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α-Conotoxin MII Blocks Nicotine-Stimulated Dopamine Release in Rat Striatal Synaptosomes

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Activation of presynaptic nicotinic acetylcholine receptors (nAChRs) can induce the release of neurotransmitters such as dopamine and norepinephrine in the CNS. Accumulating evidence suggests that distinct nAChR subtypes are involved; however, it has been difficult to determine the subunit composition of these receptors, in part because of the lack of a sufficient variety of selective nAChR ligands. We present experimental data that at least two different nAChR complexes are involved in dopamine release, one of which has an α3/β2 subunit interface.

The recently discovered peptide α-conotoxin MII is a potent and selective inhibitor of rat nAChRs containing an interface formed by α3 and β2 subunits. We used this peptide to examine nicotine-stimulated release of dopamine from rat striatal synaptosomes and of norepinephrine from hippocampal synaptosomes. MII (100 nM) blocks 34–49% of the nicotine-stimulated dopamine release, but not dopamine release evoked by elevated [K^+]. Furthermore, two peptides structurally related to α-conotoxin MII, namely α-conotoxin MI (selective for α1β1γδ nAChRs) and α-conotoxin ImI (selective for α7-containing nAChRs), have no effect on nicotine-stimulated dopamine release. The results indicate that one third to half of the dopamine release in the striatal preparation is mediated by nAChRs with an α3/β2 subunit interface. In contrast, ≤10% of nicotine-stimulated release of norepinephrine from hippocampal synaptosomes is modulated by nAChRs with α3/β2 subunit interfaces.

Key words: α-conotoxin MII; dopamine release; striatum; nicotine; α3β2 receptor; acetylcholine; norepinephrine release; hippocampus

Modulation of dopamine-mediated neurotransmission is believed to be fundamental to the addicting properties of substances such as cocaine, amphetamine, and morphine (Koob, 1992; Di Chiara, 1995). Striking evidence has been obtained recently that nicotine's addictive properties are highly mechanistically related to these drugs of abuse. Specifically, nicotine activation of presynaptic nicotinic acetylcholine receptors (nAChRs) releases dopamine in critical brain reward circuits (Pontieri et al., 1996). Nicotine-stimulated dopamine release has been demonstrated *in vitro*; however, there has not been a definitive characterization of the relevant molecular components.

nAChRs are believed to be heteropentameric ion channel complexes generally composed of two or more different subunits (α and β). Molecular data indicate that the mammalian CNS has a variety of different nAChR subunits. To date, seven different α subunits (α2–α7, α9) and three different β subunits (β2–β4) have been defined by cloning.

Although the presence of nAChRs on cell soma and dendrites has been recognized for some time, more recent data have demonstrated their presence on presynaptic terminals (Wonnacott, 1997). Activation of these presynaptic nAChRs can induce neurotransmitter release. Thus, nicotinic agonists have been shown to elicit the release of several different neurotransmitters, including dopamine from striatum and frontal cortex (Rapier et al., 1988; Grady et al., 1992; El-Bizri and Clarke, 1994), norepinephrine from hippocampus (Rowell and Winkler, 1984; Wilkie et al., 1993; Sacaan et al., 1995; Clarke and Reuben, 1996), glutamate from cortex, medial habenula nucleus, and hippocampus (Vidal and Changeux, 1993; McGehee and Role, 1995; Gray et al., 1996), GABA from interpeduncular nucleus (Mulle et al., 1991), and acetylcholine from cortex and hippocampus (Rowell and Winkler, 1984; Lapchak et al., 1989).

Interestingly, it appears that distinct subtypes of presynaptic nAChRs regulate the release of different neurotransmitters. For example, nicotine-stimulated glutamate and acetylcholine release is blocked by α-bungarotoxin, suggesting that these nAChRs possess an α7 subunit (McGehee and Role, 1995). In contrast, nicotine-stimulated dopamine release is not blocked by α-bungarotoxin (Grady et al., 1992). Furthermore, the nAChRs modulating norepinephrine release differ pharmacologically from those modulating the release of glutamate, acetylcholine, or dopamine (Sacaan et al., 1995; Clarke and Reuben, 1996).

The modulation of dopamine release by nicotinic acetylcholine circuitry is of central importance because of its significance for problems of addiction as well as because of its relevance to psychosis. However, the specific nAChR subtype(s) that mediates dopamine release remain unidentified. A major operational obstacle has been the lack of subtype-specific ligands. In this report, we describe the use of the newly characterized α-conotoxin MII (Cartier et al., 1996a,b; Harvey et al., 1997), a peptide that potently and selectively blocks α3β2 nAChRs, to investigate nicotine-induced dopamine release. The results indicate that at least two different nAChR receptor complexes may mediate striatal dopamine release and that one of these receptors contains an α3/β2 subunit interface.

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MATERIALS AND METHODS

Materials. [^{3}H]dopamine (~30 Ci/mmol) (dihydroxyphenyl-ethylamine, 3,4 [7-^{3}H]) and [^{3}H]norepinephrine (~42 Ci/mmol) (norepinephrine, levo-[ring-2,5,6-^{3}H]) were obtained from Dupont NEN, Boston, MA (#NET-131 and #NET-678, respectively). ^{3}H-labeled radioligands were aliquoted in 5 and 14.1 μCi amounts, respectively, and stored under argon at −80°C. (−)Nicotine hydrogen tartrate was obtained from Sigma (St. Louis, MO; #N5260). Pargyline HCl (#D-026) and mecamylamine HCl (#M-106) were from obtained from Research Biochemicals International (Natick, MA). Before use, all drugs were prepared fresh in superfusion buffer (SB) consisting of (in mM): 128 NaCl, 2.4 KCl, 3.2 $CaCl_2$, 1.2 KH_2PO_4, 0.6 $MgSO_4$, 25 HEPES, 10 D-glucose, 1 L-ascorbic acid, and 0.1 pargyline, and 0.1 mg/ml BSA, pH adjusted to 7.5 with NaOH. α-Conotoxin MII was synthesized as described previously (Cartier et al., 1996a).

Methods. Male Sprague Dawley rats, weighing 200–400 gm, were maintained on a 12:12 hr light/dark cycle. Rats were drug-naive and housed three per cage, and food and water were available *ad libitum*.

Synaptosomal preparation and ^{3}H-labeled radioligand preloading. Synaptosomes were prepared essentially as described by Clarke and coworkers (El-Bizri and Clarke, 1994; Clarke and Reuben, 1996). For each experiment, two rats were decapitated, and both striata or hippocampi from each (total wet tissue weight 180–240 mg) were excised immediately and dissected on an ice-chilled platform and placed in dissection buffer consisting of 0.32 M sucrose and 5 mM HEPES, adjusted to pH 7.5 with NaOH. Unless otherwise indicated, buffers used in the synaptosomal preparation were at 4°C. Tissues were homogenized in dissection buffer (0.02 ml/mg wet tissue weight) by 12 up-and-down strokes of a 0.25 mm clearance glass Teflon homogenizer operating at 900 rpm. The homogenate was centrifuged at 1000 × *g* for 10 min at 4°C. The pellet was discarded, and the supernatant was recentrifuged at 12,000 × *g* for 20 min at 4°C. The final crude P2 synaptosomal fraction was resuspended in SB (0.5 ml/100 mg wet tissue weight) containing 0.12 μM [^{3}H]dopamine for striatal tissue or 0.2 μM [^{3}H]norepinephrine for hippocampal tissue and incubated at 37°C for 10 min. The loaded synaptosomes were centrifuged at 1000 × *g* for 5 min at room temperature (24°C), and the pellet was resuspended gently in 2.0 ml of SB. For "calcium-free" release studies, $CaCl_2$ in the SB was replaced by 3.2 mM $MgCl_2$ and 2.25 mM Na_4EGTA before adjusting pH. For high [K^+]-labeled stimulated release solution, [K^+] was elevated by 20 mM.

Superfusion. The assay system had 12 identical channels. Each channel had a length of 0.8 mm intradermal Teflon tubing (#5-8696, Supelco, Bellefonte, PA) connected to a three-way subminiature solenoid valve (#161T031, Neptune Research, West Caldwell, NJ), which was used to switch in pulses of buffer containing nicotine or 20 mM KCl. Teflon tubing connected the solenoid valve to a stainless steel filter unit (#09-753-10A, Fisher, Houston, TX) through a Teflon PTFE male luer adapter (#DN-06391-90, Cole-Parmer, Niles, IL). Each filter unit was filled with a 13 mm diameter A/E glass fiber filter (#09-730-51, Fisher) to catch the synaptosomes. The outlet port of the filter unit was connected to a peristaltic pump (#H-07553-70, Cole-Parmer) by platinum-cured silicone tubing (#H-96410-13, Cole-Parmer). The pump continuously pulled the superfusate through the filter at a rate of 0.5 ml/min. Teflon tubing and Teflon-coated parts were used upstream of the synaptosomes to avoid plasticizers such as Tinuvin 770 (a common light and UV radiation stabilizer used in a wide range of plastics) known to block neuronal nAChRs (Papke et al., 1994).

The 12 channel system enabled several assays to be performed in parallel simultaneously. Before loading the filters with synaptosomes, channels were rinsed with distilled water and then superfusate buffer (SB alone or SB plus antagonist), with care taken to ensure that the tubing leading to the three-way solenoid switching valve was fully loaded with superfusate buffer plus agonist (nicotine or KCl). Then 2 ml of the ^{3}H-labeled radioligand-loaded synaptosomes were diluted fourfold with SB and pumped into the filtration apparatus.

After a preliminary superfusion period of 20 min, seventeen 2 min fractions per channel were collected in polypropylene minivials (#2060, Outpatient Services, Petaluma, CA) containing 4.0 ml of scintillation fluid (#88245305, Cytoscint, ICN Pharmaceuticals, Costa Mesa, CA). After an initial collection period of 11–12 min, a 1 min (0.5 ml) pulse of SB with or without agonist was delivered simultaneously to all channels by switching on the solenoids. After the collection period, the filters holding the synaptosomes were removed to determine the residual radioactivity. A liquid scintillation counter (Beckman LS9800, 57.2% efficiency) was used to monitor tritium levels.

Data analysis. It has been shown previously that tritium released by nAChR agonists or by depolarizing concentrations of KCl is directly proportional to total radioligand released (Rapier et al., 1988). Thus, throughout this paper, levels of tritium release is assumed to correspond directly to amounts of radioligand release.

Release is calculated as (dpm in the peak fractions minus the baseline release)/baseline release. Baseline release is defined as the average of two pre- and two postrelease fractions. Release is normalized as a percentage of total agonist-stimulated release. Agonist-stimulated release with superfusate containing different α-conotoxin concentrations was compared with that of controls without toxin and analyzed for statistically significant mean differences using a *t* test on raw (non-normalized) data with SPSS software (SPSS, Chicago, IL).

RESULTS

The effects of α-conotoxin MII on nicotine-stimulated [^{3}H]dopamine release

(−)-Nicotine has been shown previously to increase [^{3}H]dopamine release from rat striatal synaptosomes in a concentration-dependent manner with an estimated EC_{50} of 1.6×10^{-7} M (El-Bizri and Clarke, 1994). As shown in Figure 1, nicotine (3 μM) stimulates the release of [^{3}H]dopamine. Furthermore, the release is calcium-dependent and fully blocked by the nonselective, noncompetitive antagonist mecamylamine.

As shown in Figure 1*E*, α-conotoxin MII blocked part, but not all, of 3 μM nicotine-stimulated [^{3}H]dopamine release. This block was dose-dependent and statistically significant at concentrations ≥ 1 nM (Fig. 2). α-Conotoxin MII (100 nM) produced 34% block. This partial block was seen in 13 separate synaptosomal preparations (43 data points) and was highly significant ($p < 0.001$). One micromolar MII, however, failed to produce significantly more block than 100 nM MII ($p = 0.2$), suggesting that maximum (selective) block was achieved at ≤100 nM concentration. At low nanomolar concentrations, α-conotoxin MII has been shown to be specific for rat α3β2 receptors expressed in *Xenopus* oocytes (IC_{50} = 0.5 nM). At concentrations >100 nM, α-conotoxin MII begins to have measurable effects on other oocyte-expressed nAChR subtypes. For example, the IC_{50} value for α4β2 nAChRs (the α/β-type receptor with the next highest affinity for α-conotoxin MII) is ~400 nM (Cartier et al., 1996a,b; Harvey et al., 1997). Thus, these results indicate that α-conotoxin MII blocks nicotine-stimulated dopamine release by blocking nAChRs with an α3/β2 subunit interface (see Discussion).

α-Conotoxin MII effects on depolarization-induced release; effect of other α-conotoxins on nicotine-stimulated dopamine release

To investigate further the specificity of the block of release by α-conotoxin MII, we next assessed its effects on elevated [K^+]-induced dopamine release. Striatal synaptosomes were loaded with [^{3}H]dopamine and preincubated for 20 min with or without α-conotoxin MII. Synaptosomes were then depolarized with a 1 min pulse of SB that contained high (22.4 mM) [K^+] with or without toxin. [K^+]-stimulated release under control conditions was defined as 100%. α-Conotoxin MII (100 nM), which significantly blocks nicotine-stimulated dopamine release, had no effect on depolarization-stimulated dopamine release (106 ± 4%, $p = 0.3$). The experiment was performed three times with six replicates per experiment.

Next, we tested to determine whether structurally related α-conotoxins that target non-α3β2 nicotinic subtypes (Table 1) could also block nicotine-stimulated dopamine release. Neither α-conotoxin MI, a toxin specific for the muscle subtype of nicotinic receptor, nor α-conotoxin ImI, which is specific for the α7

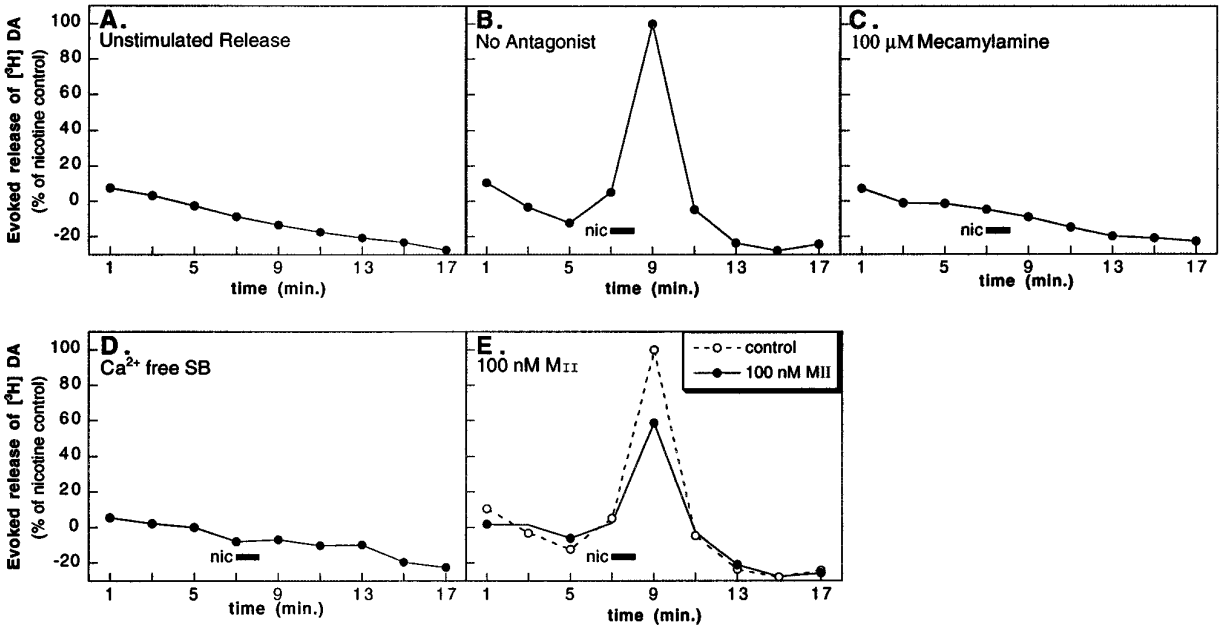


Figure 1. Nicotine-stimulated dopamine (*DA*) release. Rat striatal synaptosomes loaded with [^{3}H]dopamine were perfused with SB with or without antagonist for 20 min before sample collection. The amount of [^{3}H]dopamine release at $t = 9$ min in the absence of nicotine pulse is defined as zero, and the nicotine-stimulated release is defined as 100%. Data are plotted on the *x*-axis as the time midpoint of each fraction. *A*, Unstimulated release of [^{3}H]dopamine. *B*, [^{3}H]dopamine released by a 1 min pulse of 3 μM nicotine (*horizontal bar* in this and all subsequent panels). *C*, Mecamylamine (100 μM) blocked all of the nicotine-evoked [^{3}H]dopamine release. *D*, No nicotine-stimulated release of dopamine was evident when Ca^{2+} is absent from the SB ($CaCl_2$ replaced by equimolar $MgCl_2$ and 2.25 mM Na_4EGTA added). *E*, MII (100 nM) blocked 40% of nicotine-stimulated [^{3}H]dopamine release.

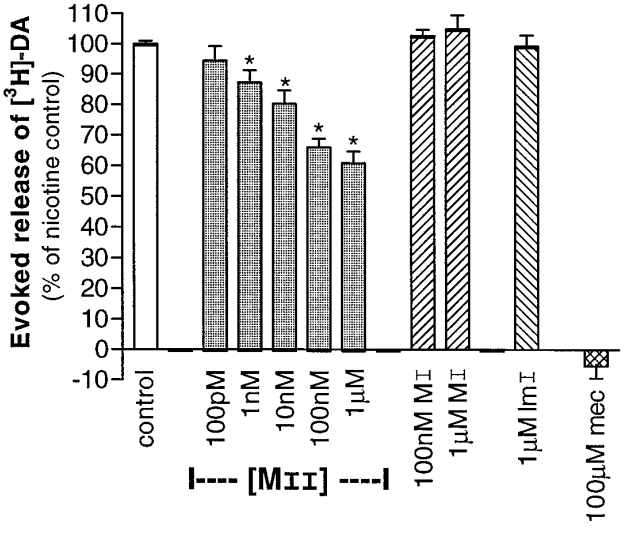


Figure 2. α-Conotoxin MII blocks nicotine-evoked dopamine release. Rat striatal synaptosomes were loaded with [^{3}H]dopamine and then preincubated for 20 min with various concentrations of α-conotoxins before a 1 min pulse of 3 μM nicotine plus toxin. Data are plotted as a percentage of nicotine-stimulated release (which is defined as 100% for control conditions, in which no antagonist was present). Concentrations of MII ≥ 1 nM produced significant block (*) of nicotine-stimulated [^{3}H]dopamine release (1 nM, $p < .05$; 10 nM, $p = .01$; 100 nM and 1 μM, $p < .001$). Mecamylamine (mec; 100 μM) essentially completely blocked evoked dopamine release. In contrast, neither 1 μM MI ($p = 0.4$) nor 1 μM ImI ($p = 0.3$) had detectable effect. Experiments were performed 3 to 13 times with 3 to 6 replicates per experiment.

subtype of nicotinic receptor (Johnson et al., 1995), had any effect on release (Fig. 2). Thus, the partial block of nicotine-evoked dopamine release by α-conotoxin MII was the only significant inhibition of dopamine release by any α-conotoxin.

Effects of α-conotoxin MII on different concentrations of nicotine-stimulated [^{3}H]dopamine release

Previous investigators who have examined the effects of κ-bungarotoxin on nicotine-stimulated dopamine release have reported variable results. One group reported that κ-bungarotoxin (100 nM) inhibited striatal dopamine release by 50% (Wonnacott et al., 1995). In contrast, other investigators reported that κ-bungarotoxin (100 nM) completely blocked striatal dopamine release (Schulz and Zigmond, 1989; Grady et al., 1992). One difference between these studies is that the experiments in which 50% inhibition was observed used 3 μM nicotine, whereas the experiments in which complete block was produced used 50 or 100 μM nicotine. This raises the possibility that high concentrations of nicotine reveal a distinct population of nAChRs with high affinity for κ-bungarotoxin (Wonnacott et al., 1995), although other experimental differences among laboratories may account for the results.

To be able to compare our results more fully with results reported previously using κ-bungarotoxin, we tested the effects of α-conotoxin MII on dopamine release stimulated by 160 nM, 3 μM, and 100 μM nicotine (Fig. 3*A*). α-Conotoxin MII (100 nM) caused significant but partial block of the [^{3}H]dopamine release at all nicotine concentrations tested. However, unlike the large differences in inhibition observed with κ-bungarotoxin, differences among the amount of block observed when nicotine levels change almost three orders of magnitude are relatively modest (Fig. 3*B*).

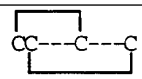
Effects of α-conotoxin MII on nicotine-stimulated norepinephrine release

It has been reported previously that in synaptosomal preparations, nicotine releases hippocampal norepinephrine less potently than it does striatal dopamine (EC_{50} = 6.5 vs 0.16 μM) (Clarke and Reuben, 1996). We used both 3 μM and 100 μM nicotine to

Table 1. Selectivity of α-conotoxins

α-CTx	Sequence*	nAChR	IC_{50} (nM)	Reference
MII	GCCSNPVCHLEHSNLC	α3β2	0.5	(Cartier et al., 1996a,b)
MI	GRCCHPACGKNYSC	α1β1δγ	10	(Johnson et al., 1995)
ImI	GCCSDPRCAWRC	α7	220	(Johnson et al., 1995)

The disulfide bonding pattern is as follows:



The structurally related α-conotoxins are selective for the indicated nAChR subunits expressed in *Xenopus* oocytes. Clones for neuronal subunits were from rat and clones for muscle subunits were from mouse.

*The C-terminal α-carboxyl group of all these peptides is amidated.

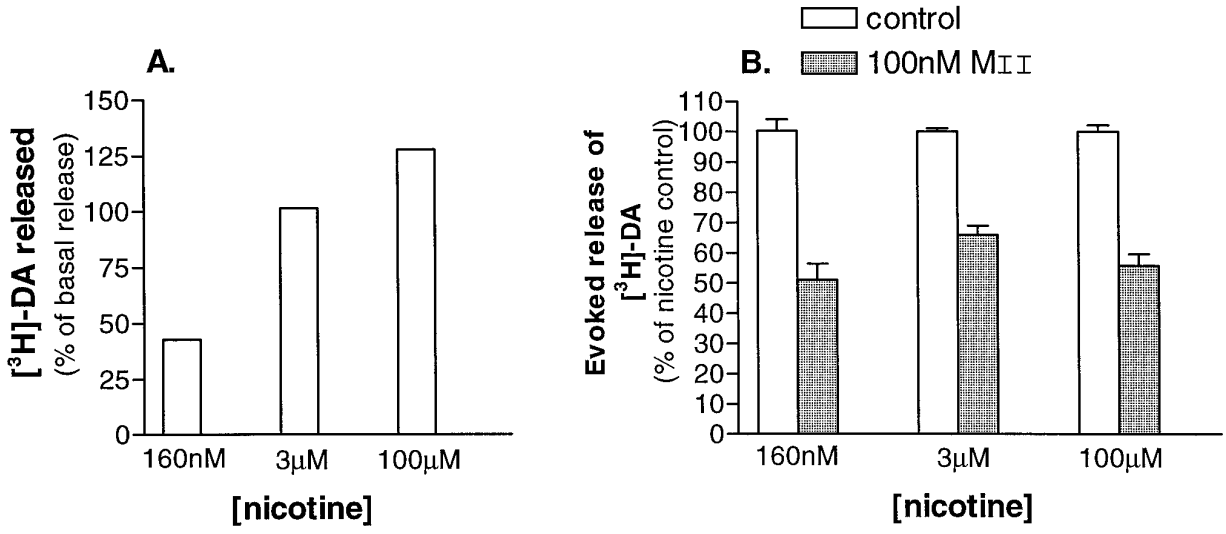


Figure 3. α-Conotoxin MII blocks a fraction of nicotine-evoked dopamine release at various nicotine concentrations. *A*, Increasing concentrations of nicotine evoked progressively higher levels of [^{3}H]dopamine release. The average amount of evoked [^{3}H]dopamine release relative to basal [^{3}H]dopamine release is shown. Nicotine at 100 μM releases approximately threefold more dopamine than does 160 nM nicotine. *B*, The effect of 100 nM α-conotoxin MII (*shaded bars*) was assessed over the range of nicotine concentrations used in *A*. Data are plotted as a percentage of nicotine-evoked release (which at each nicotine concentration is defined as 100%). Block by α-conotoxin MII ranged from 34 to 49% and was highly significant at all nicotine concentrations tested ($p < 0.001$). Experiments were performed 3 to 13 times with 3 to 8 replicates per experiment.

assay the effects of α-conotoxin MII. Nicotine-evoked norepinephrine release is completely blocked by the nonspecific antagonist mecamylamine, and norepinephrine release is dependent on the presence of extracellular calcium (Fig. 4). In contrast to nicotine-stimulated dopamine release, α-conotoxin MII blocked significantly less or failed to block norepinephrine release (Fig. 5). When the block of neurotransmitter release is compared, 100 nM α-conotoxin MII (a concentration that primarily acts on α3β2 receptors) blocks significantly more dopamine than norepinephrine release across all tested nicotine concentrations ($p < 0.001$ for all comparisons).

DISCUSSION

We have shown that α-conotoxin MII causes a high-affinity, but partial, block of nicotine-stimulated dopamine release from rat striatal synaptosomes. In contrast, α-conotoxin MII does not block dopamine release evoked by depolarization, indicating the toxin's specificity for nicotine-stimulated neurotransmitter release. α-Conotoxin MII blocks rat α3β2 nAChRs expressed in *Xenopus* oocytes with an IC_{50} of 0.5 nM. α-Conotoxin MII also blocks other nAChR subunit combinations, but at concentrations that are two to four orders of magnitude higher, with the order of potency: $\alpha3\beta2 \gg \alpha7 > \alpha4\beta2 > \alpha2\beta2 \cong \alpha3\beta4 > \alpha1\beta1\delta\gamma > \alpha4\beta4 \geq \alpha2\beta2$ (Cartier et al., 1996a,b; Harvey et al., 1997). At low nanomolar concentrations, α-conotoxin MII blocks essentially only α3β2 receptors, strongly suggesting that the block of nicotine-stimulated dopamine release seen at these α-conotoxin MII concentrations is attributable to block of native α3β2-containing nAChRs. Maximal MII block of nicotine-stimulated dopamine release is seen at 100 nM concentration with little additional block at 1 μM concentration. These results suggest that some, but not all, nicotine-stimulated dopamine release is attributable to α3β2-containing nAChRs. Other nAChR subtypes presumably mediate the remainder of the response. Thus, more than one subtype of neuronal nAChR appears responsible for nicotine-stimulated dopamine release.

In contrast to nicotine-stimulated dopamine release, nanomolar concentrations of MII block substantially less or fail to block nicotine-stimulated norepinephrine release from rat hippocampal synaptosomes. Thus, α3β2-containing nAChRs appear to play a smaller or no role in nicotine-stimulated norepinephrine release from synaptosomes. It should be noted, however, that in slice preparations of hippocampus, a much larger percentage of nor-

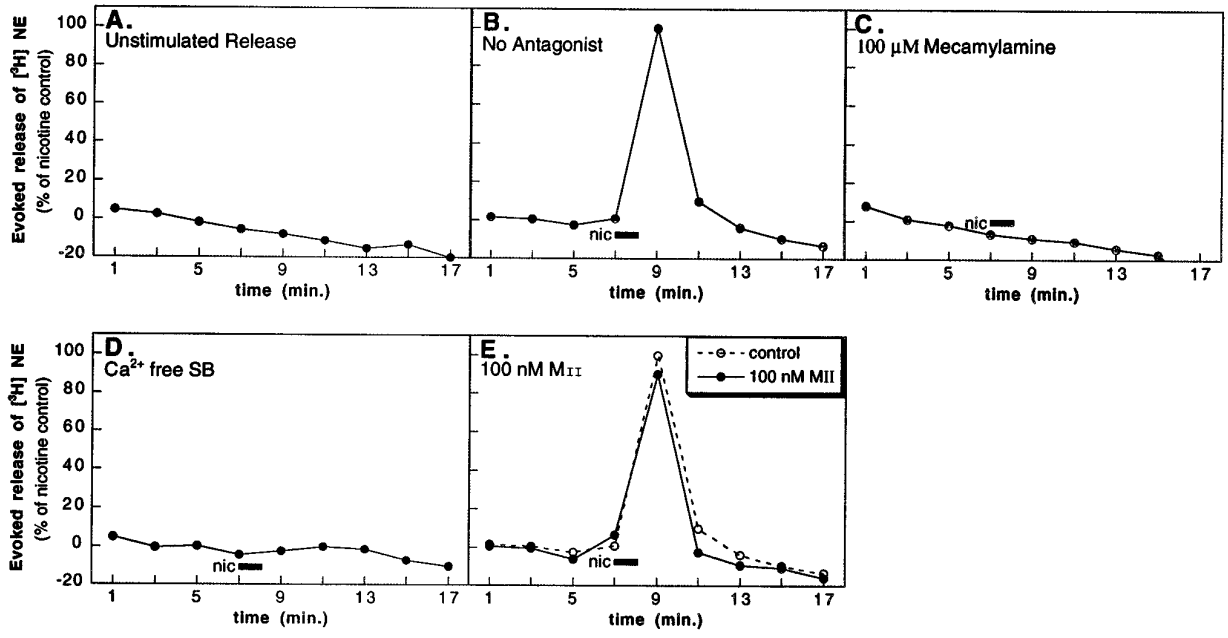


Figure 4. Nicotine-stimulated norepinephrine (*NE*) release. Rat hippocampal synaptosomes were loaded with [^{3}H]norepinephrine and perfused with buffer with or without antagonist for 20 min before sample collection. Nicotine-stimulated release is defined as 100%, and the corresponding basal release at $t = 9$ min is defined as zero. Data are plotted as described in Figure 1. *A*, Unstimulated release of [^{3}H]norepinephrine. *B*, [^{3}H]norepinephrine release after a 1 min pulse of 100 μM nicotine (*horizontal bar* in this and all subsequent panels). *C*, Nicotine-stimulated release in the presence of the nicotinic antagonist mecamylamine. *D*, Nicotine-stimulated release in the absence of Ca^{2+}. *E*, α-Conotoxin MII (100 nM) blocks ~10% of nicotine-evoked release of [^{3}H]norepinephrine.

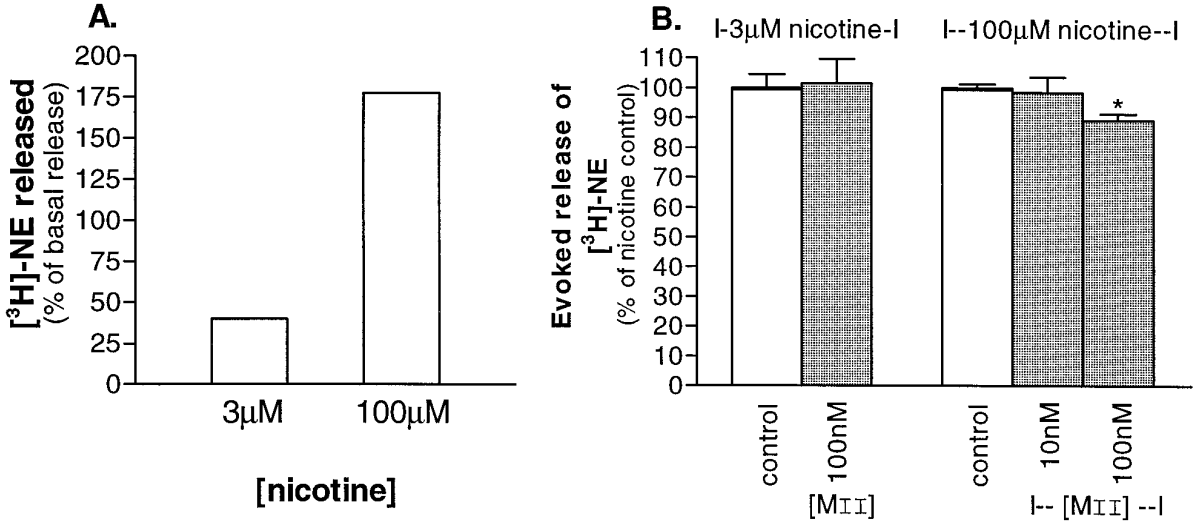


Figure 5. α-Conotoxin MII's effect on nicotine-stimulated norepinephrine release from rat hippocampal synaptosomes. *A*, The average amount of nicotine-evoked relative to unstimulated (basal) [^{3}H]norepinephrine release. Nicotine at 100 μM concentration releases four- to fivefold more [^{3}H]norepinephrine than it does at 3 μM. *B*, Synaptosomes were preincubated with or without antagonist for 20 min and then exposed to a 1 min pulse of either 3 μM or 100 μM nicotine ± antagonist. Control nicotine-stimulated [^{3}H]norepinephrine release is defined as 100% at each respective nicotine concentration. MII at 100 nM fails to significantly block release evoked by 3 μM nicotine ($p = 0.4$). Using 100 μM nicotine, 10 nM MII does not significantly block release ($p = 0.25$), but 100 nM MII blocked 10% of release ($p < 0.05$). Note that 100 nM MII blocks significantly more dopamine than norepinephrine release ($p < 0.001$ for all nicotine concentrations tested; compare Fig. 2). Experiments were performed three to four times with four to six replicates per experiment.

epinephrine is released by nicotine, e.g., ~400% over basal (Sacaan et al., 1995) versus the ~200% over basal release from synaptosome hippocampal preparations reported in this and other studies (Clarke and Reuben, 1996). Nicotine-stimulated release of either norepinephrine or dopamine from synaptosomes is not affected by the sodium channel blocker tetrodotoxin (Clarke and Reuben, 1996). In contrast, tetrodotoxin does block the majority of nicotine-stimulated norepinephrine release from hippocampal slices (Sacaan et al., 1995), suggesting that at least some of the norepinephrine release is not attributable to nAChRs located on nerve terminals, but instead is dependent on propagated action potentials. It will be of interest in future studies to

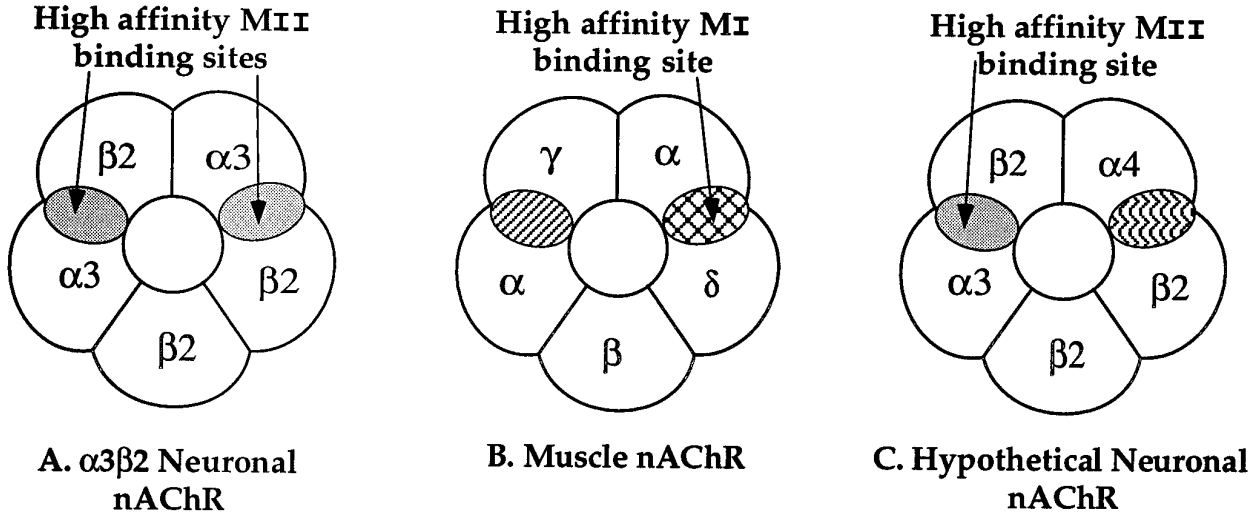


Figure 6. Neuronal nAChRs are believed to be pentameric. *A*, Heterologous expression of a single α with a single β subunit yields receptors with two ligand binding sites at α/β interfaces. More complex receptors, i.e., those with more than one type of α and/or non-α subunit, are possible as illustrated by the muscle receptor (*B*) and hypothetical neuronal receptor (*C*). α-Conotoxins can discriminate between ligand binding sites at subunit interfaces. Thus, α-conotoxin MI binds more tightly to the α/δ than the α/γ interface of the muscle nAChR with 10,000-fold difference (Kreienkamp et al., 1994; Groebe et al., 1995) (*B*). Similarly, α-conotoxin MII blocks α3β2 nAChRs 800-fold more potently than α4β2 nAChRs (Cartier et al., 1996a,b). Thus, MII might be expected to selectively block the receptor shown in *C* by binding with high affinity to the α3/β2 interface as opposed to the α4/β2 interface.

assess the effect of α-conotoxin MII on tetrodotoxin-sensitive norepinephrine release.

Previous studies have addressed the identity of nAChR subtypes involved in nicotine-stimulated dopamine release. Several factors have made it difficult to unambiguously identify the relevant subtypes. First, there is a lack of subtype-specific antagonists for neuronal nAChRs. One antagonist, a minor component of *Bungarus* venom known as κ-bungarotoxin, has been used with some success in the study of nicotine-stimulated dopamine release. Under certain conditions, κ-bungarotoxin preferentially blocks α3β2 receptors (Luetje et al., 1990), although the presence of venom purification contaminants has led to inconsistent findings (Fiordalisi et al., 1994). In addition, it has been shown that κ-bungarotoxin blocks several subtypes of neuronal nAChRs, which was not initially appreciated because of the complex kinetics of this ligand (Papke et al., 1993). The ability of κ-bungarotoxin to selectively block α3β2 receptors or block multiple subtypes of nAChRs, depending on the conditions used, may help account for the reported differences in efficacy of this toxin in blocking nicotine-stimulated dopamine release.

Another approach used to examine nAChR subtypes has been to assess the potency of various nicotinic agonists in stimulating dopamine release and compare these potencies to the ability of these agonists in activating nAChRs heterologously expressed in *Xenopus* oocytes. One complicating factor with this approach is the possibility that more than one subtype of nAChR underlies nicotine-stimulated dopamine release. In such a case, the potency of a nicotinic agonist in stimulating dopamine release in brain tissue would not correlate with the agonist's potency in activating a single nAChR subtype. An even more complicated possibility is that native neuronal nAChRs are not simply a combination of a single type of α and β subunit. For example, in chick ciliary ganglia, it appears that some neuronal nAChRs are composed of at least four types of subunits: α3, α5, β2, and β4 (Conroy and Berg, 1995). In general, nicotinic receptors require the binding of two molecules of acetylcholine to trigger channel opening. In muscle, the receptor-ligand binding sites are pharmacologically nonequivalent, being composed of an α1/δ and an α1/γ interface. These two interfaces have different affinities for agonists. Similarly, both the α and β subunits contribute to agonist sensitivity of neuronal nAChRs (Luetje and Patrick, 1991). Furthermore, it has been shown that nAChRs with two types of α-subunits have different affinities for ACh than receptors with a single type of α subunit (Ramirez-Latorre et al., 1996; Wang et al., 1996). Thus, the presence of complex receptors in CNS tissue may not readily allow comparison of agonist potency with simple combinations of α and β subunits in nAChRs expressed in *Xenopus* oocytes.

A particular advantage of α-conotoxin antagonists is their ability to discriminate among nonsymmetrical ligand binding interfaces on nAChRs. The best-studied example is the binding of α-conotoxin MI to the muscle nicotinic receptor (Fig. 6*B*). In mouse muscle, α-conotoxin MI displays a four order-of-magnitude selectivity for the α1/δ versus the α1/γ binding site (Kreienkamp et al., 1994; Groebe et al., 1995). Nevertheless, α-conotoxin MI blocks muscle receptor function with an IC_{50} comparable to its K_d for the α1/δ binding site, indicating that binding of only one toxin molecule is sufficient to block channel activation (Martinez et al., 1995). We have also demonstrated recently that α-conotoxin MII has two binding sites on α3β2 and α3β4 receptors expressed in *Xenopus* oocytes, and binding of only one toxin molecule to a receptor is sufficient to block receptor function (Cartier et al., 1996b).

α-Conotoxin MII has a lower IC_{50} for α3β2 than that for other receptor subunit combinations by two to four orders-of-magnitude. Thus, α-conotoxin MII, in theory, has the ability to potently block any receptor containing an α3/β2 subunit interface regardless of what other α and β subunits may be in the receptor complex (Fig. 6). A receptor containing only one α3β2 interface (Fig. 6*C*) would be expected to have an IC_{50} approximately equal to the K_d of MII for the α3/β2 interface. It is possible, however, that the presence of other subunits could cause some structural alterations at the α3/β2 subunit interface, leading in turn to a somewhat altered affinity for MII.

The results of the present study are consistent with the involvement of an nAChR that contains at least one $\alpha3/\beta2$ subunit interface in nicotine-stimulated dopamine release. Additional dissection of the subunit composition of nAChRs involved in nicotine-stimulated dopamine release would be facilitated by additional nAChR subunit-specific antagonists. We are in the process of isolating and characterizing such agents.

Nicotinic acetylcholine receptors have been implicated in the treatment or pathophysiology of several neuropsychiatric disorders including schizophrenia (Freedman et al., 1994, 1997), Alzheimer's disease (Nordberg et al., 1989, 1990), Parkinson's disease (Janson et al., 1988; Reavill, 1990; Grandinetii et al., 1994), and Tourette's syndrome (Sandberg et al., 1988, 1989). Currently available neuropsychiatric medications work primarily by inhibiting neurotransmission through postsynaptic receptor antagonism or by inhibiting neurotransmitter reuptake or catabolism (Schatzberg et al., 1995). In particular, most presently used antipsychotic medications inhibit dopamine signaling by blocking postsynaptic dopamine receptors. The possibility of selectively modulating the presynaptic release of dopamine has significant therapeutic implications. Our data, which suggest that dopamine release is mediated by two or more distinct subtypes of nAChRs, indicate that the cholinergic modulation of dopamine release may be highly complex, presenting opportunities for pharmacological intervention. Thus, by blocking presynaptic nAChRs, it might prove possible to attenuate, but not completely abolish, dopamine release. In principle, this could lead to an antipsychotic strategy with fewer side-effects than complete postsynaptic blockade.

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