensitize more at the highest rates (Dani et al 2000). Kinetic parameters including desensitization depend on the subunit composition, and modulatory processes, such as protein kinases, influence the nAChRs subtypes differently and selectively (Fenster et al 1999a; Paradiso and Brehm 1998). Depending on dynamic modulatory influences, different nAChR subtypes might become particularly susceptible to desensitization, and the modulatory processes can vary from synapse to synapse. Thus, modulatory processes acting upon nAChRs could provide a continually varying, powerful, computational mechanism for manipulating how information is processed at synapses.

The diversity of nAChRs and their distribution are
important parameters of the nicotinic cholinergic systems. Creating drugs that discriminate nAChRs based on their subtype or location would be important for combating specific problems—for example, AD (Wang et al 2000) or forms of epilepsy (Dani 2000; Steinle & al 1995).

Main Cholinergic Projections

Before looking further into the roles of nAChRs, we need to consider some of the basic properties of the cholinergic innervation. Cholinergic systems provide diffuse innervation to practically all of the brain, but a relatively small number of cholinergic neurons innervate each neural area (Kasa 1986; Woolf 1991). Despite the sparse innervation, cholinergic activity drives or modulates a wide variety of behaviors. By initially acting on nAChRs, nicotine or nicotinic innervation can increase arousal, heighten attention, influence rapid eye movement sleep, produce states of euphoria, decrease fatigue, decrease anxiety, act centrally as an analgesic, transiently normalize sensory gating in schizophrenic patients, and influence a number of cognitive functions (Adler & al 1999; Everitt & Robbins 1997; Levin 1992; Marubio & al 1999; Rose & Levin 1991). It is thought that cholinergic systems particularly affect discriminatory processes by increasing the signal-to-noise ratio and by helping to evaluate the significance and relevance of stimuli.

Although cholinergic neurons are distributed along the axis from the spinal cord and brain stem to the basal telencephalon, there are two major cholinergic project subsystems that can be identified. One cholinergic system arises from neurons in the pedunculopontine tegmentum and the laterodorsal pontine tegmentum, providing widespread innervation mainly to the thalamus and midbrain areas and also descending innervation that reaches to the brain stem. The second major cholinergic system arises in the basal forebrain and makes broad projections mainly throughout the cortex and hippocampus. In general, a relatively few cholinergic neurons make sparse projections that reach broad areas. Thus, the activity of a rather small number of cholinergic neurons can influence relatively large neuronal structures.

Nicotinic Mechanisms in the CNS

The most widely observed synaptic role of nAChRs in the CNS is to influence neurotransmitter release. Figure 2 depicts presynaptic nAChRs, which have been found to increase the release of nearly every neurotransmitter that has been examined (Albuquerque & al 1997; Alkondon & al 1997a; Gray & al 1996; Guo & al 1998; Jones & al 1999; Li & al 1998; McGehee & al 1995; McGehee & Role 1995; Radcliffe & Dani 1998; Radcliffe & al 1999; role and Berg 1996; Wonnacott 1997). Exogenous application of nicotinic agonists can enhance and nicotinic antagonists can often diminish the release of ACh, dopamine, norepinephrine, and serotonin as well as glutamate and GABA. In many cases, the \( \alpha \)7-containing nAChRs, which are highly calcium permeable, mediate the increased release of neurotransmitter, but in other cases different nAChR subtypes are involved. In rat hippocampal slices and cultures, presynaptic \( \alpha \)7-containing nAChRs were shown to initiate a calcium influx that, consequently, enhances glutamate release from presynaptic terminals (Albuquerque & al 1997; Gray & al 1996). Intense nicotinic stimulation was able to enhance glutamate release on multiple time scales, extending from seconds to a few minutes (Radcliffe & Dani 1998). Similar enhancements of release were obtained with a higher probability at GABAergic presynaptic terminals (Radcliffe & al 1999). The forms of enhancement lasting several minutes or more require that the incoming calcium acts as a second messenger to modify glutamatergic synaptic transmission indirectly. Properly localized calcium influx mediated by nAChRs initiates enzymatic activity (such as protein kinases and phosphatases) that is known to modify glutamatergic synapses.

Figure 3 depicts nAChRs at a preterminal location, where they can also alter the release of various neurotransmitters. Particularly at GABAergic synapses, activation of preterminal nAChRs has been found to depolarize the membrane locally, leading to activation of voltage-dependent channels that directly mediate the synaptic calcium influx underlying enhanced GABA release (Alkondon & al 1997a; Lena & al 1993). The agonist-induced effect mediated by preterminal nAChRs was inhibited by tetrodotoxin, which blocks sodium channels and, thereby, prevents the regenerative voltage activation of calcium channels in the presynaptic terminal. Axonal nAChRs may also modulate transmitter release and local excitability in another way. As represented in Figure 3, strategically located nAChRs might enable an action potential to invade only a portion of the axonal arbor. By directly exciting or by shunting the progress of an action potential at a bifurcation, axonal nAChRs could initiate or alter the spread of neuronal excitation.

Figure 4 depicts direct, fast nicotinic synaptic transmission. Fast nicotinic transmission has been detected as a small excitatory input at several neuronal areas. In the hippocampus, fast nicotinic transmission onto GABAergic interneurons has been reported (Alkondon & al 1998; Frazier & al 1998; Hefft & al 1999). In developing visual cortex, nicotinic transmission can be evoked onto both glutamatergic pyramidal cells and GABAergic interneurons (Roerig & al 1997). Because nicotinic synapses have a low density, they are difficult to detect experimentally in...
brain slice preparations. Where it has been reported, fast nicotinic transmission is a minor component of the excitatory input, which is overwhelmingly glutamatergic. It is likely that nicotinic transmission is present at low densities in more neuronal areas than the few that have been presently reported. Although direct nicotinic excitation of a neuron usually does not predominate, it could influence the excitability of a group of neurons owing to the broad cholinergic projections into an area. Thus, beyond their specific roles at discrete synapses, nAChRs also modulate neuronal circuits in a broader sense.

An example of nicotinic modulation of circuit excitability was seen in the hippocampal slice (Ji and Dani 2000). While recording from CA1 pyramidal neurons, local application of nicotinic agonist onto interneurons caused both inhibition and disinhibition. Activating nAChRs on interneurons that directly innervated pyramidal neurons caused inhibition of the pyramidal neuron, and activating interneurons that innervated mainly other interneurons caused disinhibition. The disinhibition probably occurred because the nicotinic agonist activated interneurons that then inhibited other interneurons (Alkondon et al. 1999). Consequently, GABAergic inhibitory activity decreased in the area; thus, some pyramidal neurons were temporarily released from their inhibitory inputs (i.e., disinhibition).

The results in the hippocampus indicate that, owing to the broad projections by cholinergic neurons, nicotinic activity can influence not just synaptic events but also the excitability of neuronal areas (Ji 2000; Ji and Dani 2000). By influencing circuits, nAChRs may modulate the rhythmic activity in the hippocampus or in other regions. A property of hippocampal circuits is that they enter into different rhythmic oscillations. For example, theta rhythms and gamma rhythms often occur during paradoxical sleep or while awake rats explore their environments (Vanderwolf 1969). The periods of circuit activity that accompany these rhythms provide opportunities for synaptic plasticity that underlies learning and memory (Huerta and Lisman 1993). Because interneurons are important determinants of the rhythmic oscillations and they receive cholinergic afferents that can modulate the rhythms (Csicsvari et al. 1999; Dragoi et al. 1999), it seems likely that nAChRs influence the patterns of activity in the hippocampus and elsewhere.

Nicotinic AChRs also have roles during development and neuronal plasticity (Broide and Leslie 1999; Role and Berg 1996). The density of nAChRs varies during the course of development, and nAChRs can contribute to activity-dependent calcium signals. For example, presynaptic α7-containing nAChRs increased the release of glutamate preferentially onto postsynaptic NMDA receptors in the developing rat auditory cortex, but not in the mature cortex (Aramakis and Metherate 1998). By enhancing glutamate release particularly at the locations of NMDA receptors, nAChRs might help to modulate activity-dependent synaptic plasticity that is often initiated by postsynaptic NMDA receptors (Malenka and Nicoll 1999). Nicotinic regulatory, plasticity, and developmental influences are particularly important when considering the etiology of disease. Biological changes that inappropriately alter nicotinic mechanisms could immediately influence the release of many neurotransmitters and alter circuit excitability. However, nicotinic dysfunction could have long-term developmental consequences that are expressed later in life.

In summary, the tremendous diversity of nAChRs provides the flexibility necessary for them to play multiple, varied roles. Broad, sparse cholinergic projections ensure that nicotinic mechanisms modulate the neuronal excitability of relatively wide circuits. Although fast nicotinic transmission is not the predominant driving force, it can contribute excitatory input to many synapses at one time. Presynaptic and preterminal nAChRs modulate the release of all the major neurotransmitters that have been tested. During the progression of AD, cholinergic inputs degenerate and the number of nAChRs in some areas decreases (Nordberg 1994; Perry et al. 1995). Loss of nicotinic mechanisms, which modulate the gain and fidelity of synapses and modulate the excitability of circuits, are likely to contribute to the overall cognitive deficits associated with AD.

Work from this laboratory is supported by National Institutes of Health grants from the National Institute on Drug Abuse (Nos. DA09411 and DA12661) and from the National Institute of Neurological Disorders and Stroke (No. NS21229).

Aspects of the work were presented at the symposium “Nicotine Mechanisms in Alzheimer’s Disease,” March 16–18, 2000, Fajardo, Puerto Rico. The conference was sponsored by the Society of Biological Psychiatry through an unrestricted educational grant provided by Janssen Pharmaceutica LP.

References


Alkondon M, Albuquerque EX (1993): Diversity in nicotinic acetylcholine receptors in rat hippocampal neurons. I. Phar-


Wada E, McKinnon D, Heinemann S, Patrick J, Swanson LW (1990): The distribution of mRNA encoded by a new member of the neuronal nicotinic acetylcholine receptor gene family (α5) in the rat central nervous system. *Brain Res* 526:45–53.


