ABSTRACT

The presence of distinct nicotinic acetylcholine receptor (nAChR) subtypes in specific central nervous system (CNS) areas offers the possibility of developing targeted therapies for diseases involving the affected brain region. Parkinson’s disease is a neurodegenerative movement disorder characterized by a progressive degeneration of the nigrostriatal system. α6-containing nAChRs (designated α6* nAChRs) have a relatively selective localization to the nigrostriatal pathway and a limited number of other CNS regions. In addition to a unique distribution, this subtype has a distinct pharmacology and specifically interacts with α-conotoxinMII, a toxin key in its identification and characterization. α6* nAChRs are also regulated in a novel manner, with a decrease in their number after nicotine treatment rather than the increase observed for α4* nAChRs. Striatal α6* receptors were functional and mediate dopamine release, suggesting that they have a presynaptic localization. This is further supported by lesion studies showing that both α6* nAChR sites and their functions are dramatically decreased with dopaminergic nerve terminal loss, in contrast to only small declines in α4* and no change in α7* receptors. Although the role of nigrostriatal α6* nAChRs is only beginning to be understood, an involvement in motor behavior is emerging. This latter observation coupled with the finding that nicotine protects against nigrostriatal damage suggest that α6* nAChRs may represent unique targets for neurodegenerative disorders linked to the nigrostriatal system such as Parkinson’s disease.

Currently available therapeutics for Parkinson’s disease, a neurodegenerative disorder characterized by rigidity, tremor, and bradykinesia, include administration of the dopamine precursor L-dopa and/or dopamine agonists (Olanow, 2004; Samii et al., 2004). These drugs partially compensate for the decline in striatal dopamine that arises because of the loss of substantia nigra dopaminergic neurons. However, long-term dopamine replacement therapy leads to motor and psychiatric complications, and there is a loss of efficacy with time, probably because of disease progression. There is therefore an urgent need for novel therapeutics for better management of Parkinson’s disease.

Acetylcholine can also regulate striatal dopamine levels by stimulating nicotinic acetylcholine receptors (nAChRs). These receptors are localized on nigrostriatal dopaminergic nerve terminals where they control dopamine release (Wonnacott, 1997; MacDermott et al., 1999; Gotti and Clementi, 2004). Since striatal nAChRs seem to be involved in both motor control and neuroprotection against nigrostriatal damage (O’Neill et al., 2002; Quik, 2004), identification of the receptor subtypes is important for understanding basal ganglia function under physiological conditions and pathological states such as Parkinson’s disease.

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Asterisks denote nAChRs containing the indicated α and/or β subunit and additional undefined subunits.

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ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; CNS, central nervous system; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.
α4, or α6 subunit, coupled together with a β2 or β4 subunit. The α5 and β3 subunits do not seem to interact with cholinergic ligands but modulate receptor function (Lukas et al., 1999; Gotti and Clementi, 2004). Because receptors composed of different subunits vary in drug sensitivities and functional characteristics, it is important to identify the subtypes present in different brain regions. Interestingly, receptors containing the α6 subunit (designated α6* nAChRs) are concentrated in the visual and catecholaminergic pathways, including the dopaminergic nigrostriatal pathway (Whiteaker et al., 2000; Gotti and Clementi, 2004; Quik, 2004), suggesting that they play a unique role in these systems.

Herein we review current findings on α6* nAChRs in the brain, with a focus on the dopaminergic nigrostriatal pathway. We first discuss the unique characteristics of the α6* nAChR subtype, exemplified by its selective interaction with the marine snail peptide α-conotoxinMII. The use of this toxin has allowed for α6* nAChR localization in the brain, which is distinct compared with other nAChR populations and seems to be primarily presynaptic in the striatum, although it may be localized to other neuronal elements in different brain regions. The combination of a unique pharmacology and distribution may allow for selective therapeutic targeting of α6* nAChRs for CNS disorders involving this subtype.

**Selective Interaction of α6* nAChRs with α-ConotoxinMII**

Although evidence for receptors containing an α6 subunit was first reported in 1990 (Lamar et al., 1990), several years elapsed before α6* receptor characteristics were described primarily because of difficulties in their expression and identification (Gerzanich et al., 1997; Fucile et al., 1998; Kuryatov et al., 2000). Functional α6* nAChRs expression was first achieved in oocytes with the chick α6 and human β4 subunits (Gerzanich et al., 1997) and later in human BOSC 23 cells with a purely avian α6β4* nAChR (Fucile et al., 1998). The chick α6 subunit also formed functional heteromeric nAChR with chick β2, although at a much lower abundance (Fucile et al., 1998). Functional expression of an α6* nAChR composed of only mammalian subunits was subsequently achieved, although not without difficulty (Kuryatov et al., 2000). Receptors composed of the human α6 and β4 subunits expressed only poorly in oocytes, whereas injection of α6 with β2 resulted in the formation of nonfunctional epitabidine binding aggregates; however, functional human α6β4β3, as well as α6β2α5 nAChRs, were observed (Kuryatov et al., 2000). Efficient expression was obtained when the N-terminal extracellular portion of α6 was joined to the remaining portions of either α3 or α4 and expressed with either the β2 or β4 subunits (Kuryatov et al., 2000; Dowell et al., 2003; Evans et al., 2003). Furthermore, the addition of the β3 subunit significantly improved expression of these chimeric α6* receptors (Dowell et al., 2003). Altogether, these data show that α6 subunits can assemble in helical expression systems with the β2 or β4, as well as the α5 and β3, subunit.

A striking pharmacological feature of α6* nAChRs is their high-affinity interaction with the naturally occurring snail toxin α-conotoxinMII (Kuryatov et al., 2000; McIntosh et al., 2004). This toxin binds to α6* nAChRs in a slowly reversible manner, which makes it a useful ligand for receptor identification and characterization. Indeed, receptor studies with [125I]α-conotoxinMII demonstrated a high-affinity (Kd, ~0.8 nM) nAChR in rodent, monkey, and human brain (Whiteaker et al., 2000; Quik et al., 2001; Quik et al., 2004). In addition, α-conotoxinMII also potently interacts with α3* nAChRs because of the high sequence homology to the α6 subunit (~75%). This presents a problem for receptor identification in tissues that contain both of these subtypes and has led to a search for compounds that can distinguish between α3* and α6* nAChRs (Table 1). One such agent is α-conotoxin PIA, a toxin from Conus purpurascens, which is ~75-fold more selective for heterologously expressed chimeric α6α3β2* versus α3α2 nAChRs (Dowell et al., 2003). α-ConotoxinMII analogs exhibited an even greater selectivity (up to 2000-fold) for α6* compared with α3* nAChRs (McIntosh et al., 2004). The IC50 values of these peptide analogs for chimeric α6β2* nAChRs in functional assays are all in the picomolar to nanomolar range and correlate well with those obtained from [125I]α-conotoxinMII competition binding assays in mouse striatum (Fig. 1) that contains α6* and not α3* nAChRs (Whiteaker et al., 2002; Champtiaux et al., 2003; McIntosh et al. 2004).

It is worth noting that α-conotoxinMII-sensitive α6* receptors also have high affinity for methyllycaconitine, a plant alkaloid historically considered selective for α7 nAChRs. Putative α6α4α5β2 nAChRs present on dopamine neurons in rat substantia nigra and ventral tegmental area are completely inhibited by 1 nM methyllycaconitine (Klink et al., 2001). Moreover, data from knockout mice suggest that these α-conotoxinMII- and methyllycaconitine-sensitive nAChRs do not contain α7 subunits (Klink et al., 2001). Methyllycaconitine is also a potent inhibitor of α-conotoxinMII-sensitive-mediated dopamine release in striatum (Mogg et al., 2002; Karadshesh et al., 2004). Thus, methyllycaconitine potently interacts with both α7 and α6* nAChRs.

**TABLE 1**

<table>
<thead>
<tr>
<th>α-Conotoxin</th>
<th>α-Conotoxin Sequence</th>
<th>Fold Preference of α6β2 &gt; α3β2</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIC</td>
<td>GCCSRPACGNQH1C</td>
<td>α6 ~ α3</td>
<td>McIntosh et al. (2002)</td>
</tr>
<tr>
<td>PIA</td>
<td>RDPCCSNPVCT9RN9C</td>
<td>~75</td>
<td>Dowell et al. (2003)</td>
</tr>
<tr>
<td>MII</td>
<td>GCCSNPVCHLHSNLC</td>
<td>~3 to 6</td>
<td>Carter et al. (1996); Dowell et al. (2003); McIntosh et al. (2004)</td>
</tr>
<tr>
<td>MII [H9A]</td>
<td>GCCSNPVCALEHSNLC</td>
<td>~75</td>
<td>McIntosh et al. (2004)</td>
</tr>
<tr>
<td>MII [L15A]</td>
<td>GCCSNPVCHLHSNAC</td>
<td>~40</td>
<td>McIntosh et al. (2004)</td>
</tr>
</tbody>
</table>
Fig. 1. α-ConotoxinMII binding in mouse brain correlates with chimeric α6/α3β2β3 rather than α3β2 nAChRs. Black bars indicate the Kᵈ of α-conotoxinMII and analogs (McIntosh et al., 2004) and α-conotoxin PIA (P. Whiteaker and J. M. McIntosh, unpublished data) determined using mouse brain homogenates. The white and gray bars indicate the IC₅₀ of block by α-conotoxins of rat chimeric α6/α3β2β3 and α3β2 nAChRs expressed in Xenopus oocytes, respectively (Dowell et al., 2003; McIntosh et al., 2004). Note that in each case the Kᵈ is to the IC₅₀ for α6/α3β2β3 but not α3β2 nAChRs.

Altogether, these results show that α6* nAChR receptor sites represent a class of neuronal nAChRs with a unique pharmacological profile. Moreover, α-conotoxinMII and related peptides from Conus seem to be excellent tools to investigate their characteristics and function.

Selective CNS Distribution of α6* nAChRs

mRNA Studies

mRNA localization studies show that the α6 nAChR transcript exhibits a very restricted distribution in rodent and monkey brain (Table 2). α6 mRNA labeling is particularly strong in catecholaminergic nuclei, including the substantia nigra, ventral tegmental area, and locus coeruleus, as well as in the medial habenula and interpeduncular nucleus, with a less intense signal in other brain nuclei (Le Novere et al., 2002). Since α-conotoxinMII interacts with α6* and α3* nAChRs in heterologous expression systems, the question arises whether the toxin binds to both of these subtypes in mammalian brain. Studies with nAChR subunit knockout mice showed that 125I-α-conotoxinMII binding was not significantly decreased in striatum of α3 (–/−) mice but was virtually eliminated in α6 (−/−) mice, suggesting that the toxin binds to only α6 nAChRs in mouse striatum (Champtiaux et al., 2002; Whiteaker et al., 2002). 125I-α-conotoxinMII binding was also abolished in other brain regions of α6 (−/−) mice but only partially reduced in the medial habenula and interpeduncular nucleus. In addition, 125I-α-conotoxinMII was partially decreased in these latter regions in α3 (−/−) mice and unchanged in striatum and other regions. Altogether, these data indicate that 125I-α-conotoxinMII binds to both α6* and α3* receptors in the medial habenula-interpeduncular pathway but only to α6* sites in striatum and other CNS regions in mouse brain. A different situation seems to exist in the primate CNS. Immunoprecipitation studies with subunit-targeted antibodies showed that there was appreciable α6 subunit–like immunoreactivity in monkey striatum, as well as a smaller α3 signal (Quik et al., 2005b). To conclude, only α6* nAChRs are detectable in rodent striatum, whereas both α6* and α3* nAChRs are localized in primate striatum.

Composition of α6* nAChRs in Striatum

To identify the subunit composition or other subunits co-expressed with α6, antibodies targeted to specific nAChR subunits have been used (Zoli et al., 2002; Champtiaux et al., 2003; Gotti et al., 2005; Quik et al., 2005b). These demonstrate abundant α6* but no detectable α3* nAChR expression in mouse striatum, consistent with results from nAChR subunit knockout mice (Zoli et al., 2002; Champtiaux et al., 2003; Gotti et al., 2005). They also show the presence of α4, α5, α7, β2, β3, and β4 but not α2 subunits in rodent striatum (Zoli et al., 2002; Champtiaux et al., 2003; Gotti et al., 2005). Similar studies using primate striatal tissue indicate there are species differences in nAChR subunit expression between monads and rodents. Both the α3 and α6 subunits were identified in monkey striatum, as well as α2, α4, α7, β2, and β3, but not the α5 and β4 subunits.

Dual-label immunoprecipitation shows that the subtypes common across species are α6α4β2β3 and α6β2β3, as well as α4β2 and α7 receptors (Fig. 2A; Table 3) (Zoli et al., 2002;
Fig. 2. Schematic representation (A) of the top view of the arrangement of putative neuronal nicotinic receptor subtypes common to rodent and monkey striatum. The closed diamond represents a recognition site with which both nicotine and α-conotoxinMII can interact, whereas the closed circle represents a site to which α-conotoxinMII does not bind. Schematic localization (B) of nAChR subtypes in the nigrostriatal pathway.

Champtiaux et al., 2003; Gotti et al., 2005; Quik et al., 2005b). In addition, there also seems to be a population of less abundant striatal nAChR subtypes that are unique to different species (Table 3). To date, these include α4α5β2 receptors on dopamine terminals in mouse but not monkey (Zoli et al., 2002; Champtiaux et al., 2003) and α3β2* receptors present in monkey but not mouse (Quik et al., 2005b). Identification of the precise mix of nAChR subtypes in striatum is important because it may allow for selective targeting with drugs that uniquely interact with these populations.

**Striatal α6 nAChR Stimulation and Dopamine Release**

Studies to evaluate the role of α6β2* nAChRs have focused on mammalian striatum because of the relatively high receptor density, the availability of assays to study their function, and putative links to addiction and neurodegenerative disorders. An approach that has proved particularly useful for studying function of striatal nAChRs is nicotine-evoked dopamine release. Stimulation of presynaptic striatal nAChRs results in dopamine release that is mediated by subtypes that are blocked by α-conotoxinMII and those that are not (Grady et al., 2002). In rodents, α-conotoxinMII-sensitive dopamine release is most likely mediated through α6β2* nAChRs and represents ~40% of the total response (Kulak et al., 1997; Kaiser et al., 1998; Salminen et al., 2004b), whereas α-conotoxinMII-resistant release occurs through α4β2* nAChRs and represents ~60% of the response (Fig. 3).

This conclusion is supported and extended by findings from nAChR knockout mice. Total nicotine-stimulated dopamine release is eliminated in β2 (−/−) mice, indicating an absolute requirement for the β2 subunit (Champtiaux et al., 2003; Salminen et al., 2004b). Evoked release is also abolished in double knockout α4 (−/−) α6 (−/−) mice and significantly affected in α4 (−/−) or α6 (−/−) mice, suggesting a mandatory presence for either the α4 or α6 subunit (Champtiaux et al., 2003; Salminen et al., 2004b). Results with α5 (−/−) and β3 (−/−) mice suggest a modulatory role for these subunits (Salminen et al., 2004b). In contrast, deletion of the β4 and α7 nAChRs had no effect on release. Since the α2 and α3 subunits are not present in mouse striatum, these combined results substantiate a role for receptors containing the α4, α5, α6, β2, and β3 nAChR subunits in nicotine-evoked dopamine release from mouse striatum (Cui et al., 2003; Salminen et al., 2004a; Gotti et al., 2005).

The use of α-conotoxinMII, coupled with nAChR knockout mice, has allowed for further identification of the receptors that mediate dopamine release. α-ConotoxinMII completely blocks nicotine-stimulated dopamine release in α4 (−/−) mice, showing that a component of release is mediated by α6* nAChRs (Salminen et al., 2004b). Conversely, α-conotoxinMII does not block residual dopamine release in α6 (−/−) mice, demonstrating an α4* nAChR-sensitive component (Champtiaux et al., 2003). Altogether, the above studies, coupled with immunoprecipitation data (Zoli et al., 2002; Champtiaux et al., 2003) and studies with α6*-selective conotoxins (McIntosh et al., 2004; Azam and McIntosh, 2005), suggest that α-conotoxinMII-sensitive sites in mice represent α6β2β3 and α6α4β2β3 subtypes, whereas α-conotoxinMII-resistant receptors are α4β2 and α4α5β2 nAChRs.

The situation in primate striatum bears resemblance and
some differences compared with rodents. In monkey striatum, the greater portion (70%) of nicotine-evoked dopamine release is mediated through α9-conotoxinMII-sensitive (6* and/or 3* subtypes, whereas α9-conotoxinMII-resistant or 4* receptors mediate only 30% release; these proportions were reversed in rodents (Fig. 3) (Kulak et al., 1997; Grady et al., 2002; McCallum et al., 2005a). The α9-conotoxinMII-sensitive release most likely occurs in response to stimulation of 6* and possibly 3* receptors in monkeys. Note the greater proportion of 6* nicotinic receptor-evoked dopamine release compared with that in response to 4* receptor activation. [125I]α-ConotoxinMII was used to label 6* nicotinic receptors, whereas 4* binding sites were determined using [125I]epibatidine in the presence of 100 nM α-conotoxinMII. These differences in function between monkeys and mice correlate with the 6* and 4* receptor levels in the two species.

### TABLE 3

<table>
<thead>
<tr>
<th>Species Comparison</th>
<th>nAChR Subtype</th>
<th>Presence in Striatum</th>
<th>Striatal Localization</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Similarities</strong></td>
<td>α6(σβ3)</td>
<td>Yes</td>
<td>Dopaminergic terminals</td>
<td>Zoli et al. (2002); Champtiaux et al. (2003); Gotti et al. (2005); Quik et al. (2005)</td>
</tr>
<tr>
<td>α9β3</td>
<td>Yes</td>
<td>Dopaminergic terminals</td>
<td>Zoli et al. (2002); Champtiaux et al. (2003); Quik et al. (2005)</td>
<td></td>
</tr>
<tr>
<td>α4β2</td>
<td>Yes</td>
<td>Dopaminergic terminals, other</td>
<td>Zoli et al. (2002); Champtiaux et al. (2003); Quik et al. (2005)</td>
<td></td>
</tr>
<tr>
<td>α7</td>
<td>Yes</td>
<td>Glutamatergic terminals, other</td>
<td>Zoli et al. (2002); Jones and Wonnacott (2004); Gotti et al. (2005); Quik et al. (2005)</td>
<td></td>
</tr>
<tr>
<td><strong>Differences</strong></td>
<td>α4β2</td>
<td>Yes</td>
<td>Dopaminergic terminals, other</td>
<td>Zoli et al. (2002); Champtiaux et al. (2003)</td>
</tr>
<tr>
<td>α3β2</td>
<td>No</td>
<td>Dopaminergic terminals, other</td>
<td>Quik et al. (2005)</td>
<td></td>
</tr>
<tr>
<td>α6β2</td>
<td>No</td>
<td>Not known</td>
<td>Quik et al. (2005)</td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 3.** Comparison of α6* and α4* nAChR-evoked dopamine release and receptor levels in monkey and mouse striatum. Nicotine-evoked [3H]dopamine release was done in the absence (total release) and presence of 50 nM α-conotoxinMII to distinguish release mediated by different nAChR subtypes. Release remaining in the presence of α-conotoxinMII, defined as α-conotoxinMII-resistant, is most likely mediated by 4* nAChRs; the difference between total and α-conotoxinMII-resistant [3H]dopamine release is defined as α-conotoxinMII-sensitive and represents release mediated by 6* nAChRs in mouse and by 6* and possibly 3* receptors in monkeys. Note the greater proportion of 6* nicotinic receptor-evoked dopamine release compared with that in response to 4* receptor activation. [125I]α-ConotoxinMII was used to label 6* nicotinic receptors, whereas 4* binding sites were determined using [125I]epibatidine in the presence of 100 nM α-conotoxinMII. These differences in function between monkeys and mice correlate with the α6* and α4* receptor levels in the two species.

α6* versus α4* nAChRs in primates than rodents. The localization and function of α6* nAChRs in the visual, habenular-interpeduncular, and other pathways remain to be investigated.

### Down-Regulation of α6* nAChRs with Long-Term Nicotine Treatment

Until fairly recently, there was a general consensus that nicotine treatment up-regulates nAChRs (Wonnacott, 1990; Gotti and Clementi, 2004). However, converging studies now suggest that this pertains primarily to 4β2* nAChRs. This is one of the most prevalent CNS subtypes, and effects on these sites may have masked changes in other populations.
Indeed, chronic nicotine administration does not alter $\alpha3\beta4^*$ nAChRs in the central or peripheral nervous system (Flores et al., 1997; Davila-Garcia et al., 2003; Nguyen et al., 2003), whereas $\alpha7$ nAChRs are generally unaffected and sometimes modestly increased across brain regions (Pauly et al., 1991, 1996). In contrast, recent studies indicate that $\alpha6^*$ nAChRs are decreased in mouse striatum after several weeks of nicotine treatment administered via drinking water or by chronic jugular infusion (Salminen et al., 2004a; Lai et al., 2005). The reduced receptor number is associated with a decline in $\alpha6^*$ nAChR-evoked $[^3H]$dopamine release, indicating that the receptor loss is of functional significance (LSalminen et al., 2004a; ai et al., 2005). Although some studies have not reported a decline in $\alpha6^*$ sites after nicotine treatment, this may relate to the route of administration, species, age, and/or method of $\alpha6^*$ receptor determination (Nguyen et al., 2003; Parker et al., 2004).

Altogether, these findings show that nicotine treatment differentially influences nAChR subtypes, with increases, decreases, or no change. This disparate regulation suggests that distinct mechanisms control receptor expression. The increase in $\alpha4\beta2^*$ sites may be due to nicotine-induced receptor desensitization that resembles an apparent receptor blockade, with a compensatory increase to ameliorate the functional loss. Nicotine may also decrease the turnover rate of already assembled nAChRs and/or act intracellularly on receptor precursors to enhance their maturation (Sallette et al., 2005). The down-regulation of $\alpha6^*$ nAChRs with chronic nicotine treatment suggests that this subtype is controlled in a fashion analogous to that for neurotransmitter receptors (Creese and Sibley, 1981; Wonnacott, 1990). This differential control by nicotine may occur through an interaction with specific residues on the $\alpha$ subunits, comparable to the regulatory microdomains identified on the $\beta2$ versus $\beta4$ subunits (Sallette et al., 2004).

Overall, the presence of multiple nAChR populations, including $\alpha64\beta2/3$, $\alpha6\beta2/3$, $\alpha4\beta2$, as well as $\alpha7$ and possibly others (Fig. 1; Table 2), provides the potential for a complex regulation by nicotine in striatum. There may thus be widely divergent nAChR-mediated functional changes in striatum after nicotine exposure in smokers or individuals on chronic nicotine therapy.

### Loss of Striatal $\alpha6^*$ nAChRs with Nigrostriatal Damage

Denervation studies are commonly used to investigate receptor localization and function. Lesion experiments involving $\alpha6^*$ nAChRs have focused on the nigrostriatal system because of the availability of toxins that selectively destroy this pathway and the relevance to neurodegenerative disorders, such as Parkinson’s disease. Initial studies using monkeys lesioned with the selective dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) showed that there was a dramatic decline in $[125I]\alpha$-conotoxinMII binding or $\alpha6^*$ nAChRs, with a smaller loss of $\alpha4^*$ nAChRs (Quik et al., 2001). The nAChRs declines correlated with those in the dopamine transporter, a marker of nerve-terminal integrity, suggesting that $\alpha6^*$ receptors are located presynaptically. Experiments to investigate the link between lesion-induced receptor declines and function showed there was a regionally selective compensation in both $\alpha6^*$ and $\alpha4^*$ receptor-mediated $[^3H]$dopamine release with nigrostriatal damage (McCallum et al., 2005a,b).

There was also a decline in nAChRs with nigrostriatal damage in rodents, but in this species there were similar losses in both $\alpha6^*$ and $\alpha4^*$ subtypes (Zoli et al., 2002; Champaiaux et al., 2003; Quik et al., 2003). Interestingly, striatal nAChR function in mice was not decreased until the dopamine transporter was reduced by $-20\%$ (Quik et al., 2003), again suggesting some form of functional adaptation.

The results of lesion studies suggest that $\alpha6^*$ nAChRs are localized to dopaminergic terminals in the striatum, with a loss of $\alpha6\alpha4\beta2/3$ and $\alpha6\beta2/3$ nAChRs after nigrostriatal damage in both primates and rodents (Fig. 2B). These latter studies also showed declines in $\alpha4\beta2^*$ nAChRs (Zoli et al., 2002; Champaiaux et al., 2003; Salminen et al., 2004b; Gotti et al., 2005; Quik et al., 2005b). These receptor losses seem to be biologically relevant, with a decline in dopamine release after nigrostriatal damage, although significant functional compensation occurred, particularly in primates. These adaptive mechanisms may be responsible, at least in part, for the observation that Parkinson’s disease symptoms only develop after $>80\%$ declines in striatal dopamine.

#### $\alpha6^*$ nAChRs in Human Brain: Declines in Parkinson’s Disease

Multiple nAChRs have been identified in human CNS, including $\alpha4^*$, $\alpha7^*$, and more recently $\alpha6^*$ subtypes (Court et al., 2000; Gotti and Clementi, 2004; Quik, 2004). These latter studies show that $[125I]\alpha$-conotoxinMII binds with high affinity ($\sim0.5\text{ nM}$) to numerous regions in human brain that include, in order of decreasing intensity of labeling, optic tract, nucleus accumbens, caudate, and putamen, consistent with results in rodents and primates (Quik, 2004; Bohr et al., 2005). In addition, $[125I]\alpha$-conotoxinMII binding sites were identified in hippocampus, globus pallidus, frontal cortex, thalamus, and cerebellum, distinct from results in rodent and nonhuman primates. This may reflect binding to $\alpha3^*$ nAChRs in human brain, although it is also possible that $\alpha6^*$ nAChRs are present in these latter regions in humans. Such an interpretation is consistent with in situ hybridization results with $\alpha3$-subunit mRNA probes, which identified the $\alpha3$ transcript in the cortex, hippocampus, and thalamus (Rubboli et al., 1994; Guan et al., 2002). In addition, studies using $\alpha3$-subunit directed antibodies show that $\alpha3$-like immunoreactivity is present in these same regions (Guan et al., 2002).

Since the nigrostriatal pathway degenerates in Parkinson’s disease, the question arose whether $\alpha6^*$ nAChRs are decreased as in experimental models. Indeed, there were 50 to 90% reductions in $[125I]\alpha$-conotoxinMII sites in Parkinson’s disease striatum, the region containing dopaminergic nerve terminals (Quik et al., 2004; Bohr et al., 2005). In human brain, this decline in $[125I]\alpha$-conotoxinMII binding did not parallel the dopamine nerve terminal loss as closely as anticipated, with greater losses of the transporter compared with nAChRs (Quik et al., 2004). These data suggest that $[125I]\alpha$-conotoxinMII sites may be located both pre- and postsynaptically in human striatum.

The composition of $[125I]\alpha$-conotoxinMII receptors, that is, whether they contain $\alpha3$ and/or $\alpha6$ subunits in human brain,
is currently not known. One study reported a decrease in a3-like immunoreactivity in Parkinson's disease striatum (Guan et al., 2002), although others found no change in a2–a7, b2, and b3 nACHR subunit immunoreactivity (Martin-Ruiz et al., 2002) despite undisputed declines in striatal nACHRs using radioligand binding studies. Thus, although there are clearly alterations in both a4* and a6*/a3 (a-conotoxinMII-sensitive) subtypes in Parkinson's disease striatum, their composition requires further study.

Functional Consequence of Striatal a6 nACHR Stimulation

nACHRs are important in a host of CNS functions, including learning, attention, addiction, reinforcement, and motor activity (Picciotto, 2003; Gotti and Clementi, 2004; Quik, 2004; Wonnacott et al., 2005). Although the specific nACHR subtypes remain to be elucidated, accumulating studies suggest a role for a4* nACHRs. In addition, the relatively dense distribution of a6* nACHRs in brain regions linked to these and other functions suggest this latter subtype may also play a role (Whiteaker et al., 2000; Quik et al., 2001; Moretti et al., 2004). For instance, a6* nACHRs in the optic tract and its target regions may be important in vision; the occurrence of a6* receptors in nucleus accumbens suggests a possible involvement in addiction, whereas their presence in striatum may imply a role in locomotor activity.

Putative Symptomatic Effect of Nicotinic Receptor Stimulation on Motor Function

Nicotine and nicotinic agonist administration has long been known to modulate movement in rodents (Picciotto, 2003). Extensive studies indicate a role for striatal a4* nACHRs, most likely through release of striatal dopamine (Grady et al., 1992; Wonnacott, 1997; Ryan et al., 2001). More recent experiments indicate that a6* subtypes may also be involved since nicotine-induced locomotor activity is attenuated in mice treated with a6 antisense (Le Novere et al., 1999). a6* nACHRs may represent a somewhat more select target for modulating motor behaviors since these receptors exhibit a more restricted localization than the a4* subtype, which is widespread throughout the brain (Gotti and Clementi, 2004; Quik, 2004).

In neurodegenerative disorders such as Parkinson's disease, there is a loss of presynaptic dopamine terminals and an accompanying decline in nACHRs (Quik et al., 2004; Bohr et al., 2005). This raises the possibility that stimulation of residual a6* (as well as a4*) nACHRs could enhance release from remaining terminals to result in symptomatic improvement in motor symptoms. A question that arises is how effective a6* (and/or a4*) nACHR stimulation is when studies show there is ~50 to 90% decline in the receptors in Parkinson's disease striatum (Quik et al., 2004; Bohr et al., 2005). Interestingly, our recent work in monkeys with nigrostriatal damage shows that striatal a6* (and also a4*) nACHR function is at normal levels despite 50% receptor declines (McCallum et al., 2005a,b). These compensatory changes in function in the presence of significant nACHR losses suggest that subtype-selective agonists would be beneficial despite nigrostriatal damage. In addition, the use of nicotine or subtype-selective nicotinic receptor agonists may offer the advantage that released dopamine from the nerve terminal represents a more physiologic mode of stimulation of postsynaptic dopamine function than that which occurs in response to administration of L-dopa or dopamine agonists. There may be advantages in synchronizing postsynaptic dopamine receptor stimulation with presynaptically evoked action potentials that are not conserved with directly acting dopamine agonists. Indeed, previous work has shown that administration of nACHR agonists to monkeys in combination with L-dopa allowed for a reduction in L-dopa dosage without a loss in the antiparkinsonian efficacy of L-dopa (Schneider et al., 1998). The reduction in L-dopa dose may result in a decline in debilitating side effects, including dyskinesias and psychiatric disturbances, while maintaining the therapeutic response.

To date, studies investigating effects of nicotine administration for Parkinson's disease therapy have been very limited and have yielded mixed results. Reductions in tremor and/or bradykinesia, as well as other improvements in motor performance, have been observed in some studies but not others (Ishikawa and Miyatake, 1993; Fagerstrom et al., 1994; Ebersbach et al., 1999; Kelton et al., 2000; Vieregge et al., 2001; Lemay et al., 2004). This inconsistency may relate to the small number of patients in the different studies as well as the short duration of nicotine treatment (a few weeks). The mode of nicotine administration has also been quite variable and includes i.v. infusion and/or use of the nicotine patch, gum, or lozenge. These dosing regimens are quite distinct, and the particular one selected may impact the behavioral response. For instance, chronic delivery with the nicotine patch will lead to steady-state nicotine levels that may result in greater receptor desensitization than intermittent regimens such as the gum or lozenge (Giniatullin et al., 2005; Wang and Sun, 2005). Further study to evaluate the potential for nicotine in the symptomatic treatment of Parkinson's disease is critical.

Neuroprotective Effect of Nicotine

Another potential benefit of nACHR stimulation relates to neuroprotective effects against nigrostriatal damage. Nicotine or nicotinic agonists have been shown to attenuate a wide variety of toxic insults in culture models (O’Neill et al., 2002; Quik, 2004), including protection against MPTP-induced damage to nigral dopaminergic neurons (Jeyarasasingam et al., 2002). Unexpectedly, protection by nicotine in this system seems to be mediated via non-a7 nACHRs in contrast to protection in most culture models in which a7 nACHRs seem to be involved (O’Neill et al., 2002; Quik, 2004). The effects of nicotine and nicotinic agonist have also been tested in rodent models of nigrostriatal damage; however, the results have proved rather variable, with some studies reporting a partial protection but others not (O’Neill et al., 2002; Quik, 2004). The reasons for these inconsistencies in vivo are unknown but may relate to the acute nature of the toxic insult and/or the short duration of nicotine treatment (Costa et al., 2001; Ryan et al., 2001). It may also be related to inherent differences between rodents and humans with respect to nicotine metabolism (which is much more rapid in rodents) and/or the nACHR subtypes present in striatum. Interestingly, in a recent chronic (~1 year) study in
primates, we observed a clear-cut protective effect of nicotine against MPTP-induced striatal damage (Quik et al., 2005a).

Although the neuroprotective potential against nigrostriatal damage in Parkinson’s disease remains to be evaluated, epidemiological studies overwhelmingly demonstrate ~50% reduced incidence of Parkinson’s disease in smokers (Mores et al., 1995; Checkoway and Nelson, 1999; Allam et al., 2004). This relationship is directly correlated to the duration of smoking and the number of cigarettes smoked, and the protective effect is reduced when smoking is discontinued. Admittedly, the mechanism(s) for this inverse correlation are not known; however, the work described above in culture and animal models supports a role for nicotine in tobacco products (Quik et al., 2005a). Future long-term studies in patients should help evaluate whether nicotine attenuates Parkinson’s disease progression.

Concluding Remarks

α6* nAChRs represent a subtype with a restricted localization in the CNS, including their presence on striatal dopaminergic terminals. They also exhibit a differential regulation in response to nicotine administration with declines in their number, in contrast to the well known up-regulation of α4* nAChRs. The predominant α6* nAChR populations in both rodent and monkey striatum seem to be α6α4β2δ3 and α6αβ2β3 complexes, although the subtypes and relative proportions of α3* and α6* nAChRs in human striatum await clarification. Since α6* nAChRs regulate dopamine release and are involved in motor behaviors, they may represent unique targets for the treatment of neurodegenerative disorders characterized by nigrostriatal dopaminergic damage, such as Parkinson’s disease. Selective α6* nAChR stimulation may afford symptomatic relief by stimulating dopamine release and/or reduce Parkinson’s disease progression through a neuroprotective action (O’Neill et al., 2002; Quik, 2004).

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References


