Nicotinic Receptor Abnormalities of Alzheimer’s Disease: Therapeutic Implications

Agneta Nordberg

The neuronal nicotinic acetylcholine receptors (nAChRs) in the brain are important for functional processes, including cognitive and memory functions. The nAChRs acting as neuromodulators in communicative processes regulated by different neurotransmitters show a relatively high abundance in the human cortex, with a laminar distribution of the nAChRs of superhigh, high, and low affinity in the human cortex. The regional pattern of messenger RNA (mRNA) for various nAChR subtypes does not strictly follow the regional distribution of nAChR ligand-binding sites in the human brain. Consistent losses of nAChRs have been measured in vitro in autopsy brain tissue of Alzheimer’s disease patients (AD), as well as in vivo by positron emission tomography (PET). Measurement of the protein content of nAChRs showed reduced levels of the α4, α3, and α7 nAChR subtypes. The finding that the α4 and α3 mRNA levels were not changed in AD brains suggests that the losses in high-affinity nicotinic-binding sites cannot be attributed to alterations at the transcriptional level of the α4 and α3 genes and that the causes have to be searched for at the translational and/or posttranslational level. The increased mRNA level of the α7 nAChR subtype in the hippocampus indicates that subunit-specific changes in gene expression of the α7 nAChR might be associated with AD. The PET studies reveal deficits in nAChRs as an early phenomena in AD, stressing the importance of nAChRs as a potential target for drug intervention. PET ligands measuring the α4 nAChRs are under development. Studies of the influence of β-amyloid on nAChRs in brain autopsy tissue from patients with the amyloid precursor protein 670/671 mutation have shown that there is no direct relationship between nAChR deficits and pathology. Treatment with cholinergic drugs in AD patients indicate improvement of the nAChRs in the brain, as visualized by PET. Further studies on neuroprotective mechanisms mediated via nAChR subtypes are exciting new avenues. Biol Psychiatry 2001;49:200–210 © 2001 Society of Biological Psychiatry

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Introduction

The neuronal nicotinic acetylcholine receptors (nAChRs) are transmitter-gated ion channels that belong to a superfamily of ion channels of homologous receptors including γ-aminobutyric acid, glycine, and serotonin 3 (5-HT3) (Karlin and Akabas 1995; Paterson and Nordberg 2000; Sargent 1993, 2000). The nAChRs are obvious candidates for transducing cell surface interactions, not only for acetylcholine but also for other neurotransmitters (Kaiser et al 2000; Wonnacott 1997). Experimental data suggest that the nAChRs might act as neuromodulators in communicative processes in the brain (Kaiser et al 2000; Lindström 1997; Wonnacott 1997). It is therefore of great importance to define by which mechanisms the nAChRs exert their action in the brain. The nAChRs are found to be involved in a complex range of central nervous system disorders including Alzheimer’s disease (AD), Parkinson’s disease, schizophrenia, Tourette’s syndrome, anxiety, depression, and epilepsy (Newhouse and Kelton 2000; Newhouse et al 1997; Paterson and Nordberg 2000). The exact role of nAChRs and their full potential as a therapeutic target in these diseases have yet to be clarified. Interestingly enough, a considerable body of evidence exists to suggest that the nAChRs are involved in cognitive and memory functions (Levin 2000; Newhouse and Kelton 2000; Newhouse et al 1997; Sahakian and Coull 1994).

Alzheimer’s disease is the most common form of dementia and one of the most devastating diseases of the middle aged and elderly. Although the last decade has witnessed a steadily increasing effort directed at discovery of the etiology and neuropathologic and neurochemical mechanisms involved in the disease, there is still no cure (Braak and Braak 1998; Cohen-Mansfield 2000; Fratiglioni et al 1999; Hardy 1997; Master and Beyreuther 1998; St George-Hyslop 2000). However, extensive research activities have stimulated the development of new...
treatment strategies in AD, and several drugs that improve cholinergic transmitter activity have reached clinical use (for a review, see Nordberg and Svensson 1998). The role of nAChRs in AD is discussed below, with the focus mainly on new therapeutic implications.

### nAChRs in the Human Cortex

The nAChRs are distributed in many regions of the human brain. So far the nAChR subunits α3, α4, α5, α7, β2, β3, and β4 have been cloned (Anand and Lindstrom 1990; Chini et al 1994; Fornasari et al 1990; Matter and Ballivet 2000; Raimondi et al 1991; Willoughby et al 1993). The distribution of the nAChRs and their transcripts have been mapped in vitro in the human brain, using autoradiography (Adem et al 1988, 1989; Court and Perry 1994; Sihver et al 1998b), in situ hybridization (Rubboli et al 1994; Wever et al 1994), and reverse transcription polymerase chain reaction (Hellström-Lindahl et al 1998). In vitro receptor binding studies in human autopsy brain tissue suggest that nAChRs are heterogeneous and can be rationalized to three different nAChR sites (superhigh, high, and low affinity) (Marutle et al 1998; Nordberg et al 1988; Warrman and Nordberg 1995). The distribution of high-affinity nAChRs was studied with [3H]nicotine and [3H]epibatidine as radioactive receptor ligands (Marutle et al 1998; Sihver et al 1998b). Two high-affinity nAChR sites were identified in the human cortex with [3H]epibatidine, most likely representing the α3 and α4β2 nAChR subtypes. Differences in the regional binding between [3H]nicotine and [3H]epibatidine were observed in the human brain, with a proportionally higher level of [3H]epibatidine binding sites in the thalamus and cerebellum. The difference possibly reflects a selectivity for different nAChR subtypes between the nAChR ligands, nicotine, and epibatidine—that is, a greater selectivity for [3H]epibatidine for the α3 nAChRs in the human brain (Marutle et al 1998).

The laminar distribution of nAChRs in human cortex was studied in autopsy brain tissue, using autoradiographical analysis and the nAChR ligands [3H]nicotine, [3H]epibatidine, and [3H]cytisine (Sihver et al 1998b). A general high binding was observed in layers 1, 11, and V, with particularly high levels in the layer of primary sensory motor cortex and the inferior frontal sulcus (Sihver et al 1998b). All three ligands appeared to bind to a common high-affinity binding site, which most likely represents binding to the nAChR α4 subunits. A significantly greater binding for [3H]nicotine and [3H]epibatidine than for [3H]cytisine is observed in areas of the primary motor cortex, layer 11b of the occipital cortex, and layer V of the superior temporal sulcus, most likely representing binding of an additional nAChR site. High levels of [3H]nicotine binding have been observed in layers 1 and V1 of the primary cortex, deeper layer V of the primary cortex, layer 111 of the superior temporal sulcus, and layer V1 of the parietal cortex. This suggests the presence of an additional third nAChR site in the human cortex (Sihver et al 1998b) that may be the α7 nAChR subtype.

The distribution of messenger RNA (mRNA) for different nAChR subunits has been examined in different cortical layers (Schröder et al 1995). The α4 mRNA seems to be abundant in all layers except I and IV of the frontal cortex (Schröder et al 1995). The α3 mRNA has been found to be most prominently expressed in the pyramidal neuron layers 11–V1, moderately expressed in layer 11, and minimally expressed in layer IV of the human cortex (Schröder et al 1995; Wever et al 1994). A high expression of α7 mRNA was observed in layers 11 and 111, moderate in layers V and V1, and low in layers I and IV of the human cortex (Schröder et al 1995). A significant decrease in [3H]epibatidine binding has been observed with aging in the human cortical brain regions and cerebellum (Marutle et al 1998). The levels of α4 and α7 nAChR mRNA showed a decrease with aging, whereas the levels of α3 mRNA were unchanged in the elderly brain relative to the fetal brain (Hellström-Lindahl et al 1998). Interestingly, the observed regional pattern of expression of nAChR subunit mRNA in the human brain does not directly correspond to the regional pattern of nAChR binding sites revealed by ligand-binding studies.

### nAChR Changes in AD

A consistent, significant loss of nAChRs has been observed in cortical autopsy brain tissue from AD patients relative to age-matched healthy subjects (Nordberg and Winblad 1986). More recently, we have found that the nAChR deficits in AD brains probably represent an early phenomenon in the course of the disease, which can be detected in vivo by positron emission tomography (PET) (Nordberg et al 1990, 1995, 1997). The cortical nAChR deficits significantly correlate with cognitive impairment in AD patients (Nordberg, in press; Nordberg et al 1995, 1997).

When the laminar binding distribution of [3H]nicotine, [3H]epibatidine, and [3H]cytisine was measured in AD cortical autopsy tissue, marked reductions were observed relative to control brains (Sihver et al 1999c) (Figure 1). In addition, a marked reduction in the laminar distribution of [3H]epibatidine binding was observed in control cortical tissue with aging (Figure 1). A reduction in the density of the presynaptic vesicular acetylcholine transporter binding sites measured by [3H]vesamicol was also seen in the AD cortical tissue, but the decrease in [3H]vesamicol binding was less than in nAChRs. The observation suggests that
the vesamicol binding sites may be more preserved in the existing presynaptic terminals of AD cortical tissue, thereby expressing a compensatory capacity to maintain cholinergic activity. In addition, the observation suggests that nAChRs are present on noncholinergic nerve terminals to a significant extent. Basal forebrain lesions in rats, with the selective cholinergic immunotoxin 192Ig saporin and ibotenic acid, also show the rich presence of nAChRs on noncholinergic nerve terminals (Bednar et al 1998), supporting the assumption of nAChR as a neuromodulator also in noncholinergic nerve terminals (Kaiser et al 2000).

β-Amyloid (Aβ) is also an important factor, which may initiate and promote AD (Selkoe 1999). Recently, the possible role of Aβ as a neuromodulator in the brain has stressed the possible regulatory mechanisms between Aβ and cholinergic neurotransmission and nAChRs in the brain (Auld et al 1998). We have investigated the influence of Aβ on nAChRs in autopsy brain tissue from AD patients carrying the Swedish amyloid precursor protein (APP) 670/671 mutation and in brain tissue from sporadic cases of AD (Marutle et al 1999). This mutation at codon 670/671 on the APP gene on chromosome 21 was discovered in a Swedish family, and the mutation is unique in the sense that it is the only AD mutation that has been shown to alter the APP metabolism, resulting in an overexpression of the amyloid leading to plaque formation (Mullan et al 1992). Significant reductions in the number of nAChRs were measured in cortical regions of Swedish APP 670/671 carriers (Marutle et al 1999) The reduction in nAChRs was more pronounced than in the sporadic AD cases (−37% to −57%) when compared with age-matched control subjects. The APP 670/671 carrier

Figure 1. Laminar distribution of [3H]epibatidine through the entire cortical depth of the human temporal cortex from a control and an Alzheimer’s disease (AD) patient of young age (top) and from a control and an AD patient of a higher age (bottom). The binding profiles are means ± SEMs created from three to six sections. The division of I–VI corresponds to different laminae according to the Brodmann atlas. Data are from Sihver et al (1999c).
was approximately 15 years younger than the sporadic patients. Both positive and negative correlations were observed between the number of nAChR binding sites and the number of neuronal plaques and neurofibrillary tangles, respectively, in the temporal cortex and parietal cortex of mutation carriers. This finding suggests that these processes may be closely related but not directly dependent on each other (Figure 2). Different brain regions may show different kinetics for the development of neurofibrillary tangles and neuritic plaques. We have recently characterized the nAChRs in APP 670/671 transgenic mice and found compensatory mechanisms for some of the nAChR subtypes, which correlates to behavior and pathology in the transgenic mice (Paterson et al, unpublished data).

A decrease in the protein levels of the α3 and α4 nAChR subunits was recently measured in the temporal cortex and parietal cortex of four patients (P1–P4) carrying the Swedish amyloid precursor protein (APP) 670/671 mutation. Data are from Marutle et al (1999).

Examination of the regional expression of mRNA of the nAChR α4 and α3 subunits has shown no difference in autopsy AD brain tissue in any region analyzed (Hellstro¨m-Lindahl et al 1999; Terzano et al 1998), whereas the level of the α7 mRNA was significantly higher in the hippocampus (Hellstro¨m-Lindahl et al 1999). The studies suggest that the nAChR deficits in AD brains mainly reflect posttranscriptional events (Hellstro¨m-Lindahl et al 1999; Schröder and Wever 1998). The mechanisms for changes in protein levels of nAChRs in AD are unclear. Possible factors such as amyloid peptide accumulation, hyperphosphorylation of tau protein, oxidative stress, and modification of cell membrane during the development of AD may be related to decreased protein levels of nAChRs (Farooqui et al 1995; Smith et al 1996). Interestingly enough, lipid peroxidation has been shown to decrease the number of nAChRs in PC12 cells (Guan et al 2000a).

Visualization of nAChRs in AD by PET

Significant progress has been made during recent years in developing and applying functional brain imaging techniques to allow for early diagnosis of AD and evaluation of drug treatment efficacy. Positron emission tomography might be a suitable method for functional studies of pathologic changes in the brain, not only revealing dysfunctional changes early in the course of the disease but also providing a deep insight into the functional mechanisms of new potential drug treatment strategies. A limited number of PET ligands are so far available for mapping the cholinergic system in the human brain (Table 1).

[11 C] Physostigmine, [11 C]MPA4A, and [11 C]PMP have been used in PET studies to measure acetylcholinesterase in brains of healthy volunteers (Kuhl et al 1999; Namba et al 1999; Pappata et al 1996). Positron emission tomography studies have revealed a reduced cortical acetylcholinesterase activity in AD patients (Iyo et al 1997; Kuhl et al 1999). A progressive loss of cortical acetylcholinesterase activity has been observed in AD patients with
cognitive decline (Shinotoh et al 2000). The presynaptic vesicular acetylcholine transporter vesamicol ([125I]iodobenzovesamicol) has been used in vivo as a marker of presynaptic cholinergic activity in single photon emission computed tomography (SPECT) studies (Kuhl et al 1996). Greater reduction in [125I]iodobenzovesamicol binding was observed throughout the cerebral cortex in AD patients with early onset of the disease (Kuhl et al 1996). The loss in cortical acetylcholinesterase activity was less pronounced in mildly demented AD patients relative to autopsy material and did not strictly correlate with cerebral glucose metabolism impairment (Kuhl et al 1999).

The development of methods for the synthesis of radiolabeled nicotinic ligands with short half-life [11C] has enabled the study of the binding of [11C]nicotine in both normal and AD brains by PET (Nordberg et al 1990, 1995). The use of [11C]nicotine in PET studies was reviewed by Mazière and Delforge (1995), who identified some problems with the tracer due to high nonspecific binding, rapid metabolism, and rapid washout from the brain. [11C]Nicotine has drawbacks such as rapid dissociation from the receptor ligand and strong dependence of accumulation on cerebral blood flow. Different kinetic modeling approaches have been developed to improve the analysis of the PET studies, and the dual tracer kinetic rate constant (k2*) model for [11C]nicotine compensates for some of the problems (Lundqvist et al 1998). The measurement of [11C]nicotine in the human brain in vivo by PET (k2*) agrees with the distribution of nAChRs observed in vitro radioligand binding in human brain tissue autopsy (Nordberg 2000b). A significant decrease in [11C]nicotine binding (increase in k2* value) was measured in the temporal cortex, frontal cortex, and hippocampus (Nordberg et al 1995, 1997). Selective cortical deficits in [11C]nicotine binding are often observed by PET early in the course of the AD disease (Figure 3A). A significant correlation can be observed between cognitive function and nicotinic receptor binding (k2*) (Figure 3A). The findings might be promising in that PET imaging combined with genetic testing (e.g., apolipoprotein E typing) could be used to detect subjects with a greater risk of developing AD.

The past several years have seen an expanded effort to develop PET probes for noninvasive study of nAChRs. This has also led to the search for new nAChR PET ligands. A ligand with a selectivity for the α4β2 nAChRs would be particularly preferable because the α4β2 has been recognized as the predominant subtype that is deficient in AD (for a review, see Siilver et al 2000). Several PET ligands have been synthesized, including [11C]ABT-418, [11C]MPA, and [76Br]BAP (A-85380) (Siilver et al 1998a, 1999a, 1999b) (Figure 4). [11C]MPA and [76Br]BAP (A-85380) showed a more distinct binding pattern in the rat brain relative to [11C]nicotine and [11C]ABT, as revealed by autoradiography (Figure 3B). Administration of the PET ligands to monkeys also revealed a slower binding dissociation, and higher specific binding for [11C]MPA and [76Br]BAP (A-85380) than for [11C]nicotine and [11C]ABT (Siilver et al 1999a, 1999b). The very high affinity for [76Br]A-85380, the high specific in vivo uptake seen in monkeys (Figure 3C), and thereby the possible α4β2 nAChR selectivity increase interest in using the ligand in human PET studies. The application of A-85830 analog with an [11C] label is ongoing. Positron emission tomography studies using 2-[18F]A-85380 and SPECT studies with [125I]5-A-85380 have recently been performed in monkeys (Fujita et al 2000; Siilver et al 2000). Both PET and SPECT studies reveal a high uptake of the radioligand to areas rich in α2β2 nAChR such as the thalamus (Figure 3C). Recently, several epibatidine analogs such as norchloro[18F]fluoroepibatidine (Ding et al 2000) have been developed, but it is still uncertain whether the epibatidine analogs can be applied in humans due to risk of toxicity.

Positron emission tomography studies not only allow

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### Table 1. Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) Ligands for Visualization of Cholinergic Activity in the Human Brain

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MPA, N-methyl-4-piperidylacetate; PMP, methylpiperidin-4-yl propionate; IBVM, iodobenzovesamicol; QNB, quinuclidinylbenzilate; NMPB, N-methylphenylbenzilate.
measurement of nAChRs in steady-state situation in AD, but also allow measurement of nAChRs during functional activation studies. We have recently performed studies where functional activation patterns in the brain during an episodic memory task in healthy subjects as well as in AD patients have been measured by cerebral blood flow.
changes with PET (Bäckman et al 1997, 1999). In some ongoing studies we are now measuring the changes in nAChRs in different brain regions before and during task performances such as attentional tests. This type of study will provide further insight into the regional network in the brain, where nAChRs play an important role, and how drug treatment can improve brain function.

nAChRs: Target for AD Treatment?

Transmitter replacement therapy is the mainstay treatment for AD. Cholinergic therapy is based on the assumption that low levels of acetylcholine are responsible for the cognitive decline associated with AD. Cholinesterase inhibitors including tacrine, donepezil, rivastigmine, and galantamine have in clinical studies shown palliative effects on symptoms and some trend to slow disease progression (Giacobini 2000; Nordberg and Svensson 1998; Van den Berg et al 2000). It is likely that the therapeutic benefit of cholinesterase inhibitors occurs at least in part through activation of the nAChRs, by the direct action of increased levels and/or through a direct activation of the allosteric site on the nAChR (Maelicke et al 1995, 2000).

Positron emission tomography and SPECT studies performed in AD patients under treatment with cholinergic drug therapy have shown an improvement in the cerebral blood flow and glucose metabolism (Nordberg 1999). In addition, PET studies also have revealed an improvement in nAChRs in AD patients during long-term treatment with cholinesterase inhibitors such as tacrine and NXX-066 (Nordberg 2000; Nordberg et al 1992, 1998). Since an enhanced activity of acetylcholinesterase has been measured in cerebrospinal fluid following long-term treatment with tacrine (Nordberg et al 1999), possibly as a result of an increased acetylcholinesterase gene expression, it might be an advantage to use drugs interacting with nAChRs. Nerve growth factor intraventricularly administered to AD patients for 3 months resulted in an increased \[^{11}C\]nicotine binding (Eriksdotter-Jönhagen et al 1998), whereas treatment with the 5-HT\(_3\) blocker ondansetron showed a decreased number of cortical nAChRs (Nordberg et al 1997). The few PET studies performed so far in AD patients illustrate that the nAChRs might be sensitive markers for modulatory processes induced by AD drugs.

The potential therapeutic benefit of nAChR stimulation in AD is based upon the fact that nicotine improves memory in animals, healthy subjects, and AD patients (Levin 2000; Newhouse and Kelton 2000; Newhouse et al 1997; Rusted and Warburton 1992). Activation of the nAChR modulates the release of several neurotransmitters (Kaiser et al 2000; Wonnacott 1997) that mediate important physiologic mechanisms including cognitive functions. Administration of the nicotinic antagonist mecamylamine to elderly subjects and AD patients has produced cognitive impairment (Newhouse et al 2000; Wonnacott et al 1997) that mediate important physiologic mechanisms including cognitive functions. Administration of the nicotinic antagonist mecamylamine to elderly subjects and AD patients has produced cognitive impairment (Newhouse et al 2000; Wonnacott et al 1997) that mediate important physiologic mechanisms including cognitive functions. Administration of the nicotinic antagonist mecamylamine to elderly subjects and AD patients has produced cognitive impairment (Newhouse et al 2000; Wonnacott et al 1997) that mediate important physiologic mechanisms including cognitive functions.
Cholinesterase inhibitors produce similar improvement in cognitive function in AD patients (Nordberg et al. 1998). Although epidemiologic data are somewhat conflicting about the possibility that smoking can protect against AD (Doll et al. 2000; Van Duijn et al. 1995), experimental data suggest that a neuroprotective effect against Aβ toxicity might be obtained via the nAChR (e.g., the α7 subtype) (Kihara et al. 1997; Svensson and Nordberg 1998; Zamani et al. 1997). Estrogen, which in epidemiologic studies has been shown to reduce the risk of AD (Henderson 1997), has in experimental studies in PC 12 cells shown neuroprotective effects against Aβ toxicity that are at least partly mediated by the α7 subtype nAChR (Svensson and Nordberg 1998). It is reasonable to assume that to obtain significant neuroprotective effects the drug probably must be given during a very early stage of AD, probably a presymptomatic level. There are tremendous possibilities for the development of nAChR agonists as potential therapeutic agents in AD.

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