Biochemical Pharmacology 82 (2011) 873–882

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Review

Role of α6 nicotinic receptors in CNS dopaminergic function: relevance to addiction and neurological disorders

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ARTICLE INFO

Article history:
Received 30 March 2011
Accepted 2 June 2011
Available online 13 June 2011

Keywords:
Addiction
Cyclic voltammetry
Nucleus accumbens
Parkinson's disease
Striatum

ABSTRACT

Although a relative newcomer to the nicotinic acetylcholine receptor (nAChR) family, substantial evidence suggests that α6 containing nAChRs play a key role in CNS function. This subtype is unique in its relatively restricted localization to the visual system and catecholaminergic pathways. These latter include the mesolimbic and nigrostriatal dopaminergic systems, which may account for the involvement of α6 containing nAChRs in the rewarding properties of nicotine and in movement. Here, we review the literature on the role of α6 containing nAChRs with a focus on the striatum and nucleus accumbens. This includes molecular, electrophysiological and behavioral studies in control and lesioned animal models, as well as in different genetic models. Converging evidence suggest that the major α6 containing nAChRs subtypes in the nigrostriatal and mesolimbic dopamine system are the α6β2β3 and α6α4β2β3 nAChR populations. They appear to have a dominant role in regulating dopamine release, with consequent effects on nAChR-modulated dopaminergic functions such as reinforcement and motor behavior. Altogether these data suggest that drugs directed to α6 containing nAChRs may be of benefit for the treatment of addiction and for neurological disorders with locomotor deficits such as Parkinson's disease.

Contents

Abbreviations: α-CtxMII, α-conotoxinMII; nAChR, nicotinic acetylcholine receptor.

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doi:10.1016/j.bcp.2011.06.001

1. Introduction

Knowledge concerning the presence and function of nicotinic acetylcholine receptors (nAChRs) throughout biological systems has increased significantly over the last century. Extensive evidence now indicates that multiple nAChR subtypes exist in the mammalian central and peripheral nervous systems, as well as in other tissues. The muscle nAChR, which is composed of 4 distinct subunits ($\alpha1\beta1\gamma\delta$), was the first to be studied in detail in the early 1970s because of its relative abundance in *Torpedo* electric organ and the isolation of the selective ligand α-bungarotoxin [1,2]. This was followed, several decades later, by the discovery of the $\alpha3\beta4^*$,[1] $\alpha4\beta2^*$ and α7 nAChR populations, which are mainly present in neuronal tissues [3–5]. The asterisk indicates the possible presence of other subunits in the receptor complex.

nAChRs expressing the α6 subunit were one of the last to be identified, with cloning of the α6 nAChR subunit only reported in the 1990s [6]. Progress in understanding its functional significance was slow because of difficulties in expressing receptors containing the α6 subunit (α6* nAChRs) in cell models and the lack of suitable probes for its characterization. However, subsequent advances in these areas, especially the discovery of the selective antagonist α-conotoxinMII (α-CtxMII) (Cartier et al. [18]), have greatly enhanced our basic knowledge concerning α6* nAChRs.

One point of note is that α6* nAChRs are not present in the peripheral nervous system and exhibit a relatively restricted distribution in the mammalian CNS [7–12]. This receptor shares the same basic motif as other nAChR subtypes, that is, it is a ligand gated ion channel consisting of five subunits around a central pore that lie within the membrane bilayer. Great strides have been made over the last few years in our understanding of the subunit composition of α6* nAChRs, their role in CNS dopaminergic function and the ensuing behavioral consequences. These combined findings, which are the focus of the current review, suggest that α6* nAChRs may represent key therapeutic targets for the treatment of addiction and for neurodegenerative movement disorders involving deficits in the nigrostriatal dopaminergic system.

1.1. Localization of α6* nAChRs in the brain

In situ hybridization studies done to identify the presence of α6 nAChR subunit mRNA were the first to suggest that receptors containing the α6 subunit exhibited a very restricted localization in both rodent and primate brain. The strongest α6 mRNA signal is in the retina and catecholaminergic nuclei, including the locus coeruleus, ventral tegmental area, and substantia nigra, with a weaker signal in the superior colliculus, medial habenula, interpeduncular nucleus, visual cortex and a few other small nuclei [12–16]. This limited distribution of α6 nAChR transcripts contrasts with that of the α4 and α7 nAChR mRNAs, which are widespread throughout the brain [13,14,17].

The mRNA distribution studies were followed by experiments to localize α6* nAChR protein (Table 1). Such work became feasible because of the isolation of α-CtxMII, a novel snail toxin that was first shown to bind to $\alpha3\beta2^*$ nAChRs [18] and was subsequently shown to have even higher affinity for $\alpha6\beta2^*$ nAChRs [10]. Another breakthrough was the development of α6 nAChR-subunit directed antibodies [19,20]. The combined use of these two different probes showed that α6* nAChR protein is present in the mesolimbic and nigrostriatal dopaminergic pathways, consistent with the existence of α6 mRNA in catecholaminergic nuclei. α6* nAChRs are also very prominent in structures linked to the visual system including the superior colliculus, optic tract, olivary pretectal area, and mediolateral and dorsolateral geniculate nuclei, as well as the retina [20–25]. In addition, small populations of α6* nAChRs have been identified in the rodent habenulo-interpeduncular pathway [26].

This unique anatomical distribution suggests that α6* nAChRs may play a role in behaviors linked to reward, motor activity and vision.

[1] * indicates the possible presence of other subunits in the receptor complex.

Table 1
Distribution of α6* nAChR subtypes in the mammalian CNS.

Brain region	Species	Subtype	Reference
Nigrostriatal dopaminergic system – dorsal striatum	Rodent	$\alpha6\beta2\beta3$ $\alpha6\alpha4\beta2\beta3$ $\alpha6\beta2^*$	[1–6]
	Monkey	$\alpha6\beta2\beta3$ $\alpha6\alpha4\beta2\beta3$ $\alpha6\beta2^*$	[7]
	Human	$\alpha6\beta2\beta3$ $\alpha6\alpha4\beta2\beta3$ $\alpha6\beta2^*$	[1,8–10]
Mesolimbic dopaminergic system – ventral striatum	Rodent	$\alpha6\beta2\beta3$ $\alpha6\alpha4\beta2\beta3$ $\alpha6\beta2^*$	[6]
Retina and optic tract	Rodent	$\alpha6\beta2^*$ $\alpha6\alpha3\beta2^*$ $\alpha6\alpha2\beta2^*$ $\alpha6\beta2\beta3^*$ $\alpha6\alpha4\beta2^*$ $\alpha6\alpha3\alpha4\beta2^*$	[11–13]
Habenulo-interpeduncular pathway	Rodent	$\alpha6\beta2^*$ $\alpha6\beta3\beta4^*$	[14]

Although the $\alpha6\beta2\beta3$ and $\alpha6\alpha4\beta2\beta3$ nAChRs are the primary subtypes in the mesolimbic and nigrostriatal dopaminergic systems, the $\alpha6\beta2^*$ nAChR designation is retained in the event novel subtypes are identified.

1.2. Composition of α6* nAChRs

1.2.1. CNS dopaminergic pathways

Our knowledge concerning the subunit composition of α6* nAChRs in dopaminergic pathways has grown considerably as a result of a concerted effort using multiple experimental strategies. Immunoprecipitation studies with antibodies targeting β2 nAChRs, coupled with the use of β2 knockout mice, demonstrated the presence of the β2 subunit in all α6* nAChR complexes in rodent and monkey striatum and/or nucleus accumbens (Fig. 1 and

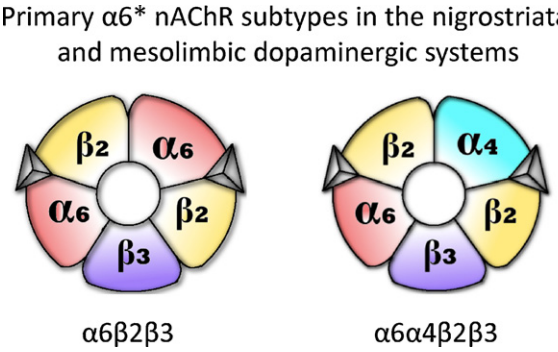

Fig. 1. Putative composition of α6* nAChRs in the nigrostriatal and mesolimbic dopaminergic systems. Cumulative results from numerous molecular and functional studies indicate that nAChRs containing the α6 subunit are pentameric channels with the subunits around a central channel or pore that lie within the membrane bilayer. The acetylcholine binding sites (triangles) are located at the interface between an α and β subunit. Although there is the potential for multiple combinations, the major nAChR subtypes in the nigrostriatal and mesolimbic dopaminergic systems appear to be composed of $\alpha6\beta2\beta3$ (left) and $\alpha6\alpha4\beta2\beta3$ (right) subunits (see also Table 1) [7–11,141,142]. The possibility exists, however, that other minor $\alpha6\beta2^*$ nAChR subtypes remain to be identified.

Table 1) [19,20,27,28]. The β3 subunit also appears to be expressed in the majority of α6* nAChR complexes (Fig. 1 and Table 1). Indirect evidence for this possibility initially stemmed from the observation that α6 and β3 mRNAs were uniquely co-localized in the same brain regions [14,29]. Subsequent dual immunoprecipitation studies using α6 and β3 nAChR subunit-directed antibodies confirmed this idea by showing that the α6 and β3 subunit proteins coexist within the same receptor complex. Lesion studies further showed that α6 and β3 nAChR subunit protein declined in parallel with nigrostriatal damage [19,20,27,28,30]. It should be noted that there is not a one-to-one correspondence in the decline of receptors expressing these subunits in nAChR knockout mice [29]. This may be due to the development of compensatory mechanisms in knockout mice not normally present in wildtype animals.

In addition to the presence of the β2 and β3 nAChR subunits, a significant proportion of α6* nAChR complexes also include the α4 subunit (Fig. 1 and Table 1) [20,27,28,31]. The relative levels of the α6β2β3 and α6α4β2β3 subtypes in the nigrostriatal and mesolimbic dopamine system are currently under investigation. One study reports the existence of roughly equal amounts of the α6β2β3 and α6α4β2β3 subtypes in the striatum [32], while another suggests a predominance of the α6α4β2β3 subtype [28]. In ventral striatum, which includes the nucleus accumbens, ventral pallidum and olfactory tubercle, the α6β2β3 and α6α4β2β3 nAChR subtypes are present in similar proportions [28]. Although the mesolimbic dopaminergic system also projects to the frontal cortex, the primary nAChRs in this latter region appear to be the α4β2* and α7, with a role for the α6β2* subtype currently uncertain [33,34].

Overall, these data indicate that the α6β2β3 and α6α4β2β3 subtypes are the major subtypes in the nucleus accumbens and striatum, although there is always the possibility that minor novel subtypes remain to be identified. Since α6 containing receptors in the mesolimbic and nigrostriatal systems presently appear to contain a β3 subunit, they will be designated as α6β2β3* nAChRs in subsequent sections of the review.

1.2.2. Visual system

Although α6* nAChRs have been studied most extensively in CNS dopaminergic pathways, it should be noted that they are in fact most densely expressed in areas linked to the visual system, as well as the superior colliculus, optic tract, geniculate nuclei and the retina, including microglial cells [21,24,25,35]. Sequential immunoprecipitation studies with select nAChR subunit antibodies indicate that considerable subtype heterogeneity exists in regions of the mammalian visual system (Fig. 1 and Table 1), with expression of α6β2*, α6α3β2*, α6α2β2*, α6β2β3*, α6α4β2*, α6α3α4β2* identified to date [11,23,24]. This diversity in α6* nAChR composition in the visual system may be important for the fine-tuning of this essential function under varying physiological and pathological conditions.

2. α6β2β3* nAChRs and the control of dopaminergic function

Although the most intense expression of α6* nAChRs is in the visual system, very little is known about their function in vision. By contrast, significant work has been done to investigate their involvement in dopaminergic activity in the terminal regions of both the mesolimbic and nigrostriatal pathways, that is, the nucleus accumbens and striatum. Two approaches that have greatly contributed to our understanding of their role in these latter regions are measurement of nAChR-stimulated ^{3}H-dopamine release from synaptosomes or slices, and electrically evoked dopamine release from slices using fast scan cyclic voltammetry.

2.1. nAChR-evoked ^{3}H-dopamine release

The concept that nAChRs could modulate dopamine release initially stemmed from the work of Giorgueieff et al., who investigated nAChR-mediated release of newly synthesized ^{3}H-dopamine from ^{3}H-tyrosine [36]. This was followed several years later by experiments showing that nicotine stimulated release of ^{3}H-dopamine from nucleus accumbens or striatal synaptosomes [37–39]. For about a decade, dopamine release in both the nucleus accumbens and striatum was thought to be regulated only by α4β2* nAChRs. However, the discovery of α-CtxMII, which interacts with α6β2β3* nAChRs, subsequently showed that activation of this subtype also stimulated ^{3}H-dopamine release [34,40,41]. In fact, the α4β2* and α6β2β3* nAChR subtypes promote dopamine release evoked by 1 μM nicotine in roughly equal proportions in rodent nucleus accumbens and striatum (Fig. 2) [34,40,41]. Furthermore, the α6β2β3* nAChR subtype plays a dominant role in nonhuman primate nucleus accumbens and striatum (Fig. 2), with 80% of release mediated by α6β2β3* subtypes and only 20% by the α4β2* nAChR population [42,43]. It should be noted that the functional data regarding our knowledge of α6β2β3* nAChRs described above, as well as in the next section, was obtained with a single, antagonist α-CtxMII, because this is currently the only selective agent available for their study. Work

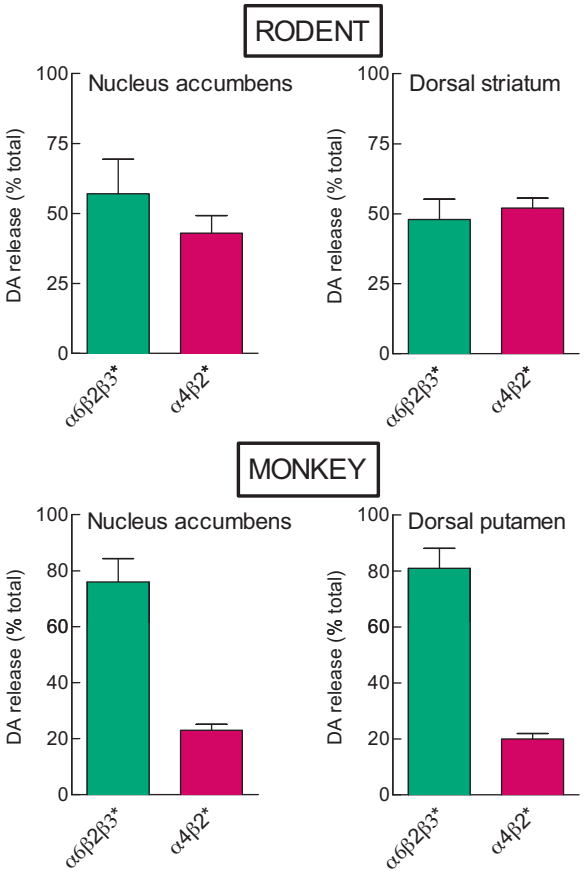

Fig. 2. Prominent contribution of the α6β2β3* nAChR subtype in stimulating ^{3}H-dopamine release from nucleus accumbens and striatal synaptosomes prepared from rodent or nonhuman primate brain. nAChR-evoked ^{3}H-dopamine was determined as previously described [34,42,63]. The results show that the α6β2β3* nAChR subtypes make a similar contribution to ^{3}H-dopamine release (stimulated by 1 μM nicotine) as the α4β2* nAChR subtypes (α4β2 and α4α5β2) in rodent nucleus accumbens and dorsal striatum. Moreover, the α6β2β3* nAChR subtypes play a dominant role in modulating ^{3}H-dopamine in nonhuman primate nucleus accumbens and dorsal putamen, with ~80% of release evoked by the α6β2β3* and only ~20% by α4β2* (α4β2 and α4α5β2) nAChR subtypes. Values represent the mean ± SEM of 5–7 animals.

with other selective antagonists, antibodies or alternate molecular probes, is necessary for confirmation of the role of α6β2* nAChRs in dopaminergic function.

2.2. Cyclic voltammetry

Another approach that has yielded significant insight concerning the role of α6β2β3* nAChRs in dopaminergic function is cyclic voltammetry, in which carbon-fiber microelectrodes are used as chemical sensors to detect electrically evoked dopamine release in tissue slices [44–50]. This approach is distinct from nAChR-stimulated ^{3}H-dopamine release as it provides a measure of action-potential evoked endogenous dopamine release. Moreover, it has the advantage that it provides information about release with single and multiple electrical pulses, which may more closely resemble tonic and burst stimulation with subsequent reward and movement.

One strategy currently being used to evaluate the role of different nAChR subtypes in modulating dopamine release involves the use of nAChR antagonists. In the mesolimbic dopaminergic system, the α6β2β3* nAChR selective antagonist α-CtxMII reduced single-pulse stimulated dopamine release by the same amount as general nAChR antagonists in nucleus accumbens slices from rodents [44] and monkeys [50]. These data indicate that the prime regulator of dopamine release in the mesolimbic dopamine pathway is the α6β2β3* nAChR.

The role of the α6β2β3* subtype was somewhat less pronounced in the nigrostriatal dopaminergic system. However, it still exerted major effects, regulating about 75% of single-pulse evoked nAChR-mediated dopamine release in the dorsal striatum of rodents (Fig. 3) [44,46,48] and ventral putamen of nonhuman primates (Fig. 3) [47,50]. Results in nonhuman primate dorsal putamen were similar to those in the mesolimbic dopamine systems with all single-pulse nAChR-mediated evoked dopamine release modulated by α6β2β3* nAChRs.

As might be expected, nAChR modulation of burst firing is much more complex, with an increase or no apparent change in dopamine release with either α6β2* or α4β2* nAChR antagonism [44–47,50,51–54]. This may be attributed to the fact that the effects of acetylcholine with burst stimulation involve a complex interplay between nAChR desensitization and activation, as well as muscarinic AChR activation, all of which would be dependent on the concentration of acetylcholine at the synaptic cleft.

Altogether these studies suggest that α6β2β3* nAChRs are major modulators of dopaminergic function in both striatum and nucleus accumbens of rodents and monkeys. The relative roles of

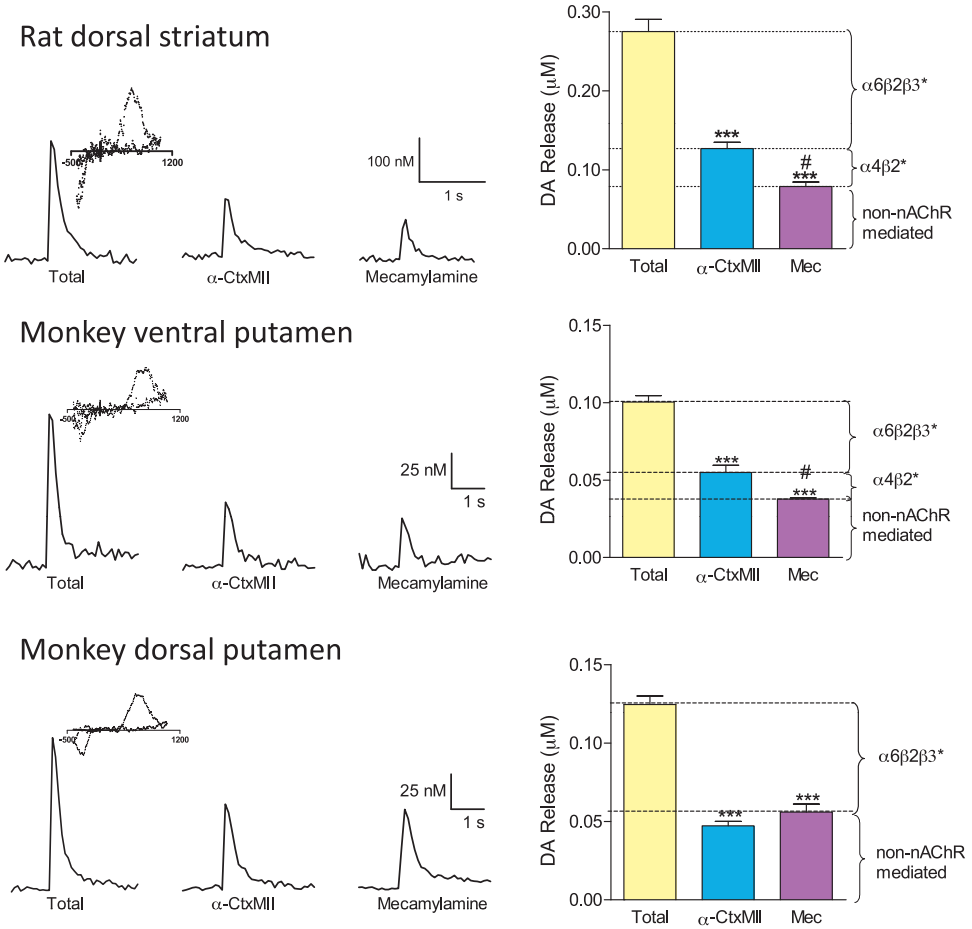

Fig. 3. α6β2β3* nAChRs are major regulators of nAChR-modulated dopamine release in the nigrostriatal system. Cyclic voltammetry was used to measure electrically stimulated dopamine release in coronal brain slices containing rat dorsal striatum (top panel), monkey ventral putamen (middle panel) or monkey dorsal putamen (bottom panel). Endogenous dopamine release was evoked by a bipolar stimulating electrode and detected with a carbon fiber recording microelectrode as described [47,49]. Representative traces of single-pulse stimulated total dopamine release in the different tissue slices are shown on the left. The traces in the middle and on the right depict dopamine release in the presence of the α6β2β3* nAChR antagonist α-CtxMII (100 nM) or the general nAChR blocker mecamylamine (100 μM), respectively. Quantitative analyses of the data show the relative contribution of the α6β2β3* and α4β2* nAChR subtypes (α4β2 and α4α5β2) in modulating dopamine release. As can be seen, 75–100% of nAChR-modulated evoked dopamine release occurs via the α6β2β3* subtype. Values represent the mean ± SEM of 3–9 animals. $^{***}p < 0.001$ indicate significance of difference from control, while $^{\#}p < 0.05$ indicates significance of difference from α-CtxMII, using a Newman–Keuls multiple comparisons *post hoc* test.
Data taken in modified form with permission [47,49].

the α6β2β3 and α4α6β2β3 subtypes are currently unclear; however, novel approaches involving concatameric α6* nAChRs [55] and/or the development of selective drugs should help in this regard. Overall, α6β2β3* nAChRs appear to play a prominent role in evoked dopamine release, and consequently drug reinforcement and motor function.

3. Long term nicotine exposure modulates α6β2β3*nAChR expression and function

Although acetylcholine or nAChR agonists acutely increase dopamine release, it is well established that continued nicotine exposure rapidly (seconds to minutes) results in a decline in release due to receptor desensitization [56,57]. Sustained nicotine exposure (days to months) leads to additional long term molecular and cellular changes which may represent mechanisms that underlie nicotine addiction and tolerance [58,59]. It is therefore important to understand the effects of chronic nicotine treatment on nAChR subtype expression and function.

Nicotine administration is well known to increase α4β2* nAChR expression throughout the brain, including the striatum, in experimental animal models and in human smokers [60–68]. Moreover, functional studies in rodents and primate show that the elevated α4β2* nAChR levels in striatum may be associated with increases or no change in receptor-mediated function (Table 2) [69,70]. By contrast, the same long term nicotine treatment paradigms decrease or do not change α6β2β3* nAChR expression in rodent and monkey striatum (Table 2) [42,63,71–73]. Nicotine-induced changes in α6β2β3* nAChR levels generally correlate with declines in ^{3}H-dopamine release from striatal and nucleus accumbens synaptosomes (Table 2). Subsequent work to distinguish between changes in the α6β2β3 and α6α4β2β3 subtypes showed that chronic nicotine dosing to mice led to a selective decline in α6α4β2β3 nAChRs with some increase in α6β2β3 nAChRs [48]. These results are consistent with data using transfected cell culture systems, which show that α6β2 or α6β2β3 nAChRs can be upregulated with long term nicotine treatment [74–77].

Cyclic voltammetry has also been used to evaluate changes in dopaminergic activity with long term nicotine dosing [46,48]. Single-pulse evoked dopamine release in the presence of α-CtxMII was still reduced with chronic nicotine treatment. However, nicotine exposure modified the pattern of burst firing observed in the presence of α-CtxMII. In untreated rats, burst firing is enhanced or not changed in the presence of α-CtxMII. By contrast, in nicotine-treated rats, there was a decrease in burst-stimulated dopamine release in the presence of α-CtxMII. The finding that nicotine exposure preferentially downregulates the α6α4β2β3 subtype in striatum [48], coupled with the release results, suggest that the α6α4β2β3 nAChR subtype is a primary regulator of evoked dopamine release under physiological (untreated control) conditions. The differential burst-stimulated release pattern with chronic nicotine treatment may be due to a more prominent role of the α6β2β3 subtype after nicotine treatment [48].

Chronic nicotine exposure also altered striatal dopaminergic function in nonhuman primate striatum. However, the changes were different from those obtained in rat striatum and also exhibited regional variations [47]. Nicotine treatment via the drinking water (3–4 months) altered both nonburst and burst-stimulated dopamine release in ventral but not dorsal putamen [47]. In addition, chronic nicotine treatment resulted in large declines in dopaminergic function in monkey nucleus accumbens with a complete loss of α6β2β3* nAChR-modulated evoked dopamine release and a depression of non-nAChR-modulated evoked dopamine release [50]. Comparable studies have not yet been done to evaluate the effects of chronic nicotine treatment on dopamine release in rat nucleus accumbens using cyclic voltammetry.

Overall, these findings indicate that chronic nicotine exposure may result in dramatic alterations in α6β2β3* nAChR-modulated dopamine release in the striatum and nucleus accumbens. These mechanisms are of relevance to the chronic nicotine exposure that occurs with smoking in humans, and may underlie the reinforcing and addictive properties of smoking.

4. Role of mesolimbic α6β2β3* nAChRs in reward and addiction

4.1. Nicotine addiction

An extensive clinical and preclinical literature over the last few decades supports the idea that nicotine in tobacco contributes to its addictive properties [78–84]. Molecular, cellular and behavioral studies using various animal models have provided significant insight into the nAChR subtypes relevant to addiction [80,85–87]. Initial work with nAChR blockers and selective lesions showed that the reinforcing effects of nicotine were mediated via nAChRs in the mesolimbic dopaminergic pathway [82,83]. The subsequent use of nAChR knockout and transgenic mice, coupled with receptor re-expression, proved critical in defining the nAChR subtypes involved. Lentiviral vector re-expression of the β2 subunit into the ventral tegmental area of β2 knockout mice demonstrated a key role for β2* nAChRs in nicotine reinforcement [88,89]. Work with genetically engineered mice containing a point mutation in α4 nAChR subunits (Leu9′ to Ala9′ in the M2 domain) that yielded receptors hypersensitive to nicotine showed that the α4β2* nAChR subtype is sufficient for nicotine-induced reward, tolerance, and sensitization [90]. Evidence for a role for α6* nAChRs stemmed from studies showing that α6 nAChR knockout mice did not self-administer nicotine, but that re-expression of the α6 subunit restored this behavior [91]. Moreover, intraventricular or local infusion of α6β2β3* nAChR selective toxins into the rat nucleus accumbens shell blocked behaviors linked to nicotine reward and withdrawal [92,93]. Studies with mice expressing hypersensitive α6β2β3* nAChRs (with α6 subunit Leu 9′ to Ser 9′ mutation) but

Table 2
Long term oral nicotine treatment decreases or does not change α6β2β3* nAChR expression and function in striatum and nucleus accumbens of rats and monkeys. By contrast, this same treatment increases or does not change α4β2* nAChR expression and function.

nAChR subtype	Species	Region	nAChR binding sites	nAChR-mediated ^{3}H-DA release	Reference
α6β2β3*	Rat	Striatum	↓	↓	[15–20]
	Monkey	Striatum	↓	↓	[19]
		Nucleus accumbens	No change	No change	[18]
α4β2*	Rat	Striatum	↑	No change	[15,19–27]
	Monkey	Striatum	↑	↑	[19]
		Nucleus accumbens	↑	↑	[18]

expressing or lacking the α4 subunit further suggest that an α6α4β2* nAChR subtype may play an important role in nicotine reward and reinforcement [94,95].

Thus, converging data from numerous animal models indicate that α6β2β3* nAChR, possibly in concert with other nAChR subtypes [96], are important for nicotine's addictive properties. These findings suggest that selective α6β2β3* nAChR agonists and antagonists would represent novel agents for the therapeutic management of nicotine addiction.

4.2. Alcohol addiction

Another widely used neuroactive substance with addictive properties is ethanol. Although its mechanism of action is most likely multifactorial, ethanol appears to exert some of its reinforcing effects by activating dopaminergic neurons in the ventral tegmental area through an interaction at nAChRs [97]. This idea is based in part on studies with varenicline, an agonist/partial agonist that acts at multiple subtypes including α3β4*, α4β2*, α6β2β3* and α7 nAChRs [97–100]. Varenicline decreases alcohol consumption in rats [101–103], as well as in heavy-drinking smokers [104]. With respect to the nAChR subtype involved, studies with genetically modified nAChR mice indicate that α4β2* nAChRs play a role in alcohol addiction [105]. In addition, α3β4* partial agonists reduce ethanol consumption and seeking in rats [106]. The α6β2β3* subtype has also been implicated based on experiments showing that intracranial injection of α-CtxMII into the ventral tegmental area decreased ethanol-induced accumbal dopamine release [107].

Thus multiple nAChR subtypes in the mesolimbic dopaminergic system may be involved in the activating and reinforcing effects of ethanol. Drugs targeting α6β2β3*, as well as α4β2* and α3β4* nAChR subtypes may thus have potential in modulating addictive behaviors associated with ethanol consumption.

5. Role of nigrostriatal α6β2β3* nAChRs in motor control

5.1. Studies in nonlesioned animal models

The presence of α6β2β3* nAChRs in the nigrostriatal pathway, coupled with the well known association between this system and movement, prompted studies to investigate a role for striatal α6β2β3* nAChRs in locomotor activity. The first report to explore this possibility was that of le Novere et al. [108] who showed that α6 antisense oligonucleotide infusion into the CNS decreased nicotine-mediated locomotor activity. In addition, transgenic mice expressing hypersensitive α6β2β3* receptors exhibit altered locomotor responsiveness with increased walking, turning, and rearing [94,109]. These genetically modified mice express an α6 subunit in which Leu280 in the M2 domain (α6 L9′S mice) was mutated to Ser rendering an α6* receptor with enhanced sensitivity to nicotine. By contrast, mice that lack the α4 nAChR subunit behaved normally despite the presence of a hyperactive α6 nAChR subunit, suggesting a critical involvement of the α6α4β2β3 nAChR subtype in motor activity [94].

These combined data indicate that striatal α6α4β2β3 nAChRs play an important role in movement. They most likely influence motor function in concert with α6β2β3, α4β2* and α7 nAChRs in the striatum, as well as other brain areas including the mesolimbic dopaminergic system [28,110,111].

5.2. Effect of nigrostriatal damage; relevance to Parkinson's disease

The next question that arises is the effect of neuronal damage on α6β2β3* nAChR expression, cellular function and behavior. This is of particular relevance to Parkinson's disease, a neurological movement disorder characterized by severe losses in nigrostriatal dopaminergic neurons. To approach this, work has been done in parkinsonian animal models in which the nigrostriatal pathway is selectively damaged with dopaminergic neurotoxins such as 6-hydroxydopamine or MPTP. Such lesions result in a decrease in α6β2β3* nAChR expression and function that closely parallels the decline in dopaminergic terminal integrity [19,20,22,112,113]. These findings indicate that α6β2β3* nAChRs are primarily localized to dopaminergic terminals in the striatum. Nigrostriatal damage also reduced α4β2* nAChRs levels and function. However, the decreases in this subtype were much smaller than those in α6β2β3* nAChRs suggesting that α4β2* nAChRs are also present on other neuronal elements in the striatum [19,20,113,114].

Results in parkinsonian animal models parallel those in Parkinson's disease brains. Large declines are observed in α6β2β3* nAChRs in striatum of Parkinson's disease patients, which correlate with the magnitude of the dopamine transporter loss [115–117]. By contrast, striatal α4β2* nAChRs are reduced to a lesser extent, in agreement with studies in rodent and nonhuman primate striatum. To further delineate effects on select nAChR populations, competition studies were done with α-CtxMII [E11A] that differentiates between α6β2β3* subtypes (Fig. 4). A preferential loss of the striatal α6α4β2β3 nAChR subtype was observed with mild to moderate nigrostriatal damage, while the α6β2β3 subtype was only decreased with more severe nigrostriatal damage. This was observed across species, that is, in the striatum of parkinsonian rodents and in monkeys, as well as with Parkinson's disease (Fig. 4) [32]. These data suggest that drugs targeting both the α6α4β2β3 and α6β2β3 nAChR subtypes may be useful with moderate nigrostriatal damage, but that α6β2β3 nAChRs drugs may be more suitable with severe degeneration.

5.3. Role for selective nAChR drugs in Parkinson's disease

Studies have been done in parkinsonian animal models, which suggest that nicotine or nAChR agonist treatment may provide therapeutic benefit in Parkinson's disease. Extensive evidence has shown that nicotine protects against nigrostriatal damage through an interaction at nAChRs in several parkinsonian animal models including mice, rats and monkeys [118–120]. Further studies investigating the nAChR subtypes that contribute to this effect suggest that protection against nigrostriatal damage may be linked to the striatal α6α4β2β3 nAChR population [121].

There have also been reports that nicotine may reduce a serious side effect of L-dopa treatment for Parkinson's disease, that is, L-dopa-induced abnormal involuntary movements (AIMs) or dyskinesias [122–126]. There are currently only limited treatment options for these abnormal movements of the head, trunk and/or limbs, which develop in most patients on L-dopa therapy [127]. Recent work shows that nicotine treatment ameliorates L-dopa induced dyskinesias in parkinsonian mice, rats and monkeys by acting at nAChRs [128–131]. Because nicotine is nonselective and may result in side effects in addition to the desired response, studies were done to determine whether more selective nAChR agonists improve these abnormal movements in a rat model of L-dopa-induced dyskinesias. The drugs tested include varenicline, an agonist that interacts with multiple nAChRs such as the α4β2*, α6β2β3* and α7 subtypes [98,132], and iodo-A-85380, an agonist that interacts with α4β2* and α6β2* nAChRs [133]. Both these compounds reduced L-dopa-induced dyskinetic-like movements by 50% in parkinsonian rats [134]. Since these drugs reduced AIMs more effectively in rats with partial compared to near-complete nigrostriatal damage, these data suggest that presynaptic α4β2* and α6β2β3* nAChRs on dopaminergic terminals may play a role in reducing L-dopa-induced AIMs but also with a contribution from other nAChRs located throughout the brain.

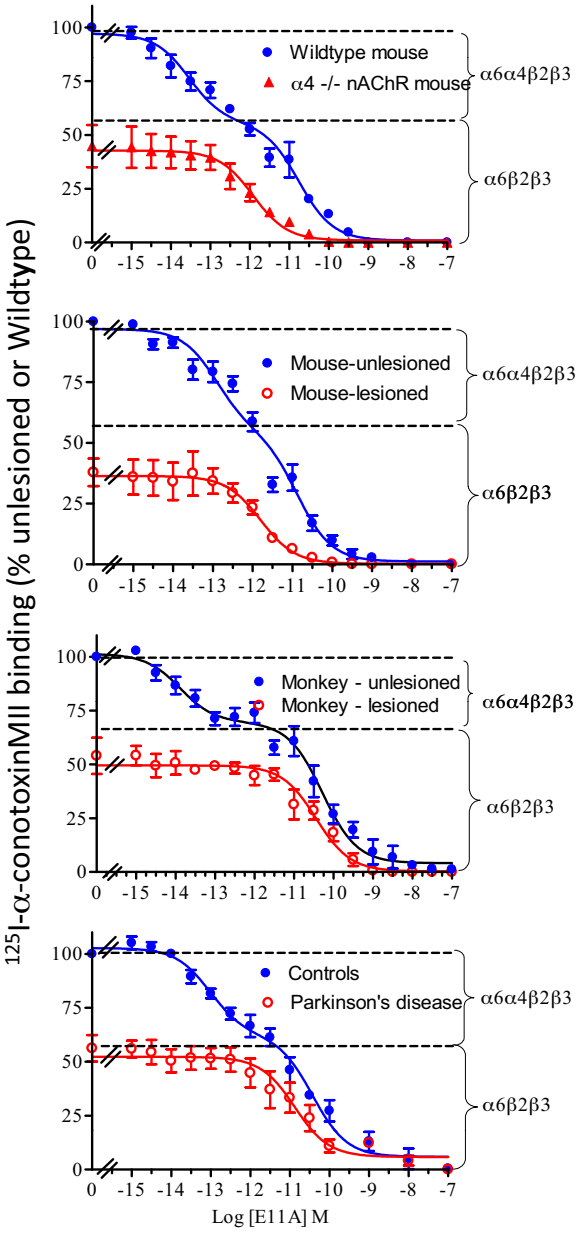

Fig. 4. Preferential loss of the α6α4β2β3 nAChR subtype in striatum with nigrostriatal damage. The top panel depicts ^{125}I-α-CtxMII competition curves in the presence of varying concentrations of α-CtxMII [E11A] in the striatum of wildtype and α4 nAChR knockout mice. Biphasic curves are observed in wildtype mouse striatum, but a monophasic curve in α4 knockout mice. These findings suggest the presence of two major α6β2β3* nAChR populations, with the higher affinity site being the α6α4β2β3 subtype and the lower affinity site the α6β2β3 receptor. A point of note is that the K_i of the lower affinity site in wildtype mice was not identical to that in the knockout mice. This may be due to differences in molecular configurations of the α6β2* interfaces, variable post-translational modifications (phosphorylation or glycosylation) or possibly additional subtype combination with knockout of the α4 subunit. The second panel depicts similar competition experiments using striatum from control and parkinsonian mice. Interestingly, the curves in the lesioned mice are identical to those in the α4 knockout mice, suggesting a loss of the α6α4β2β3 subtype. The third and fourth panels depict similar competition experiments from striatum of control and parkinsonian monkeys, and from control human and Parkinson's disease cases. The biphasic inhibition curves obtained in control striatum become monophasic with nigrostriatal damage, again suggesting a loss of the α6α4β2β3 subtype. Values represent the mean ± SEM of 4–8 mice, 4–6 monkeys, and 5 control and 4 Parkinson's disease cases.
Taken in modified form with permission [32].

The mechanisms whereby nAChR agonists improve L-dopa-induced AIMs remain to be elucidated. Current experimental evidence suggests that they most likely involve long term molecular changes in the brain since several days of agonist administration is required to reduce AIMs [134]. The site of action of these long term changes is currently not known. One might anticipate that agonists exert their beneficial effect by initially modulating nigrostriatal function. This may be followed by secondary actions in other CNS pathways, for instance, in the serotonergic system [135–137]. Evidence for this idea is derived from studies showing that L-dopa-induced AIMs are substantially reduced in rodents with serotonergic lesions. The idea that multiple brain regions may subsequently mediate the effects of nAChR activation is also suggested from an extensive literature showing that deep brain stimulation of the subthalamic nucleus is very effective in reducing dyskinesias in Parkinson's disease patients [138].

6. Role for α6* nAChRs in the visual system

The pronounced expression of diverse α6* nAChRs in the mammalian visual system and retina (Table 1) suggests that the α6* nAChR subtype most likely has a significant role in vision [11,35]. Experiments done to evaluate the effect of visual deprivation on nAChRs showed no changes in α6* nAChR expression in rats reared in the dark [35], although eye enucleation did reduce α6* nAChRs in superior colliculus and lateral geniculate nucleus [23]. However, the function of α6* nAChRs in vision is currently unknown. Acetylcholine acting at nAChRs has been shown to modulate neuronal excitability in the visual cortex [139], while studies with nAChR blockers suggest there may be a permissive role for cholinergic input in visual function [140].

The intense localization of α6* nAChRs in the visual system, coupled with the importance of vision in daily life, suggests that this could represent a promising new research area for future studies.

7. Summary

Accumulating literature points to a significant and possibly predominant role for α6β2β3* nAChRs in modulating dopaminergic function in the mesolimbic and nigrostriatal dopaminergic pathways. Since these systems are critically involved in behaviors linked to addiction/reward and the control of movement, α6β2β3* nAChRs may represent novel therapeutic targets for addiction and for the treatment of movement disorders such as Parkinson's disease.

Acknowledgements

This work was supported by NIH grants NS42091, NS59910, NS65851, DA003194 and DA012242, grants from the Michael J. Fox Foundation and from the California Tobacco Related Disease Research Program 17RT-0119.

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