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Subunit Composition and Pharmacology of Two Classes of Striatal Presynaptic Nicotinic Acetylcholine Receptors Mediating Dopamine Release in Mice

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ABSTRACT

Pharmacological evaluation of nicotine-stimulated dopamine release from striatum has yielded data consistent with activation of a single population of nicotinic acetylcholine receptors (nAChR). However, discovery that α-conotoxin MII (α-CtxMII) partially inhibits the response indicates that two classes of presynaptic nAChRs mediate dopamine release. We have investigated the pharmacology and subunit composition of these two classes of nAChR. Inhibition of nicotine-stimulated dopamine release from mouse striatal synaptosomes by α-CtxMII occurs within minutes; recovery is slow. The IC_{50} is 1 to 3 nM. α-CtxMII–sensitive and -resistant components have significant differences in pharmacology. The five agonists tested were more potent at activating the α-CtxMII–sensitive nAChRs; indeed, this receptor is the highest affinity functional nAChR found, so far, in mouse brain. In addition, cytisine was more efficacious at the α-CtxMII–sensitive sites. Methyllycaconitine was 9-fold more potent at inhibiting the α-CtxMII–sensitive sites, whereas dihydro-β-erythroidine was a 7-fold more potent inhibitor of the α-CtxMII–resistant response. Both the transient and persistent phases of nicotine-stimulated dopamine release were partially inhibited by α-CtxMII with equal potency. The subunit composition of functional nAChRs, was assessed in mice with null mutations for individual nAChR subunits. The β2 subunit is an absolute requirement for both classes. In contrast, deletion of β4 or α7 subunits had no effect. The α-CtxMII–sensitive response requires β3 and is partially dependent upon α4 subunits, probably α6β3β2 and α4α6β3β2, whereas the α-CtxMII–resistant release requires α4 and is partially dependent upon α5 subunits, probably α4β2 and α4α5β2.

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Nicotinic cholinergic receptors (nAChRs) are expressed both peripherally and centrally. Many, if not most, brain nAChRs are located presynaptically, where they serve to modulate neurotransmitter release (Wonnacott, 1997; Nishi et al., 2000). Activation of presynaptic nAChRs may elicit neurotransmitter release in the absence of a propagated signal (Vizi and Lendvai, 1999), and may increase action-potential induced transmitter release and, consequently, enhance synaptic fidelity (McGehee et al., 1995; Gray et al., 1996).

Neuronal nAChR subunits form pentameric complexes, closely resembling the nAChR found at the neuromuscular junction (Leonard and Bertrand, 2001). Some of these receptors are homomeric (α7), but most are heteromeric. Subunit composition of heteromeric nAChRs expressed in *Xenopus laevis* oocytes or transfected cell lines has profound effects on agonist potency and efficacy as well as sensitivity to antagonists (Luetje and Patrick, 1991; Harvey and Luetje, 1996;

ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; α-CtxMII, α-conotoxin MII; ACh, acetylcholine iodide; A85380, 3-[2-(*S*)-azetidinylmethoxy]pyridine; DEC, decamethonium chloride; DHβE, dihydro-β-erythroidine; MLA, methyllycaconitine; bp, base pair(s).

Ramirez-Latorre et al., 1996; Chavez-Noriega et al., 1997; Kuryatov et al., 2000; Rush et al., 2002). Dopaminergic neurons in the substantia nigra that project to the striatum express detectable concentrations of mRNA for α3, α4, α5, α6, α7, β2, and β3 subunits, as well as traces of β4 (Klink et al., 2001; Azam et al., 2002). Given that neuronal nAChRs are pentameric, the finding that the mRNAs for eight different nAChR subunits are expressed in dopaminergic neurons suggests that many structurally and functionally diverse nAChR subtypes might be expressed in these neurons.

Pharmacological evaluation of nicotinic agonist-evoked dopamine release from striatal synaptosomes of rodents has been consistent with activation of a single population of receptors (e.g., Rowell, 1995; Grady et al., 1997; Wonnacott, 1997). However, the demonstration that α-conotoxin MII (α-CtxMII) partially inhibits nicotinic agonist-induced dopamine release from striatal synaptosomes (Kulak et al., 1997; Kaiser et al., 1998; Grady et al., 2001) indicates that at least two nAChR populations, referred to as α-CtxMII–resistant and -sensitive nAChRs, are expressed in striatal dopaminergic terminals. Recent studies have attempted to identify the subunit compositions of the α-CtxMII–resistant and -sensitive nAChRs. The original characterization of α-CtxMII demonstrated that the α3β2-nAChR subtype was the most sensitive to inhibition by α-CtxMII of several nAChRs subtypes expressed in *X. laevis* oocytes (Cartier et al., 1996) (for nAChR nomenclature, see Lukas et al., 1999). However, α3 subunits do not contribute substantially to the α-CtxMII binding sites found in nigrostriatal pathways (Whiteaker et al., 2002). Therefore, other subtypes that have high affinity for α-CtxMII must exist in mouse brain. Indeed, α6*-nAChRs expressed in oocytes have high affinity for α-CtxMII (Kuryatov et al., 2000; Dowell et al., 2003) and ^{125}I-α-CtxMII binding in nigrostriatal pathways is absent in α6 null mutant mice (Champtiaux et al., 2002). Furthermore, striatal α-CtxMII binding is decreased in β3- (Cui et al., 2003) and α4-null mutant mice (Marubio et al., 2003), indicating that these subunits are required for some receptor subtypes that bind α-CtxMII with high affinity. Indeed, Champtiaux et al. (2003) reported the results of immunoprecipitation studies that used α4-, α6-, and β2-null mutant mice, as well as 6-hydroxydopamine–treated wild-type mice and concluded that three heteromeric nAChRs (α4β2*, α6β2*, and α4α6β2*) are expressed in dopaminergic nerve terminals. Consistent with these results, Zoli et al. (2002) concluded that nicotinic binding sites expressed in rat include α4β2, α4α5β2, α6β2(β3), and α4α6β2(β3). Function of nAChRs, measured by dopamine release, has been reported for α4-, α6-, β2-, and β3-null mutant mice, as well as α4α6 double-null mutants (Grady et al., 2001; Champtiaux et al., 2003; Cui et al., 2003). Selective loss of α-CtxMII–sensitive function is seen in α6−/− and β3−/− mice. Selective loss of α-CtxMII–resistant dopamine release is found in α4−/− mice, as well as virtually complete loss of all agonist-stimulated release activity in β2−/− and α4α6−/− mice. These results indicate that binding sites represent functional receptors.

We have evaluated the function of presynaptic nAChRs on striatal dopaminergic terminals derived from α4−/−, α5−/−, α7−/−, β2−/−, β3−/−, and β4−/− mice. Consistent with previous reports, our data suggest that as many as four different functional nAChRs are expressed in mouse striatal tissues. Two of these nAChRs are very sensitive to α-CtxMII inhibition (probably α4α6β2β3, α6β2β3) and two are not (probably α4β2, α4α5β2). In addition, α-CtxMII–sensitive and -resistant nAChRs have significant differences in pharmacological properties. The α-CtxMII–sensitive subtypes have very high affinity function, nearly full activity when stimulated with cytisine, and a different profile of antagonist inhibition.

Materials and Methods

Materials. [^{3}H]Dopamine was obtained from either PerkinElmer Life and Analytical Sciences (Boston, MA) (3,4-[ring-2,5,6-^{3}H] at 30–60 Ci/mmol) or Amersham Biosciences (Piscataway, NJ) (7,8-^{3}H at 40–60 Ci/mmol). HEPES and sucrose were products of Roche Applied Science (Indianapolis, IN). Sigma Chemical, Co. (St. Louis, MO) was the source for the following compounds: acetylcholine iodide (ACh), ascorbic acid, atropine sulfate, A85380, bovine serum albumin, cytisine, decamethonium chloride (DEC), dihydro-β-erythroidine (DHβE), diisopropyl fluorophosphate, (±)-epibatidine hydrochloride, methyllycaconitine (MLA), (−)-nicotine tartrate, nomifensine, and pargyline. α-CtxMII was synthesized as described previously (Cartier et al., 1996). All other chemicals were reagent grade. Econo-Safe scintillation fluid was purchased from Research Products International (Mt. Prospect, IL).

Animals. Animal care and experimental procedures were in accordance with the guidelines and approval of the Animal Care and Utilization Committee of the University of Colorado, Boulder. Female C57BL/6 mice (60–90 days of age), as well as all genotypes of the subunit null mutant mice, were bred at the Institute for Behavioral Genetics, University of Colorado (Boulder, CO), housed five to a cage with same-sex littermates, and maintained on a 12-h light/12-h dark cycle (lights on from 7 AM to 7 PM), at 22°C, with free access to food (Teklad Rodent Diet; Harlan, Madison, WI) and water. All nAChR subunit null mutant mice were bred onto the C57BL/6 strain for the indicated number of generations: β2 (Picciotto et al., 1995) six to eight generations; α4 (Ross et al., 2000), one generation; α5 (Salas et al., 2003), six generations; β4 (Xu et al., 1999), four to six generations; α7 (Orr-Urtreger et al., 1997), eight to nine generations; and β3 (Cui et al., 2003), nine generations. All breeding was conducted by mating heterozygotic male and female mice. Offspring were weaned at 25 days of age and housed with same-sex littermates, 2 to 5 to a cage, thereafter.

Genotype of above-mentioned mice was determined by polymerase chain reaction analysis of DNA obtained from tail clippings of mice at 40 days of age. DNA was extracted from these tail clippings using the DNeasy kit from QIAGEN (Valencia, CA) and analyzed by polymerase chain reaction for assignment of genotype.

The following primers were used to determine β2 genotype: β2 wild-type 5′, 5′-GCTTCAAACCTCTCCACGTC-3′; β2 wild-type 3′, 5′CGCCATAGAGTTGGAGCACC-3′; LacZ 5′, 5′ CACTACGTCTGAACGTCGAAAA-3′; and LacZ 3′, 5′- CGGGCAAATAATATCGGTGGC-3′. Amplification was achieved through the following protocol: 94° for 10 min, 94° for 30 s, 65° for 30 s (touch down, 2°/two cycles to 55°), 72° for 1 min; 30 cycles at 94° for 30 s, 55° for 30 s, and 72° for 1 min; 72° for 7 min; hold at 4°. Samples were then subjected to electrophoresis through a 1.5% agarose gel. The approximate sizes of the wild-type and mutant bands were 400 and 600 bp, respectively.

The following primers were used to determine α4 genotype: α4 wild-type 5′, 5′-CAATGTACACAACCGCTCAC-3′; α4 wild-type 3′, 5′-ACTGCTATTGGGTGGGTGAC-3′; Neo 5′, 5′-CTTGGGTGGAGAGGCTATTC-3′; and Neo 3′, 5′-AGGTGAGATGACAGGAGATC-3′. Amplification was achieved through the following protocol: 95° for 7 min, 25 cycles of 94° for 1 min, 55° for 2 min, 72° for 3 min, 72° for 7 min; hold at 4°. Samples were then subjected to electrophoresis through a 1.5% agarose gel. The approximate sizes of the wild-type and mutant bands were 385 and 280 bp, respectively.

The following primers were used to determine α5 genotype:

α5 wild-type 5′, 5′-CACTGTCACTTGGACGCAGCC-3′; α5 wild-type 3′, 5′-GTTCCCCTTGCTCCCCATTGC-3′; Neo-1, 5′-CTTTTTGTCAAGACCGACCTGTCCG-3′; and Neo-2, 5′-CTCGATGCGATGTTTCGCTTGGTG-3′. Amplification was achieved through the following protocol: 94° for 10 min, 94° for 30 s, 65° for 30 s (touch down, 2°/two cycles to 55°), 72° for 1 min; 30 cycles at 94° for 30 s, 55° for 30 s, and 72° for 1 min; 72° for 7 min; hold at 4°. Samples were then subjected to electrophoresis through a 1.5% agarose gel. The approximate sizes of the wild-type and mutant bands were 380 and 290 bp, respectively.

The following primers were used to determine β3 genotype: β3 5′, 5′-GGGCTCTCTCATGACCAAGG-3′; β3 3′, 5′-GTATCTGATGGACTCAGAGGCC-3′; LacZ 5′, 5′-CACTACGTCTGAACGTCGAAAA-3′; and LacZ 3′, 5′-CGGGCAAATAATATCGGTGGC-3′. Amplification was achieved through the following protocol: 94° for 10 min; 30 cycles of 94° for 1 min, 60° for 1 min, 72° for 1 min; 72° for 7 min; hold at 4°. Samples were then subjected to electrophoresis through a 1.5% agarose gel. The approximate sizes of the wild-type and mutant bands were 850 and 600 bp, respectively.

The following primers were used to determine β4 genotype: β4 forward, 5′-TGTAGAGCGAGCATCCGAACA-3′; β4 wild-type reverse, 5′-TCTCTACTTAGGCTGCCTGTCT-3′; and β4 mutant reverse, 5′-AGTACCTTCTGAGGCGGAAAGA-3′. Amplification was achieved through the following protocol: 94° for 10 min, 94° for 30 s, 65° for 30 s (touch down, 2°/two cycles to 55°), 72° for 1 min; 30 cycles at 94° for 30 s, 55° for 30 s, and 72° for 1 min; 72° for 7 min; hold at 4°. Samples were then subjected to electrophoresis through a 1.5% agarose gel. The approximate sizes of the wild-type and mutant bands were 300 and 150 bp, respectively.

The following primers were used to determine α7 genotype: α7 wild-type forward, 5′-CCTGGTCCTGCTGTGTTAAACTGCTTC-3′; α7 wild-type reverse, 5′-CTGCTGGGAAATCCTAGGCACACTTGAG-3′; and Neo (reverse), 5′-GACAAGACCGGCTTCCATCC-3′. Amplification was achieved through the following protocol: 94° for 10 min; 30 cycles of 94° for 1 min, 60° for 1 min, 72° for 1 min; 72° for 7 min; hold at 4°. Samples were then subjected to electrophoresis through a 1.5% agarose gel. The approximate sizes of the wild-type and mutant bands were 444 and 750 bp, respectively.

Synaptosome Preparation. After a mouse was sacrificed by cervical dislocation, its brain was removed and placed immediately on ice, and the striatum was dissected free of the remainder of the brain tissue. The striatal tissue from each mouse was homogenized in 0.5 ml of ice-cold 0.32 M sucrose buffered with 5 mM HEPES, pH 7.5. The P2 synaptosomal pellet was prepared by centrifugation at 1000*g* for 10 min followed by centrifugation of the first supernatant at 12,000*g* for 20 min. For many experiments, a P1 pellet was used that omitted the first lower speed centrifugation. Experiments with P1 or P2 pellets gave identical results. The pellets were resuspended in 0.8 ml (P2) or 1.6 ml (P1) of "uptake buffer": 128 mM NaCl, 2.4 mM KCl, 3.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM HEPES, pH 7.5, 10 mM glucose, 1 mM ascorbic acid, and 0.01 mM pargyline.

Uptake of [^{3}H]Dopamine. Synaptosomes were incubated at 37° in uptake buffer for 10 min before addition of 100 nM [^{3}H]dopamine (1 μCi for every 0.2 ml of synaptosomes), and the suspension was incubated for an additional 5 min.

Perfusion and Release. All experiments were conducted at room temperature using methods described previously (Grady et al., 1997, 2001). In brief, aliquots of synaptosomes (80 μl) were distributed onto filters and perfused with the perfusion buffer (uptake buffer containing 0.1% bovine serum albumin and 10 μM nomifensine) at 0.6 or 1 ml/min for 10 min before fractions were collected. For experiments using ACh as agonist, the synaptosomes were treated with diisopropyl fluorophosphate (10 μM) during the last 5 min of the uptake procedure, and atropine (1 μM) was added to the perfusion buffer. Fractions (0.3 ml) were collected every 18 or 30 s depending on the perfusion speed, and radioactivity was determined by scintillation counting (1600TR Liquid Scintillation Spectrometer; PerkinElmer Life and Analytical Sciences) after addition of Econo-Safe (Research Products International). Instrument efficiency was 45%.

Data Analysis. Data were fit to equations below using the curve-fitting algorithm of SigmaPlot 5.0 for DOS (SPSS Inc., Chicago, IL). Perfusion data were plotted as counts per minute versus fraction number. Fractions collected immediately before and after the peak were used to calculate baseline as a single exponential decay. The calculated baseline was subtracted from the data obtained when agonists were added. Fractions that exceeded baseline by 10% or more were summed to give total released cpm. Counts per minute released above baseline were normalized to baseline to give units of release [(cpm − baseline)/baseline] (Grady et al., 1997, 2001). When agonist exposure is prolonged (minutes), nicotinic agonist-induced dopamine release can be divided into two components, transient (rapidly desensitizing) and persistent (slowly desensitizing) (Grady et al., 1997). Transient and persistent release parameters were calculated by fitting the data to a double exponential decay equation (Grady et al., 1997). Onset of and recovery from the effects of α-CtxMII were calculated from single exponential equations: release = $R_0(e^{-kt}) + R_r$ and release = $R_0(1 - e^{-kt}) + R_r$, where R_0 is initial release and R_r is the portion of release uninhibited by α-CtxMII. Agonist dose response data were fit to the Hill equation using SigmaPlot 5.0 DOS version (SPSS Inc.) (Grady et al., 2001). Errors for EC_{50} values were assessed by fitting the data to the Hill equation with R_{max} and n_H fixed to values obtained from the initial fit to the Hill equation. Errors for ratios were calculated using Taylor's expansion. IC_{50} values for inhibition of release were calculated by fitting the data to single-site inhibition (release = $R_0/(1 + [An]/IC_{50})$ where R_0 is uninhibited release and [An] is the antagonist concentration) (Grady et al., 2001). K_i values were determined by fitting the data using SigmaPlot 5.0 DOS version and assuming inhibitors were competitive (release = $(R_{max})([L\text{-nicotine}]/(EC_{50}(1 + [I]/K_i) + [L\text{-nicotine}]))$ using the EC_{50} values for nicotine previously determined (Table 1).

Results

Effect of α-CtxMII on Dopamine Release. To investigate the kinetics of inhibition by α-CtxMII, a concentration of 30 nM α-CtxMII was chosen for preliminary experiments of inhibition of dopamine release from mouse striatal synaptosomes based on studies done with different nAChR subtypes expressed in *X. laevis* oocytes (Cartier et al., 1996). This concentration has been reported to inhibit nicotinic receptors with high affinity for α-CtxMII and minimally affect those with lower affinity, such as the α4β2 subtype of nicotinic receptor (Cartier et al., 1996). Figure 1 presents the onset of, and recovery from, α-CtxMII inhibition. Synaptosomes were treated with 30 nM α-CtxMII for 0, 0.5, 1, 2, 5, or 10 min before exposure to L-nicotine (1 μM) plus α-CtxMII (30 nM) for 1 min. [^{3}H]Dopamine release under these conditions was compared with that elicited by L-nicotine alone. α-CtxMII produced its maximum effect quickly, with a $t_{1/2}$ of 0.69 min. For recovery experiments, synaptosomes were exposed to 30 nM α-CtxMII for 5 min followed by buffer without α-CtxMII for the indicated time intervals before the L-nicotine stimulation. The release evoked by L-nicotine under these conditions was compared with that evoked from synaptosomes that had not been exposed to α-CtxMII. Recovery is quite slow; calculated $t_{1/2}$ is 25.7 min. α-CtxMII had no stimulatory or inhibitory effect on the baseline release of [^{3}H]dopamine and no effect on release evoked by 20 mM K^+ (data not shown). From these data, it was determined that 5 min of

exposure was sufficient to ensure maximal inhibition, and that for a short exposure to agonist (1 min or less), recovery from inhibition should not occur even if α-CtxMII is not included with the agonist.

An inhibition curve for determining the potency and efficacy for α-CtxMII is shown in Fig. 2. For this curve, release was evoked with 10 μM L-nicotine for 18 s. The IC_{50} value is 2.2 ± 0.5 nM; maximum inhibition is 31%. Experiments done using different concentrations of L-nicotine (data not shown) revealed that the IC_{50} values for α-CtxMII did not differ with varying L-nicotine concentration. The mean of IC_{50} values determined at six L-nicotine concentrations (ranging from 0.1–30 μM) was 2.8 ± 0.9 nM. This result does not imply that α-CtxMII is acting noncompetitively, but rather reflects the fact that samples were exposed to α-CtxMII before L-nicotine, and the toxin has a slow off-time (Fig. 1 and Whiteaker et al., 2000).

Pharmacology of α-CtxMII–Sensitive and -Resistant nAChRs. The potency and efficacy of a number of nicotinic agonists for [^{3}H]dopamine release mediated by the α-CtxMII–sensitive and -resistant nAChRs were investigated by constructing dose response curves with and without prior exposure to a maximally effective concentration of α-CtxMII (50 nM) for 5 min (Fig. 3); a to e show response to ACh, A85380, cytisine, epibatidine, and nicotine, respectively, for α-CtxMII–resistant and -sensitive portions of stimulated dopamine release. The data were fit to the Hill equation, and the parameters calculated are presented in Table 1. The rank order of potency for α-CtxMII–sensitive release (epibatidine > A85380 > cytisine > ACh > nicotine) differed slightly from the rank order for α-CtxMII–resistant release (A85380 > epibatidine > cytisine > ACh > nicotine) in that epibatidine and A85380 changed positions in the rank order. However, the EC_{50} values for all of the agonists were significantly lower for the α-CtxMII–sensitive release and the ratio of EC_{50} values for α-CtxMII–resistant to α-CtxMII–sensitive dopamine release for the five agonists varied considerably (from 2 for nicotine to 15 for epibatidine and cytisine). The rank order of efficacy, or maximal release (R_{max}), differed for the two components of the release process: (α-CtxMII–sensitive: epibatidine > nicotine > A85380 ≅ ACh > cytisine; α-CtxMII–resistant: epibatidine > ACh > nicotine ≅ A85380 ≫ cytisine). Compared with ACh, nicotine and A85380 were approximately equally efficacious, and epibatidine was somewhat more efficacious, for both α-CtxMII–sensitive and -resistant release. However, cytisine was a partial agonist for the α-CtxMII–resistant release (33% as efficacious as acetylcholine) with a low Hill coefficient. In contrast, for the α-CtxMII–sensitive response, it was nearly as efficacious as acetylcholine (85%).

Three nicotinic antagonists, DEC, MLA, and DHβE, were investigated for their effects on the α-CtxMII–resistant and -sensitive portions of [^{3}H]dopamine release. Inhibition of dopamine release by each antagonist was evaluated using three L-nicotine concentrations (1, 3, and 10 μM) and the results are shown in Fig. 4. All of the antagonists totally blocked both α-CtxMII–sensitive and -resistant release at each nicotine concentration. The antagonists seem to be competitive inhibitors, as demonstrated by the finding that the IC_{50} values for all three antagonists increased with increasing agonist concentration. The IC_{50} values and K_i values, calculated assuming competitive inhibition, are presented in Table 2. K_i values for MLA and DHβE differed significantly for inhibition of α-CtxMII–sensitive and -resistant dopamine release stimulated by nicotine. The rank order of antagonist potency also differed when K_i values were compared (α-CtxMII–sensitive release, MLA < DHβE < DEC; α-CtxMII–resistant release, DHβE < MLA < DEC). In addition, the ratios of K_i values differed considerably; MLA is a 9-fold more potent inhibitor of α-CtxMII–sensitive release, and DHβE is a 7-fold more potent inhibitor of α-CtxMII–resistant release.

Effect of α-CtxMII on Transient and Persistent Release. Agonist-stimulated dopamine release occurs in two phases, a larger but more rapidly desensitizing phase (transient release) and a sustained low level release (persistent release) (Rowell, 1995; Grady et al., 1997). Persistent release

TABLE 1
EC_{50} values and R_{max} values for α-CtxMII–sensitive and -resistant agonist-stimulated dopamine release

	EC_{50}	R_{max}	n_H
	μM	*units*	
α-CtxMII-resistant			
A85380	0.0038 ± 0.0001	8.19 ± 0.11	1.13 ± 0.05
Acetylcholine	1.02 ± 0.06	9.45 ± 0.31	0.91 ± 0.07
Cytisine	0.47 ± 0.09	3.10 ± 0.55	0.41 ± 0.08
(±)-Epibatidine	0.012 ± 0.001	13.18 ± 1.08	0.75 ± 0.12
L-Nicotine	1.61 ± 0.19	8.48 ± 0.39	1.35 ± 0.26
α-CtxMII-sensitive			
A85380	0.0013 ± 0.0001***	3.55 ± 0.20	0.90 ± 0.12
Acetylcholine	0.15 ± 0.08***	3.51 ± 0.59	0.86 ± 0.53
Cytisine	0.031 ± 0.011**	3.00 ± 0.21	1.01 ± 0.40
(±)-Epibatidine	0.00078 ± 0.00021***	4.98 ± 0.52	0.76 ± 0.24
L-Nicotine	0.77 ± 0.27*	4.20 ± 0.65	0.63 ± 0.25

	EC_{50} Ratios Resistant/Sensitive	R_{max} (Relative to ACh)	
		Resistant	Sensitive
A85380	2.9 ± 0.2	0.87 ± 0.04	1.01 ± 0.18
Acetylcholine	6.8 ± 3.6	1.00 ± 0.05	1.00 ± 0.24
Cytisine	15.2 ± 6.1	0.33 ± 0.06	0.85 ± 0.16
(±)-Epibatidine	15.4 ± 4.3	1.39 ± 0.12	1.42 ± 0.28
L-Nicotine	2.1 ± 0.8	0.90 ± 0.05	1.20 ± 0.21

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by t test comparing EC_{50} values for α-CtxMII–resistant and -sensitive agonist-stimulated dopamine release.

is supported by very low concentrations of agonist with EC_{50} values similar to those measured for α-CtxMII–sensitive dopamine release. Therefore, it seemed possible that the α-CtxMII–sensitive release might coincide with persistent release, whereas α-CtxMII–resistant release might be equivalent to the transient release. Figure 5 compares synaptosomes perfused with α-CtxMII (30 nM) for 5 min before treatment with L-nicotine (1 μM) and α-CtxMII for 10 min with those perfused with buffer before L-nicotine alone. Treatment with α-CtxMII decreased transient release, elicited by 1 μM L-nicotine, by 45%, and persistent release by 55%. Therefore, both receptor classes support both phases of release. IC_{50} values for α-CtxMII inhibition of transient and persistent release were determined by exposing synaptosomes to various concentrations of α-CtxMII for 5 min before and during stimulation with 1 μM L-nicotine for 10 min. Two plots were constructed from the results of these experiments (Fig. 6), the first as the transient response obtained from the double-exponential curve fit procedure and the second as the persistent response, also derived from the curve fit. The IC_{50} values are similar: 1.3 ± 1.1 nM for the transient response and 1.1 ± 0.8 nM for the persistent response, not different from the value determined for short exposures to nicotine 2.2 ± 0.5 nM (see Fig. 2).

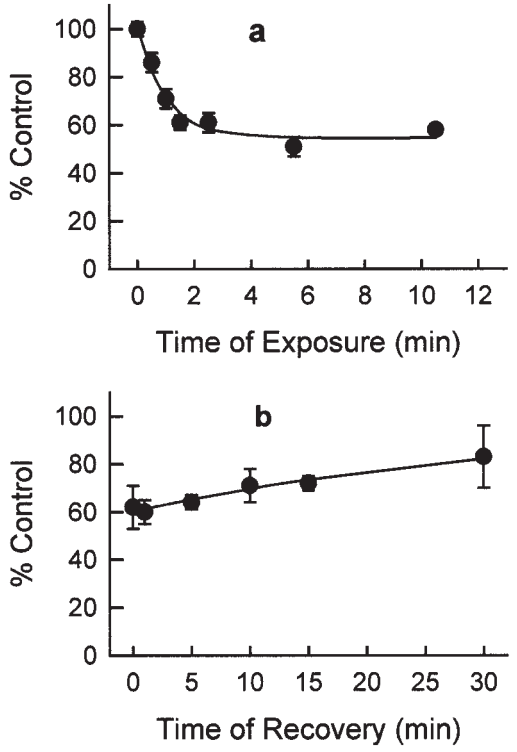

Fig. 1. Onset of inhibition and recovery from inhibition by α-CtxMII. a, dopamine release was stimulated by L-nicotine (1 μM for 1 min) with the indicated length of exposure to α-CtxMII (30 nM). The amount of dopamine release was compared with parallel filters where release was stimulated by nicotine alone without exposure to α-CtxMII (0 min of exposure time). The K_{onset} is 1.01 ± 0.21/min; $t_{1/2}$ = 0.69 min. b, synaptosomes were perfused for 5 min with α-CtxMII (30 nM) followed by perfusion buffer for the indicated interval. Dopamine release was then stimulated by L-nicotine (1 μM for 1 min) and compared with filters perfused for the same length of time without the exposure to α-CtxMII. The $K_{recovery}$ is 0.027 ± 0.002/min; $t_{1/2}$ = 25.7 min.

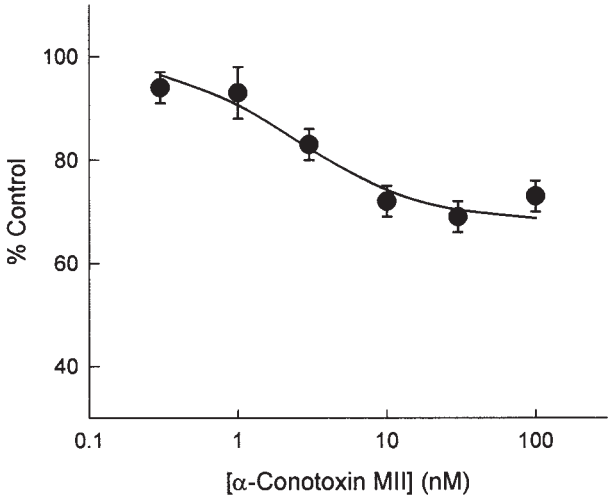

Fig. 2. Inhibition of dopamine release by α-CtxMII. Synaptosomes were perfused with α-CtxMII of the indicated concentration for 5 min just before an 18-s exposure of L-nicotine (10 μM). n = 5 experiments. IC_{50} = 2.2 ± 0.5 nM; maximum inhibition = 31.0 ± 3.8%.

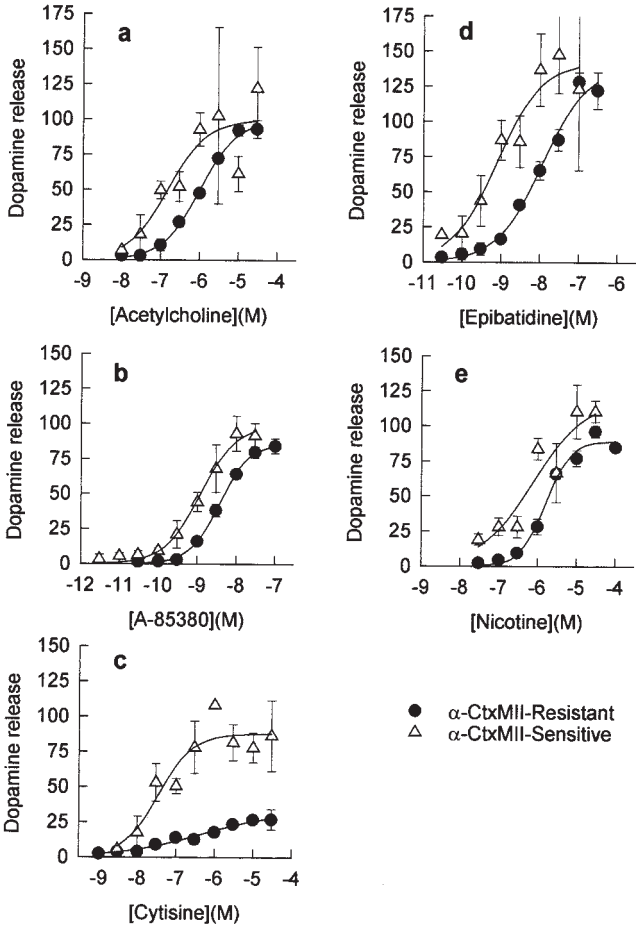

Fig. 3. Stimulation of dopamine release by various nicotinic agonists. Synaptosomes were perfused with buffer or α-CtxMII (50 nM) for 5 min before an 18-s exposure to the indicated concentration of agonist. Dopamine release resistant to inhibition by α-CtxMII was determined by release stimulated after exposure to α-CtxMII. The amount of nAChR-mediated dopamine release sensitive to α-CtxMII was determined from the difference between release evoked with and without prior exposure to α-CtxMII. All data are shown as percentage maximal response to ACh. Lines are curve fits to the Hill equation. n = 3 to 9 animals assayed per point. Errors shown are SEM. a, b, c, d, and e show release stimulated by ACh, A-85380, cytisine, epibatidine, and nicotine, respectively.

Effects of Subunit Deletion on α-CtxMII–Resistant [^{3}H]Dopamine Release. For measurement of α-CtxMII–resistant dopamine release, samples of synaptosomes from the wild-type (+/+), heterozygous (+/−), and homozygous (−/−) null mutant mice for six nAChR subunits were assayed with exposure to α-CtxMII before stimulation of dopamine release by acetylcholine, the natural agonist. Synaptosomes were treated with α-CtxMII (50 nM) for 5 min before agonist stimulation. This procedure ensured maximal inhibition by α-CtxMII. Figure 7 presents the dose-response curves for ACh-induced α-CtxMII–resistant dopamine release. None of the R_{max} values determined in wild-type mice were different from that determined in C57BL/6 mice (Table 1). All α5 genotypes had an EC_{50} value for ACh that was slightly lower

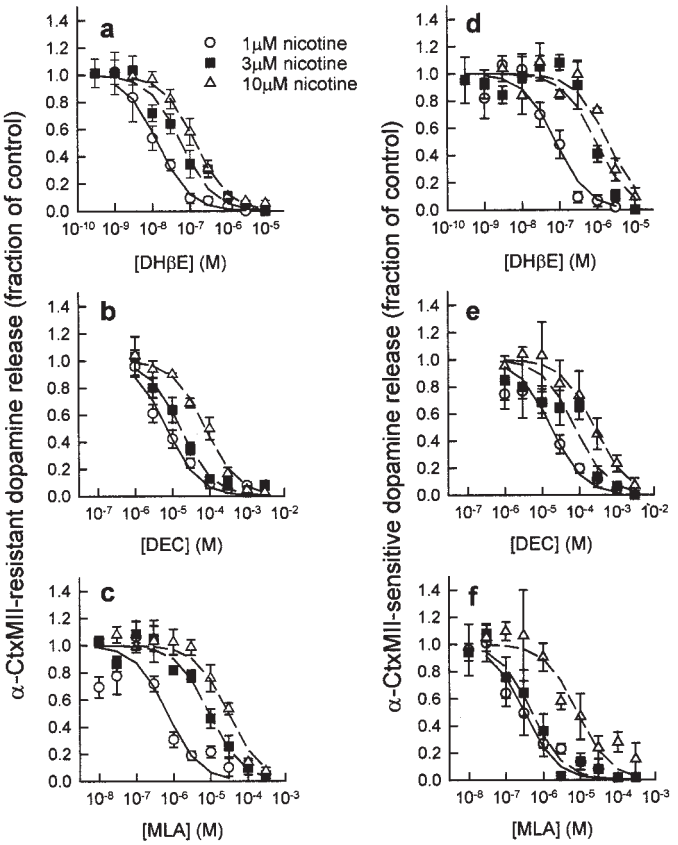

Fig. 4. Inhibition of nicotine-stimulated dopamine release by nicotinic antagonists. a, b, and c present data on inhibition by DHβE, DEC, and MLA, respectively, of α-CtxMII–resistant dopamine release determined from synaptosomes perfused with α-CtxMII (50 nM) for 5 min before an 18-s exposure to the indicated concentration of L-nicotine with or without antagonist. d, e, and f show data for inhibition by DHβE, DEC, and MLA, respectively for the portion of dopamine release sensitive to α-CtxMII, determined by difference. $n = 3$ to 4 animals assayed per point; error bars show S.E.M.

TABLE 2

IC_{50} values and calculated K_i values for antagonist inhibition of nicotine-stimulated dopamine release

	Inhibition of α−CtxMII–Resistant Response	Inhibition of α−CtxMII–Sensitive Response
	μM	*μM*
DHβE		
@ 10 μM NIC	0.141 ± 0.013	1.86 ± 0.68
@ 3 μM NIC	0.059 ± 0.015	0.92 ± 0.34
@ 1 μM NIC	0.014 ± 0.003	0.079 ± 0.029
K_i value	0.020 ± 0.004	0.122 ± 0.040*
DEC		
@ 10 μM NIC	78.9 ± 11.0	257 ± 40
@ 3 μM NIC	16.8 ± 3.6	77.1 ± 40.4
@ 1 μM NIC	7.2 ± 2.3	17.5 ± 7.1
K_i value	9.6 ± 2.5	12.0 ± 3.5
MLA		
@ 10 μM NIC	30.3 ± 5.7	8.32 ± 3.16
@ 3 μM NIC	9.24 ± 2.02	0.49 ± 0.16
@ 1 μM NIC	0.69 ± 0.41	0.32 ± 0.14
K_i value	1.02 ± 0.93	0.070 ± 0.018***

*, $P < 0.05$; ***, $P < 0.001$ by t test comparing K_i values for inhibition of α-CtxMII–resistant and -sensitive nicotine-stimulated dopamine release.

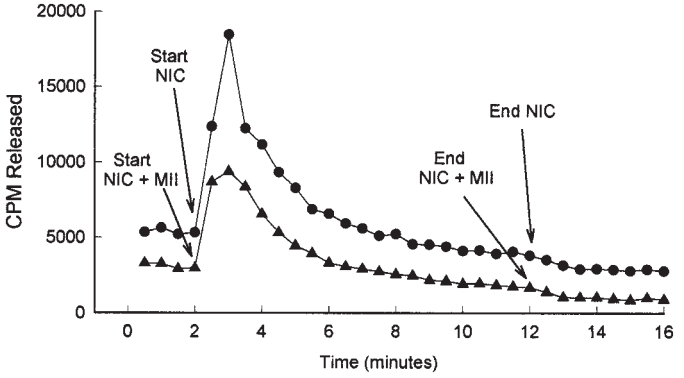

Fig. 5. Effect of α-CtxMII on transient and persistent nicotine-stimulated dopamine release. Representative data from two filters are shown. Striatal synaptosomes previously loaded with [^{3}H]dopamine were perfused with buffer at 0.6 ml/min for 10 min before collecting fractions of 30 s each. The control filter (no exposure to α-CtxMII) has been offset by 2000 cpm for clarity. Perfusion with α-CtxMII (30 nM) was started 5 min before L-nicotine for the filter with α-CtxMII treatment. Perfusion with α-CtxMII plus L-nicotine (1 μM) or L-nicotine alone was started and ended where indicated. Transient release from the synaptosomes without α-CtxMII treatment was 4.88 units; in the α-CtxMII–treated synaptosomes, transient release was reduced to 2.68 units (a 45% decrease). The persistent phase of release was 0.76 units in the untreated synaptosomes, whereas in the treated synaptosomes, there were 0.34 units of persistent release (a 55% decrease).

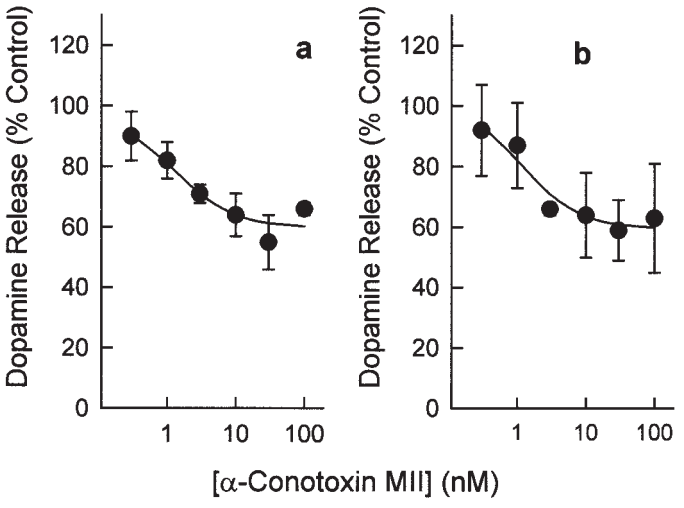

Fig. 6. Inhibition of transient and persistent dopamine release by α-CtxMII. Aliquots of synaptosomes were perfused (0.6 ml/min) for 5 min with varying concentrations of α-CtxMII, after which time they were perfused with α-CtxMII of the same concentration containing 1 μM L-nicotine for 10 min. Perfusion was continued with buffer only for an additional 5 min. Fractions were collected every 30 s. See Fig. 5 for representative experiment. Data are presented as percentage of control. Transient dopamine release was determined by curve fit. The IC_{50} value is 1.3 ± 1.1 nM with a maximum inhibition of 38 ± 10%. Persistent release, also determined by curve fit, resulted in an IC_{50} value of 1.1 ± 0.8 nM with a maximum inhibition of 44 ± 10%.

than that measured for C57BL/6 mice; no other genotypes differed from the C57BL/6 mice. Four of the null mutants showed changes in R_{max} for the α-CtxMII–resistant component of dopamine release. Both β2-and α4-null mutation abolished all of the α-CtxMII–resistant dopamine release whereas α5-null mutation resulted in a significant (63%) decrease in α-CtxMII–resistant dopamine release. In contrast, α-CtxMII–resistant dopamine release increased somewhat in β3−/− mice. No change was seen in this component of dopamine release in either β4−/− or α7−/− mice compared with corresponding wild-type mice. The α-CtxMII–resistant dopamine release was similar in mice heterozygous for each of these subunits compared with the appropriate wild-type mice, except for β2+/− mice, which tended to be lower, but this effect was not significant for this data set. None of the gene deletions resulted in a change in the EC_{50} value for ACh compared with the wild-type for each mutation. Table 3 presents a summary of the effects of different null mutations on the EC_{50} values, R_{max} values and Hill coefficients.

Effects of Subunit Deletion on α-CtxMII–Sensitive [^{3}H]Dopamine Release. For measurement of α-CtxMII–sensitive dopamine release, parallel samples of synaptosomes were assayed without exposure to α-CtxMII along with those with exposure to α-CtxMII (see above paragraph). Release sensitive to α-CtxMII was determined by the difference between these two conditions. Figure 8 presents the effects of the gene deletions on α-CtxMII–sensitive dopamine release. R_{max} and EC_{50} values for the six sets of wild-type mice did not differ from those determined in C57BL/6 mice (Table 1). Four of the null mutations differed in R_{max} for ACh-stimulated α-CtxMII–sensitive dopamine release. β2-null mutation resulted in complete loss of α-CtxMII–sensitive dopamine release and α4 gene deletion decreased the α-CtxMII–sensitive component of dopamine release by 55%. In α5-null mutant mice, the α-CtxMII–sensitive release was slightly elevated to 162% of wild type. The β3-null mutation resulted in a 76% decrease from wild type. Neither β4 nor α7 gene deletion altered this component of dopamine release. The β2+/− is the only heterozygous genotype that exhibited a change (50% decrease) in α-CtxMII–sensitive dopamine release. Table 4 presents a summary of the effects of different null mutations on the EC_{50} values, R_{max} values, and Hill coefficients.

Effects of Subunit Deletion on K^+-Stimulated [^{3}H]Dopamine Release. The potential effects of α4, α5, α7, β2, β3, and β4 gene deletion on dopamine release stimulated by 20 mM potassium ion (K^+) were also determined (data not shown). Neither the gene deletions nor the different genotypes changed the K^+-induced dopamine release with or without α-CtxMII pretreatment. Furthermore, baselines and counts per minute remaining on filters were not affected by any of the genotypes or null mutations (data not shown). These results indicate that each genotype in each null mutant mouse line has normal synaptic vesicle-mediated release stimulated by membrane depolarization.

Discussion

In the present study, we analyzed the pharmacology of two classes of nAChRs differentiated by the ability of αCtxMII to inhibit agonist-mediated dopamine release. In addition, the effect of deletion of nAChR subunits (α4, α5, α7, β2, β3, and β4) was evaluated to elucidate potential subunit composition of these functional receptors.

α-CtxMII inhibited nicotine-stimulated [^{3}H]dopamine release from mouse striatal synaptosomes by 35 to 45%, results similar to previous studies with rat or mouse (Kulak et al., 1997; Kaiser et al., 1998; Champtiaux et al., 2003). The IC_{50} value of 1 to 3 nM and the kinetics of onset ($t_{1/2}$ of 0.69 min

TABLE 3
The effect of genotype on α-CtxMII resistant dopamine release from mouse striatal synaptosomes
The R_{max}, EC_{50}, and n_H values of each genotype are presented. Data are presented as mean ± S.E.M.

	n	R_{max}	EC_{50}	n_H
		units	*μM*	
α4 genotype				
+/+	4	11.31 ± 0.75	1.13 ± 0.24	0.87 ± 0.09
+/−	6	9.42 ± 0.82	0.86 ± 0.24	0.91 ± 0.15
−/−	4	0.21 ± 1.06		
α5 genotype				
+/+	6	9.54 ± 0.40	0.57 ± 0.08	1.05 ± 0.11
+/−	3	8.40 ± 0.11	0.41 ± 0.02	1.59 ± 0.09
−/−	6	3.54 ± 0.25	0.42 ± 0.10	1.30 ± 0.30
α7 genotype				
+/+	7	9.22 ± 0.66	0.98 ± 0.22	0.95 ± 0.13
+/−	4	7.93 ± 0.50	0.71 ± 0.13	1.15 ± 0.18
−/−	7	8.28 ± 0.63	0.52 ± 0.13	1.11 ± 0.23
β2 genotype				
+/+	5	9.80 ± 0.87	1.02 ± 0.28	0.96 ± 0.16
+/−	5	7.72 ± 0.81	1.43 ± 0.45	1.15 ± 0.18
−/−	3	0.20 ± 0.07		
β3 genotype				
+/+	4	9.89 ± 0.56	0.82 ± 0.15	0.99 ± 0.11
+/−	4	11.10 ± 0.68	0.72 ± 0.14	0.97 ± 0.13
−/−	5	12.09 ± 0.27	0.70 ± 0.54	0.87 ± 0.04
β4 genotype				
+/+	3	9.57 ± 1.27	1.21 ± 0.54	0.80 ± 0.15
+/−	3	9.10 ± 0.81	1.29 ± 0.35	0.92 ± 0.13
−/−	3	10.00 ± 1.78	1.36 ± 0.87	0.71 ± 0.15

TABLE 4
The effect of genotype on α-CtxMII sensitive dopamine release from mouse striatal synaptosomes
R_{max}, EC_{50}, and n_H values of each genotype are presented. Data are presented as mean ± S.E.M.

	n	R_{max}	EC_{50}	n_H
		units	*μM*	
α4 genotype				
+/+	4	2.96 ± 0.13	0.11 ± 0.02	1.34 ± 0.26
+/−	6	4.11 ± 0.38	0.18 ± 0.07	0.95 ± 0.27
−/−	4	1.33 ± 0.12	0.41 ± 0.25	1.04 ± 0.25
α5 genotype				
+/+	6	3.41 ± 0.20	0.09 ± 0.02	1.41 ± 0.40
+/−	3	4.29 ± 0.25	0.09 ± 0.02	1.24 ± 0.31
−/−	6	5.53 ± 0.28	0.22 ± 0.04	1.16 ± 0.21
α7 genotype				
+/+	6	3.48 ± 0.36	0.23 ± 0.09	0.90 ± 0.25
+/−	4	5.01 ± 0.95	0.45 ± 0.35	0.71 ± 0.24
−/−	8	3.09 ± 0.43	0.19 ± 0.12	0.72 ± 0.24
β2 genotype				
+/+	5	3.40 ± 0.32	0.22 ± 0.08	1.11 ± 0.35
+/−	5	1.59 ± 0.15	0.11 ± 0.04	1.55 ± 0.76
−/−	3	0.15 ± 0.02		
β3 genotype				
+/+	4	3.10 ± 0.20	0.10 ± 0.02	1.21 ± 0.26
+/−	4	1.70 ± 0.36	0.05 ± 0.04	1.24 ± 1.05
−/−	5	0.75 ± 0.09	0.13 ± 0.05	1.65 ± 0.87
β4 genotype				
+/+	3	3.73 ± 0.24	0.19 ± 0.04	1.90 ± 0.62
+/−	3	2.76 ± 0.37	0.17 ± 0.09	1.06 ± 0.48
−/−	3	2.94 ± 0.23	0.08 ± 0.03	1.13 ± 0.37

at 30 nM) and recovery ($t_{1/2}$ of 25.7 min) are similar to those determined for functional measures of rat $\alpha6\beta2^*$ nAChRs expressed in oocytes (Dowell et al., 2003; McIntosh, 2004). Agonist-stimulated dopamine release occurs in two phases, a rapidly desensitizing phase (transient) and a sustained, low level, release (persistent) (Rowell, 1995; Grady et al., 1997), probably a result of activation of different receptor states as proposed by the two-state receptor model of Katz and Thesleff (1957). Both the transient and persistent phases of nicotine-stimulated dopamine release were partially inhibited by α-CtxMII, with identical IC_{50} values. This result indicates that both transient and persistent phases of presynaptic dopamine release are mediated by at least two subclasses of nAChRs (those that are inhibited by α-CtxMII and those that are not).

The rank order of potency of five agonists tested for stimulating α-CtxMII–sensitive and -resistant dopamine release differed only for epibatidine and A85380, suggesting that the receptors that mediate this neurotransmitter release have similar pharmacology. However, all agonists tested were significantly more potent for activation of the α-CtxMII–sensitive component and, in addition, the relative potencies for activation of α-CtxMII–sensitive and -resistant components varied considerably (from 2 for nicotine to 15 for epibatidine and cytisine), demonstrating that there are significant pharmacological differences for the two components. Epibatidine

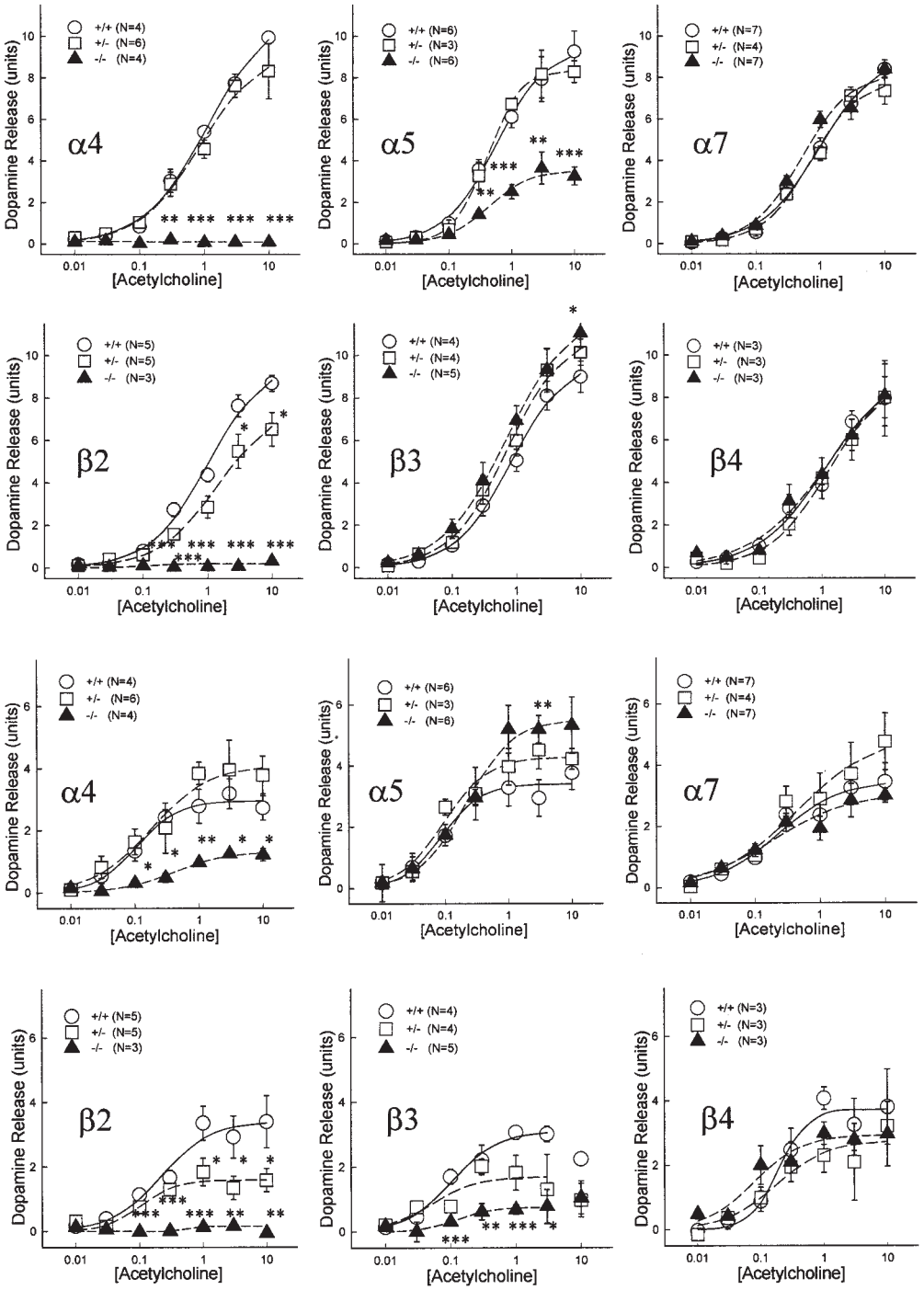

Fig. 7. Effects of subunit deletion on α-CtxMII–resistant dopamine release. Aliquots of synaptosomes prepared from the indicated genotypes of mouse striatal tissue were perfused (1 ml/min) for 5 min with α-CtxMII (50 nM) before exposure to ACh for 18 s to stimulate release. n = number of mice assayed. Data points are means ± S.E.M. One-way analysis of variance followed by least-significant-difference post hoc test indicated some significant effects of genotype; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Fig. 8. Effects of subunit deletion on α-CtxMII–sensitive dopamine release. Aliquots of synaptosomes prepared from the indicated genotypes of mouse striatal tissue were perfused (1 ml/min) for 5 min with and without α-CtxMII (50 nM) before exposure to ACh for 18 s to stimulate release. The portion of stimulated release sensitive to α-CtxMII was determined by difference. n = number of mice assayed. Data points are means ± S.E.M. One-way analysis of variance followed by least-significant-difference post hoc test indicated some significant effects of genotype; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

was the most efficacious agonist tested for both responses and cytisine the least. However, although cytisine was the least efficacious compound for stimulating both release responses, it was a weak partial agonist for the α-CtxMII–resistant release (33% of ACh) and a nearly full agonist for α-CtxMII–sensitive response (85% of ACh). The order of potency of three nicotinic antagonists tested differed for the two classes of nAChRs, and K_i values differed for DHβE and MLA. DHβE was 7-fold more potent for the α-CtxMII–resistant subtype and MLA was 9-fold more potent at inhibiting the α-CtxMII–sensitive release. Thus, the nAChRs that mediate the α-CtxMII–sensitive and -resistant components of dopamine release from mouse striatal synaptosomes clearly have different pharmacology.

Combining genetic and pharmacological tools, we were able to examine which subunits are involved in the two classes of functional, presynaptic receptors. As reported previously, mice homozygous for the β2-null mutation no longer have any functional, presynaptic nAChRs on dopaminergic terminals of the striatum (Grady et al., 2001). The β2 subunit seems, therefore, to be a necessary component of all these receptors. Mice heterozygous for the β2 subunit exhibit decreased α-CtxMII–sensitive dopamine release (47% of +/+) and only slightly decreased α-CtxMII–resistant release (79% of +/+). Perhaps under conditions of limited numbers of β2 subunits, the α-CtxMII–resistant receptors are preferentially assembled. On the other hand, the function of α-CtxMII–resistant receptors may not be decreased much because of spare receptor phenomena (i.e., when the maximum response can be elicited by activation of fewer receptors than are actually present in the wild-type mouse). In such a case, loss of some receptors may not result in the expected loss of response.

Mice homozygous for the α7- or the β4-null mutation do not differ from wild-type mice in either class of agonist-stimulated dopamine release. Consequently, neither of these subunits is an obligatory component of the dopaminergic, presynaptic receptor pool. These results are not unexpected. α-Bungarotoxin does not inhibit agonist-stimulated dopamine release from synaptosomes (Grady et al., 1997). The homomeric α7 subtype is inhibited by low nanomolar concentrations of MLA that do not significantly inhibit dopamine release (K_i values for MLA of 2.62 μM and 280 nM for the two components; Table 2). Therefore, the homomeric α7 subtype is not likely to mediate this response. The very low β4-mRNA levels in substantia nigra (Azam et al., 2002; Klink et al., 2001), indicate that the β4 subunit is not a likely component of many of the presynaptic receptors mediating dopamine release. Indeed, α-conotoxin AuIB, selective for the α3β4 subtype of nAChR (Luo et al., 1998), does not inhibit dopamine release (Grady et al., 2001).

Deletion of α4, α5, or β3 nAChR subunits have selective effects on nAChR-mediated dopamine release, indicating that each of these subunits is included in specific subsets of receptor populations.

Deletion of the α4 subunit eliminates α-CtxMII–resistant dopamine release. This result substantiates the loss of α-CtxMII–resistant dopamine release function reported in another line of α4-null mutant mice (Champtiaux et al., 2003). Therefore, α-CtxMII–resistant nAChRs presynaptic to dopaminergic terminals are all of the α4β2* subtype. In addition, the α4 subunit deletion affects a portion of the α-CtxMII–sensitive response, consistent with the decrease in α-CtxMII binding reported by Marubio et al. (2003). α4β2 nAChR does not have a high affinity for α-CtxMII (Cartier et al., 1996), thus α-CtxMII–sensitive receptors that contain α4 and β2 subunits must contain other subunits as well.

The β3 subunit seems to be a component of most of the α-CtxMII–sensitive nAChRs on dopaminergic terminals. β3 −/− mice showed substantially decreased α-CtxMII–sensitive ACh-evoked dopamine release (24% of +/+), whereas α-CtxMII–resistant ACh-stimulated response was increased (122% of +/+). These results are consistent with values obtained using nicotine as agonist (α-CtxMII–sensitive, 20% of +/+; α-CtxMII–resistant, 193% of +/+ reported by Cui et al., 2003). The increase in the α-CtxMII–resistant response is, perhaps, some type of physiological compensation.

Deletion of the α5 subunit decreased α-CtxMII–resistant release by 63%, indicating that in the wild-type mouse, a substantial portion of the α4β2* nAChR that mediates dopamine release is α4α5β2 nAChR. It is possible that in the wild-type mouse, all of the α-CtxMII–resistant nAChR is α4α5β2 nAChR and that some α4β2 nAChR is produced in α5−/− mice instead of the normal α4α5β2 nAChR. However, immunoprecipitation studies in the rat indicate that a considerable population of α4β2 without α5 does occur in dopaminergic terminals (Zoli et al., 2002). Therefore, the remaining α-CtxMII–resistant activity in α5-null mutant mice may not represent a compensatory change. The α-CtxMII–sensitive portion of dopamine release measured in the homozygous α5 null mutant is increased (162% of control), indicating that some amount of functional compensation can occur (also seen with the β3 null mutation), perhaps as a result of extra α4 subunits, which can then assemble as other receptor subtypes.

Combining the data from these six null mutant mouse lines with data published previously on α6- (Champtiaux et al., 2002, 2003) and α3- (Whiteaker et al., 2002) null mutants demonstrates that functional receptors for both the α-CtxMII–sensitive and -resistant classes are heterogeneous and that α4α5β2 nAChRs and α4β2 nAChRs comprise the α-CtxMII–resistant class of receptors, whereas α6β3β2 and α4α6β3β2 nAChRs with possibly a small amount of α6β2 or α4α6β2 nAChRs comprise the α-CtxMII–sensitive class. Data from these functional assays agree with subunit compositions determined by immunological analyses (Zoli et al., 2002).

In summary, pharmacological differences for the α-CtxMII–sensitive and -resistant nAChRs, which mediate presynaptic dopamine release, indicate that there are multiple nAChRs at dopaminergic nerve terminals, and that these different subtypes of nAChRs have different pharmacological properties. Although variation in potency, rank order, and efficacy of agonists and antagonists may not predict physiologically important functional differences for the nAChR subtypes (Le Novere et al., 2002), these differences do indicate that it may be possible to selectively activate, inhibit, or desensitize the various nAChRs. The α-CtxMII–sensitive nAChRs show a distinct and limited anatomical distribution (Whiteaker et al., 2000) and have the highest affinity functional activation for nAChRs in the brain identified to this point. All dopaminergic terminal regions display significant expression of these receptors. Within at least one of these dopaminergic regions, the striatum, it seems that the α-CtxMII–sensitive nAChR population is restricted to the

dopaminergic terminals, and this receptor subtype(s) is not found on terminals that release other neurotransmitters (Zoli et al., 2002; Champtiaux et al., 2003; Quik et al., 2003). The α-CtxMII–sensitive α6* nAChRs also seem to be sparse in the dopaminergic cell body regions, SN/VTA (Champtiaux et al., 2003). Drugs selective for various nAChR subtypes could influence selective sets of neurons and possibly certain regions of neurons. Therefore, by selective activation or inhibition of the subtypes sensitive to α-CtxMII, dopamine release might be modulated without affecting release of other neurotransmitters. This possibility of selectively targeting certain classes of neurons might produce therapeutically useful treatments for conditions in which dopaminergic neurons have a role, such as drug abuse or Parkinson's disease.

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