

α -Bungarotoxin-Sensitive Nicotinic Receptors Indirectly Modulate [3 H]Dopamine Release in Rat Striatal Slices via Glutamate Release

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ABSTRACT

Nicotinic agonists elicit the release of dopamine from striatal synaptosomes by acting on presynaptic nicotinic acetylcholine receptors (nAChRs) on dopamine nerve terminals. Both $\alpha 3\beta 2^*$ and $\alpha 4\beta 2$ nAChR subtypes (but not $\alpha 7^*$ nAChRs) have been implicated. Here, we compared nAChR-evoked [3 H]dopamine release from rat striatal synaptosome and slice preparations by using the nicotinic agonist anatoxin-a. In the more integral slice preparation, the concentration-response curve for anatoxin-a-evoked [3 H]dopamine release was best fitted to a two-site model, giving EC_{50} values of 241 nM and 5.1 μ M, whereas only the higher-affinity component was observed in synaptosome preparations ($EC_{50} = 134$ nM). Responses to a high concentration of anatoxin-a (25 μ M) in slices (but not in synaptosomes) were partially blocked by ionotropic glutamate receptor antagonists (kynurenic acid, 6,7-dinitroquinoxaline-2,3-dione) and by

$\alpha 7^*$ -selective nAChR antagonists (α -bungarotoxin, α -conotoxin-lml, methyllycaconitine) in a nonadditive manner. In contrast, the $\alpha 3\beta 2$ -selective nAChR antagonist α -conotoxin-MII partially inhibited [3 H]dopamine release from both slice and synaptosome preparations, stimulated with both low (1 μ M) and high (25 μ M) concentrations of anatoxin-a. Antagonism by α -conotoxin-MII was additive with that of $\alpha 7^*$ -selective antagonists. These data support a model in which $\alpha 7^*$ nAChRs on striatal glutamate terminals elicit glutamate release, which in turn acts at ionotropic glutamate receptors on dopamine terminals to stimulate dopamine release. In addition, non- $\alpha 7^*$ nAChRs on dopamine terminals also stimulate dopamine release. These observations have implications for the complex cholinergic modulation of inputs onto the major efferent neurons of the striatum.

The dorsal striatum is concerned with the control of movement. The principal output neurons from the striatum, the GABAergic medium spiny neurons, receive glutamatergic afferents from the cortex and thalamus and dopaminergic inputs from the substantia nigra (Smith and Bolam, 1990). Cholinergic interneurons also synapse onto the medium spiny neurons. In addition to these well-established synaptic relationships, there is increasing evidence for neurochemical cross talk between the terminals of afferent neurons via presynaptic receptors. This may provide a basis for new therapeutic approaches for the treatment of movement disorders that arise from the degeneration of neuronal subpopulations (Parkinson's and Huntington's diseases) or as a side effect of clinical treatments (tardive dyskinesia).

In vitro, glutamate stimulates the release of [3 H]dopamine from rat striatal slices (Roberts and Anderson, 1979), and

this effect appears to be mediated by both α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate and *N*-methyl-D-aspartate (NMDA) receptors present on dopaminergic nerve terminals (Wang, 1991; Desce et al., 1992). Consistent with this view, in vivo infusion into the striatum via a microdialysis probe of either NMDA (Keefe et al., 1992; Morari et al., 1993; Kendrick et al., 1996) or AMPA (Kendrick et al., 1996; Smolders et al., 1996) increased local release of dopamine. There also is strong evidence for the presence on dopamine terminals of nicotinic acetylcholine receptors (nAChRs) capable of enhancing the basal release of dopamine (Wonnacott, 1997). These nAChRs appear to be heterogeneous, composed of subtypes containing $\alpha 3$ and $\beta 2$ subunits ($\alpha 3\beta 2^*$ nAChRs; Kulak et al., 1997; Kaiser et al., 1998) and $\alpha 4$ and $\beta 2$ subunits ($\alpha 4\beta 2$ nAChRs; Sharples et al., 2000). Furthermore, locally applied (–)-nicotine in vivo has been shown to increase striatal levels of dopamine (Marshall et al., 1997) and glutamate (Toth et al., 1993) in a mecamylamine-sensitive manner. Moreover, the local application of NMDA antagonists diminished the ability of locally applied (–)-

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ABBREVIATIONS: AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AnTx-a, (\pm)-anatoxin-a; α -Bgt, α -bungarotoxin; NMDA, *N*-methyl-D-aspartate; α Ctx-MII, α -conotoxin-MII; α Ctx-lml, α -conotoxin-lml; DNQX, 6,7-dinitroquinoxaline-2,3-dione; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor.

nicotine to elicit dopamine release in vivo (Toth et al., 1992). These data lead to the hypothesis that (–)-nicotine can also act at presynaptic nAChRs on striatal glutamatergic nerve terminals to release glutamate, which in turn stimulates the release of dopamine via presynaptic ionotropic glutamate receptors on dopaminergic terminals. Recent electrophysiological recordings from striatum in situ are consistent with this argument (García-Muñoz et al., 1996).

Here, we examined the relationship between nAChRs, glutamate receptors, and dopamine release in the striatum in vitro. Comparative experiments using perfused synaptosomes (which represent isolated nerve terminals with low probability of neurochemical cross-talk) and slices (which preserve some of the anatomical integrity of the striatum) provide evidence for a component of [³H]dopamine release in slices, but not in synaptosomes, that is sensitive to glutamate receptor antagonists and α7*-selective nAChR antagonists. These results are consistent with an indirect modulation of dopamine release in striatum via α7* nAChRs on striatal glutamatergic nerve terminals.

Experimental Procedures

Materials. Adult male Sprague-Dawley rats were obtained from the University of Bath Animal House breeding colony. [7,8-³H]Dopamine (1.78 TBq/mmol) was purchased from Amersham International (Buckinghamshire, UK). α-Conotoxin-MII (αCTx-MII) was synthesized with correct disulfide bond formation as previously described (Cartier et al., 1996; Kaiser et al., 1998). (±)-Anatoxin-a (AnTx-a), 6,7-dinitroquinoxaline-2,3-dione (DNQX), and kynurenic acid were obtained from Tocris Cookson (Bristol, UK). α-Conotoxin-ImI (αCTx-ImI) was obtained from Calbiochem (San Diego, CA). Methyllycaconitine (MLA) and 4-aminopyridine were purchased from Research Biochemicals International (Natick, MA). α-Bungarotoxin (α-Bgt), mecamylamine, pargyline, and nomifensine were purchased from Sigma Chemical Co. (Poole, Dorset, UK). All other chemicals used were of analytical grade and were obtained from standard commercial sources.

Superfusion of Rat Striatal Slices and Synaptosomes. Male Sprague-Dawley rats (approximately 250 g) were sacrificed by cervical dislocation and decapitated, and brain striata (180–240 mg tissue wet wt./rat) were rapidly dissected. P2 synaptosomes were prepared by differential centrifugation as previously described (Soliakov et al., 1995). Synaptosomes were loaded with [³H]dopamine (0.1 μM, 0.132 MBq/ml) for 15 min at 37°C and superfused in open chambers (Soliakov et al., 1995). All superfusion experiments were performed in Krebs-bicarbonate buffer of the following composition: 118 mM NaCl, 2.4 mM KCl, 2.4 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, and 10 mM glucose, buffered to pH 7.4 with 95% O₂, 5% CO₂ and supplemented with 1 mM ascorbic acid, 8 μM pargyline, and 0.5 μM nomifensine to prevent dopamine degradation and reuptake.

Striatal slices (0.25 mm) were prepared as previously described (Marshall et al., 1996) using a McIlwain tissue chopper. Striatal tissue prisms were washed twice with Krebs-bicarbonate buffer and loaded with [³H]dopamine (0.1 μM, 0.132 MBq/ml) for 15 min at 37°C. After two washes, slices were resuspended in Krebs' buffer and loaded into superfusion chambers (approximately 45–50 mg of slices per chamber). Superfusion of synaptosomes (open chambers) or slices (closed chambers) was performed as previously described (Soliakov et al., 1995; Marshall et al., 1996) in a Brandel Superfusion Apparatus model SF-12 (Montreal, Quebec, Canada), using Krebs-bicarbonate buffer at 37°C and a flow rate of 0.5 ml/min; 2-min fractions were collected.

Chambers containing either synaptosomes or slices were washed for 20 min with Krebs-bicarbonate buffer, followed by a further 10

min with normal buffer or buffer containing antagonist (112 nM αCTx-MII, 1 μM αCTx-ImI, 50 nM MLA, 10 μM mecamylamine, 500 μM kynurenic acid, 100 μM DNQX). In the case of α-Bgt (40 nM) the preincubation time was extended to 1 h. Then, the nicotinic agonist AnTx-a or general depolarizing agent (KCl or 4-aminopyridine) was applied for 40 s in the presence or absence of antagonist. The 40-s drug pulse was separated from the bulk buffer flow by 10-s air bubbles.

Fractions were counted in a Packard TRI-CARB Liquid Scintillation Counter (model 1500; counting efficiency 48%). Evoked tritium release above baseline was calculated as a percent of the total radioactivity present in the synaptosomes immediately before stimulation (Soliakov et al., 1995). To estimate the radioactivity remaining in slices at the end of the experiment, superfusion was continued for 30 min with 10 mM HCl to lyse the slices (Marshall et al., 1996). Aliquots (1 ml) of the lysates were counted for radioactivity. Total radioactivity in slices at the time of stimulation was calculated as the sum of subsequently released [³H]dopamine plus lysate.

Data Analysis. The baseline was derived by fitting the following double exponential decay equation to the data using Sigma Plot Version 2.0 for Windows:

$$y = ae^{-bx} + ce^{-dx}$$

where *a*, *b*, *c*, and *d* are the curve parameters and *x* is the fraction number.

Evoked [³H]dopamine release was calculated as the area under the peak after subtraction of the baseline. Data are expressed as percents of the corresponding controls, assayed in parallel in the absence of antagonist, and are mean ± S.D. of several independent experiments, each consisting of two or three replicate chambers for each condition. One-way ANOVA (Bonferroni's *t* test) was used to determine the significance of differences from control (Sigma Stat; Jandel Scientific, Erkrath, Germany).

For analysis of the AnTx-a concentration-response curve in striatal slices, the data were fitted to a two-site model:

$$y = \{a/[1 + (k1/x)^{n1}]\} + \{b/[1 + (k2/x)^{n2}]\}$$

where *a* and *b* are the asymptotic maximums; *k*₁ and *k*₂ are the values at the inflection points (apparent EC₅₀ values), and *n*₁ and *n*₂ are the slope parameters (Hill numbers).

Results

Effect of Ionotropic Glutamate Receptor Antagonists on [³H]Dopamine Release from Striatal Slices.

The nonselective glutamate receptor antagonist kynurenic acid significantly inhibited [³H]dopamine release from striatal slices, evoked by modest concentrations of the depolarizing agents KCl and 4-aminopyridine (Fig. 1, a and b). Kynurenic acid was used at a concentration (500 μM) sufficient to maximally inhibit AMPA/kainate and NMDA receptors and decreased [³H]dopamine release by 21.0 ± 0.3% and 23.0 ± 6.0%, respectively, when slices were stimulated by 23.5 mM KCl and 1 mM 4-aminopyridine. A higher concentration (800 μM) of kynurenic acid did not produce any greater degree of inhibition. These data are consistent with previous reports (Wang, 1991; Desce et al., 1992) that presynaptic ionotropic glutamate receptors on striatal dopamine terminals positively modulate dopamine release: in the present experiments, the depolarizing agents released glutamate to activate these receptors.

Kynurenic acid produced a similar inhibition (18.0 ± 5.0%) of [³H]dopamine release from striatal slices evoked by the nicotinic agonist AnTx-a (Fig. 1c). AnTx-a was chosen because it is a potent and specific nicotinic agonist that we have

characterized extensively with respect to [^3H]dopamine release (Soliakov et al., 1995; Sharples et al., 2000). Initially, it was used at a concentration (1 μM) previously shown to elicit maximum [^3H]dopamine release from striatal synaptosomes (Soliakov et al., 1995; see Fig. 2, inset): this concentration evokes about 30% of the response achieved with 23.5 mM KCl or 1 mM 4-aminopyridine. In contrast to the results from slices, kynurenic acid had no effect on AnTx-a-evoked [^3H]dopamine release from striatal synaptosomes (Fig. 1d). These results are consistent with the hypothesis that in addition to activating nAChRs on dopaminergic nerve terminals, AnTx-a acts at nAChRs that provoke the release of glutamate, which in turn elicits [^3H]dopamine release.

To further explore this nAChR-glutamate interaction, we next examined the concentration dependence of AnTx-a-evoked [^3H]dopamine release from slices. In contrast to that for synaptosomes, the concentration-response curve had a reproducible and distinct biphasic profile and could be fitted to a two-site model, giving apparent EC_{50} values of 241 ± 36 nM and 5.1 ± 0.3 μM (Fig. 2). That AnTx-a was eliciting [^3H]dopamine release by interacting with nAChRs was established by the ability of 10 μM mecamylamine to inhibit responses across the entire concentration range examined. In contrast, although kynurenic acid (500 μM) had a small but significant effect on [^3H]dopamine release in response to 1 μM AnTx-a, as reported above, at 10 μM and 25 μM AnTx-a, kynurenic acid inhibited [^3H]dopamine release by $53.0 \pm 1.8\%$ and $45.9 \pm 5.1\%$, respectively (Fig. 2). Subsequent experiments compared responses in synaptosome and slice preparations stimulated with 25 μM AnTx-a.

In addition to kynurenic acid, the selective AMPA/kainate

receptor antagonist DNQX inhibited [^3H]dopamine release from striatal slices stimulated with 25 μM AnTx-a by $42.6 \pm 8.0\%$ (Fig. 3), and DNQX and kynurenic acid showed no additivity when applied together. Both antagonists decreased nAChR-stimulated [^3H]dopamine release to the level evoked by 1 μM AnTx-a, effectively inhibiting the second phase of the concentration-response curve. In contrast in synaptosomes, 25 μM AnTx-a produced no more [^3H]dopamine release than 1 μM AnTx-a, and neither kynurenic acid nor DNQX produced any significant inhibition of AnTx-a-evoked [^3H]dopamine release (Fig. 3, inset).

Pharmacological Characterization of nAChRs Modulating [^3H]Dopamine Release in Striatal Slices. To characterize the nAChR subtypes involved in the modulation of striatal [^3H]dopamine release, several nicotinic antagonists were compared for their abilities to inhibit release evoked by 1 μM and 25 μM AnTx-a, in both slices and synaptosomes (Fig. 4). In agreement with our previous findings (Kaiser et al., 1998), a maximally effective concentration (112 nM) of the $\alpha 3\beta 2$ -selective toxin $\alpha\text{Ctx-MII}$ (Cartier et al., 1996) partially inhibited [^3H]dopamine release evoked by AnTx-a in both slices and synaptosomes. The latter preparation was inhibited to a greater extent by $\alpha\text{Ctx-MII}$ than slices, as we have noted previously (Kaiser et al., 1998).

The $\alpha 7^*$ -selective nAChR antagonist MLA (50 nM; Alkon et al., 1992; Wonnacott et al., 1993) was perfused for 10 min: this concentration was predicted to fully block $\alpha 7^*$ nAChRs in this protocol without activating other nAChR subtypes. Unexpectedly, [^3H]dopamine release evoked by 1 μM AnTx-a was decreased 39.1 and 31.3% by MLA in synaptosomes and slices, respectively (Fig. 4). Other concentra-

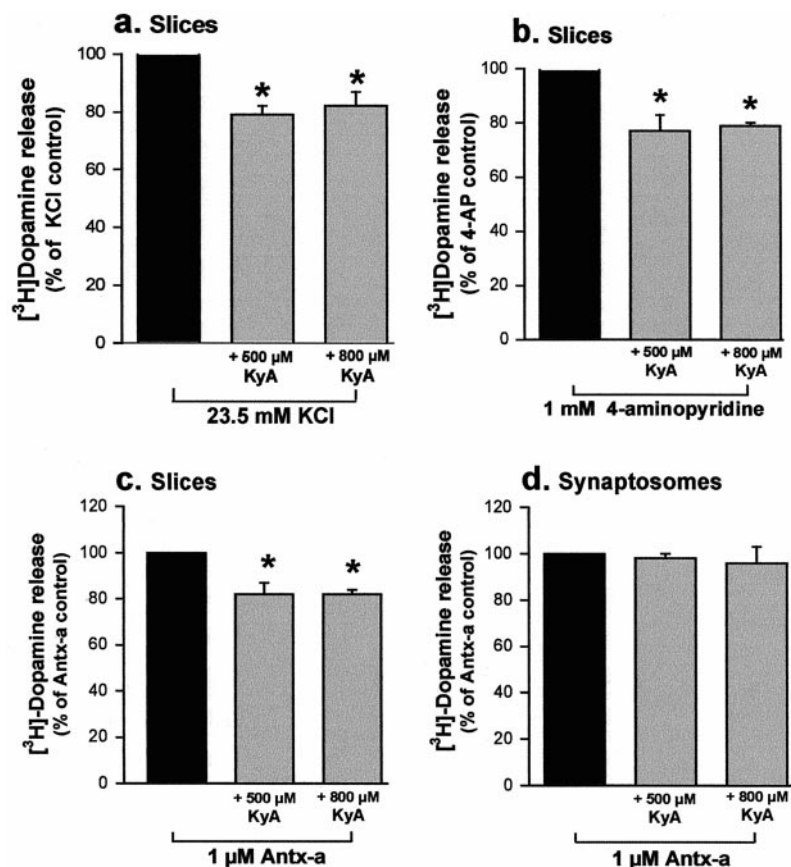


Fig. 1. Effect of kynurenic acid on [^3H]dopamine release evoked by (a) 23.5 mM KCl, (b) 1 mM 4-aminopyridine, and (c and d) 1 μM AnTx-a. Rat striatal slices (a–c) and synaptosomes (d) were loaded with [^3H]dopamine and superfused as described in *Experimental Procedures*. Kynurenic acid (KyA; 500 or 800 μM) was applied for 10 min before the administration of a 40-s pulse of depolarizing agent or agonist. Responses are expressed as a percent of the respective control in the absence of kynurenic acid; control responses, with evoked release calculated as a percent of total [^3H]dopamine taken up by the preparation, were $5.5 \pm 0.5\%$ (a), $5.5 \pm 0.5\%$ (b), $1.75 \pm 0.25\%$ (c), and $2.00 \pm 0.15\%$ (d). Error bars indicate the standard deviation for at least three independent experiments. *, significantly different from control, $P < .05$, one-way ANOVA, Bonferroni's t test.

tions of MLA were compared for their effect on [3 H]dopamine release from synaptosomes stimulated with 1 μ M AnTx-a: 10 nM MLA gave $37.1 \pm 9.9\%$ inhibition, comparable with that of 50 nM MLA, whereas 500 nM MLA produced significantly greater inhibition of $60.6 \pm 7.0\%$ (data not shown). However, responses to 25 μ M AnTx-a in synaptosomes were not significantly affected by 50 nM MLA (Fig. 4b); thus, the inhibition by 50 nM MLA disappeared with increasing agonist concentration in synaptosomes, perhaps reflecting a surmountable mode of antagonism. However, in slices this toxin inhibited the response to 25 μ M AnTx-a by 42.7% (Fig. 4a), demonstrating an increase in the percent inhibition with increasing agonist concentration.

To further explore the possible involvement of the $\alpha 7^*$ subtype of nAChRs in the nicotinic stimulation of [3 H]dopamine release from striatal slices, we examined the effect of the $\alpha 7^*$ -selective α Ctx-ImI (1 μ M; Johnson et al., 1995; Pereira et al., 1996). This toxin decreased [3 H]dopamine release from slices elicited by 25 μ M AnTx-a by 31.9% (Fig. 4a), comparable with the effect of 50 nM MLA. In synaptosomes stimulated with 25 μ M AnTx-a, 1 μ M α Ctx-ImI had no significant effect (Fig. 4b), consistent with results for (–)-nicotine-evoked [3 H]dopamine release from synaptosomes (Kulak et al., 1997). Thus, at the higher agonist concentration, an $\alpha 7$ -like component is clearly demonstrable in slices but not in synaptosomes. The involvement of $\alpha 7^*$ nAChRs was confirmed by the ability of 40 nM α -Bgt, perfused for 1 h at 37°C

before stimulation with 25 μ M AnTx-a, to inhibit [3 H]dopamine release from slices by 39.8% (Fig. 4a).

Thus, three structurally unrelated $\alpha 7^*$ -selective nAChR antagonists block [3 H]dopamine release from slices in response to the higher concentration of AnTx-a to a comparable extent (30–40%; Table 1). This level of inhibition is similar to that obtained in the presence of ionotropic glutamate receptor antagonists (Fig. 3). Coapplication of antagonists showed that neither 40 nM α -Bgt plus 100 μ M DNQX, nor 50 nM MLA plus 100 μ M DNQX, achieved any additional inhibition above that of any of the antagonists applied alone (Table 1). In contrast, α -Bgt plus 112 nM α Ctx-MII and MLA plus 112 nM α Ctx-MII resulted in clearly additive inhibition, decreasing the release of [3 H]dopamine from slices in response to 25 μ M AnTx-a by about 70% (Table 1).

Discussion

In this study, we have shown that the ionotropic glutamate receptor antagonists kynurenic acid and DNQX and the $\alpha 7^*$ -selective nAChR antagonists α -Bgt, α Ctx-ImI, and MLA partially inhibit [3 H]dopamine release from striatal slices elicited by the potent and specific nicotinic agonist AnTx-a. When applied together, glutamate receptor antagonists and $\alpha 7^*$ -selective nAChR antagonists were not additive in their inhibition and generally were without effect in corresponding experiments on synaptosome preparations. In contrast, AnTx-a-evoked [3 H]dopamine release from both slices and synaptosomes was partially inhibited by the $\alpha 3\beta 2$ -selective nAChR antagonist α Ctx-MII, and this inhibition was additive with that of $\alpha 7^*$ -selective nAChR antagonists. These findings are consistent with the hypothesis that $\alpha 7^*$ nAChRs

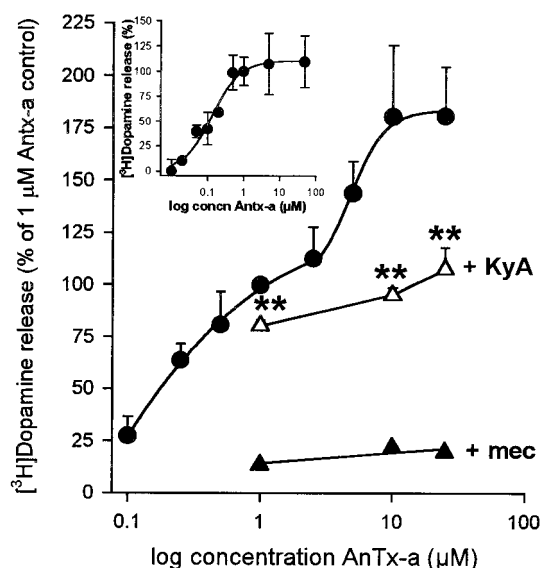


Fig. 2. Concentration dependence of AnTx-a-evoked [3 H]dopamine release. Rat striatal slices (main figure) or synaptosomes (inset) were loaded with [3 H]dopamine and superfused as described in *Experimental Procedures*. The preparation was challenged with 40-s pulses of various concentrations of AnTx-a in the absence (●) or presence of 10 μ M mecamylamine (mec, ▲) or 500 μ M kynurenic acid (KyA, Δ). Antagonists were introduced into the perfusion buffer 10 min before the agonist pulse. [3 H]Dopamine released above baseline is expressed as a percent of the response to stimulation with 1 μ M AnTx-a, taken as a control and included in every assay. Values are the mean \pm S.D. from at least three independent determinations. The concentration-response data for synaptosomes are fitted to a one-site Hill equation, giving an apparent EC_{50} value of 134 ± 26 nM, whereas the data for slices are fitted to a two-site model (see *Experimental Procedures*), giving apparent EC_{50} values of 241 ± 36 nM and 5.1 ± 0.3 μ M. **, significantly different from corresponding responses to AnTx-a in the absence of kynurenic acid, $P < .01$, one-way ANOVA, Dunnett's test.

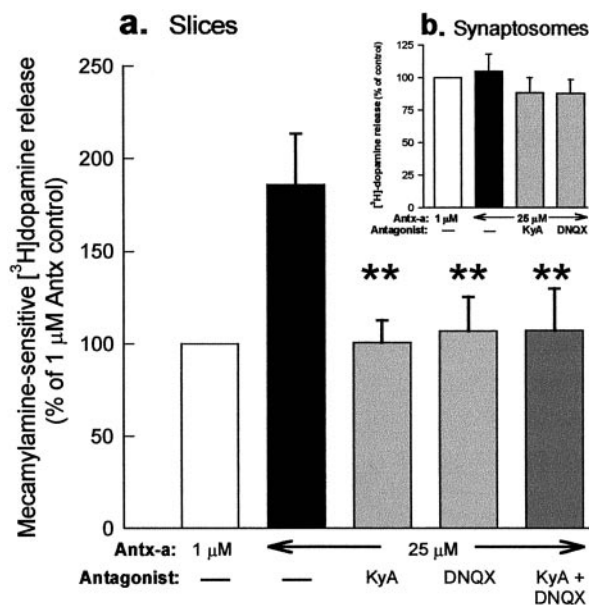


Fig. 3. Comparison of the effects of kynurenic acid and DNQX on [3 H]dopamine release evoked by 25 μ M AnTx-a. Rat striatal slices (main figure) or synaptosomes (inset) were loaded with [3 H]dopamine and superfused as described in *Experimental Procedures*. Kynurenic acid (KyA, 500 μ M) and/or DNQX (100 μ M) was applied for 10 min before the administration of a 40-s pulse of 25 μ M AnTx-a. Responses are expressed as a percent of the mecamylamine-sensitive response to 1 μ M AnTx-a, determined in parallel. Error bars indicate the standard deviation from at least four independent experiments. **, significantly different from response to 25 μ M AnTx-a in the absence of glutamate receptor antagonist, $P < .01$, one-way ANOVA, Bonferroni's t test.

are present on striatal glutamatergic terminals and promote the release of glutamate, which in turn activates presynaptic ionotropic glutamate receptors on striatal dopaminergic nerve terminals (Fig. 5).

Glutamate Heteroreceptors on Dopaminergic Terminals. The ability of the nonselective ionotropic glutamate receptor antagonist kynurenic acid to significantly decrease [3 H]dopamine release from striatal slices, evoked by the depolarizing agents KCl and 4-aminopyridine (Fig. 1, a and b) is consistent with other studies (Jin and Fredholm, 1994) and is compatible with evidence that glutamate can influence dopamine release via heteroreceptors on dopaminergic terminals (Wang, 1991; Desce et al., 1992). The similar finding using AnTx-a in place of a general depolarizing agent (Fig. 1c) suggests that nAChR activation can also evoke glutamate release. The lack of effect of kynurenic acid on AnTx-a-evoked [3 H]dopamine release from synaptosomes (Fig. 1d) supports this interpretation and, importantly, excludes the possibility that kynurenic acid was exerting a nonspecific action directly at nAChRs on dopaminergic terminals.

The comparable degree of inhibition seen with DNQX, which is selective for AMPA/kainate receptors, suggests that these ionotropic glutamate receptors are responsible for the modulation of [3 H]dopamine release described in this study. There is evidence that NMDA receptors are also present on striatal dopaminergic terminals and are capable of enhancing dopamine release (Desce et al., 1992). In that report, NMDA alone was ineffective, attributed to Mg^{2+} block of NMDA receptors, whereas activation of AMPA/kainate receptors could overcome this inhibition. Indeed, it has been proposed that ACh or (–)-nicotine, acting at presynaptic nAChRs on dopaminergic terminals, can also relieve the Mg^{2+} block of NMDA receptors, to give a synergistic effect on dopamine release when (–)-nicotine and NMDA are applied together in the presence of 1 μ M glycine (Chéramy et al., 1996). However, in the present study, NMDA receptors are unlikely to contribute because the experiments were conducted in nominally glycine-free conditions, and glycine is an obligatory coagonist of NMDA receptors (Kleckner and Dingledine, 1988; Vyklícký et al., 1990). In support of this interpretation, we have found that under the conditions used in this study, 1 mM NMDA alone failed to stimulate [3 H]dopamine release from striatal slices, and release evoked by 10 μ M (–)-nicotine was not enhanced by coapplication with

NMDA. When this experiment was repeated in the presence of 1 μ M glycine (and 1 μ M strychnine to block glycine receptors), NMDA alone was again without effect, but NMDA applied together with (–)-nicotine significantly increased [3 H]dopamine release by $52 \pm 7\%$ ($n = 4$) compared with (–)-nicotine alone (S. Kaiser and S. Wonnacott, unpublished results). Thus, we can conclude that the effects of kynurenic acid and DNQX on AnTx-a-evoked [3 H]dopamine release reported herein reflect the participation of AMPA/kainate receptors; any role of NMDA receptors was not disclosed under the experimental conditions used.

Subtypes of Presynaptic nAChRs. Impetus for this investigation came from our observation (Kaiser et al., 1998) that although AnTx-a-evoked [3 H]dopamine release in both synaptosome and slice preparations was partially inhibited by α Ctx-MII, the proportion of release attributable to $\alpha 3\beta 2^*$ nAChRs was significantly less in slices. This suggested the contribution of additional nAChRs in the more integral preparation. A comparison of the dose-response curves for AnTx-a in synaptosomes and slices shows a distinct difference in the profiles, with a second, lower-affinity component in the slice preparation (Fig. 2). The suppression of this low-affinity component by glutamate receptor antagonists and $\alpha 7^*$ nAChR antagonists, in a nonadditive manner (Figs. 2 and 4, Table 1), is consistent with an additional nicotinic component indirectly enhancing [3 H]dopamine release, via the release of glutamate. The ability to fit a two-site model to the slice data is fortuitous, reflecting the good separation of affinities for AnTx-a of the high- and low-affinity components, together with the relatively low efficacy of AnTx-a at presynaptic nAChRs on dopaminergic terminals, demonstrated in synaptosome preparations (Sharples et al., 2000). We have observed that concentration-response curves for (–)-nicotine in both synaptosome and slice preparations conform to a single-site model, but higher concentrations of nicotine (30–100 μ M) produce relatively greater amounts of [3 H]dopamine release in the slice preparation, and this increment can be blocked by DNQX (Wonnacott et al., 2000). Thus, the phenomenon described in detail here is also observed with other nicotinic agonists.

The apparent EC_{50} value for the higher-affinity component of AnTx-a-evoked [3 H]dopamine release from slices (240 nM) is similar to the EC_{50} value for synaptosomes (134 nM, Fig. 2; 110 nM, Soliakov et al., 1995). In synaptosomes, this compo-

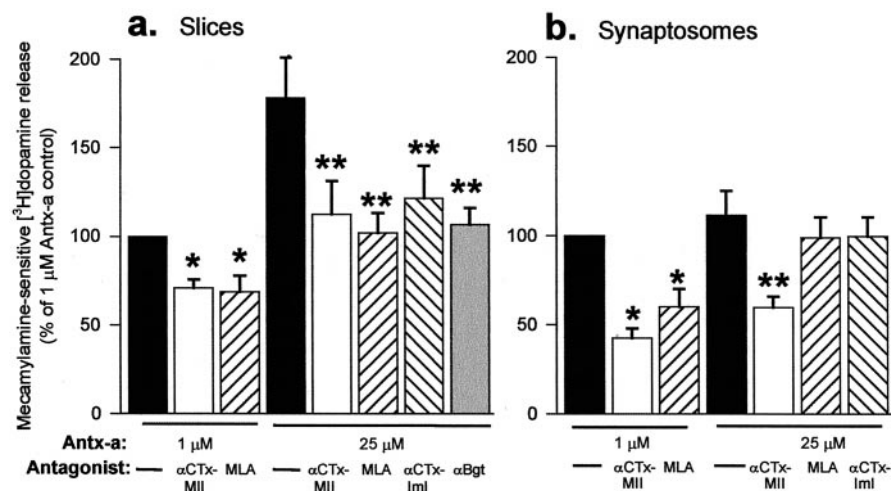


Fig. 4. Comparison of the effects of nicotinic antagonists on AnTx-a-evoked [3 H]dopamine release from slices (a) and synaptosomes (b). Rat striatal slices or synaptosomes were loaded with [3 H]dopamine and superfused as described in *Experimental Procedures*. The antagonists α Ctx-MII (112 nM), MLA (50 nM), and α Ctx-ImI (1 μ M) were applied for 10 min before stimulation with a 40-s pulse of either 1 or 25 μ M AnTx-a. Responses are expressed as a percent of the mecamylamine-sensitive response to 1 μ M AnTx-a, determined in parallel. Error bars indicate the standard deviation from at least four independent experiments. * $P < .05$, ** $P < .01$, significantly different from respective control in the absence of antagonist, one-way ANOVA, Bonferroni's t test.

nent reflects the contribution of at least two nAChR subtypes, currently considered to be $\alpha 3\beta 2^*$ and $\alpha 4\beta 2$ nAChRs (Kulak et al., 1997; Kaiser et al., 1998; Sharples et al., 2000). $\alpha 7^*$ nAChRs are not thought to be present on dopamine terminals, because α -Bgt and α Ctx-ImI fail to antagonize (-)-nicotine-evoked dopamine release from synaptosomes (Rapier et al., 1990; Grady et al., 1992; Kulak et al., 1997; A. Mogg and S. Wonnacott, unpublished observations). Therefore, inhibition by 50 nM MLA of [3 H]dopamine release evoked by 1 μ M AnTx-a was unexpected, as this concentration of MLA was assumed to be selective for $\alpha 7^*$ nAChRs (Wonnacott et al., 1993). However, Clarke and Reuben (1996) reported an IC₅₀ value of 38 nM for MLA inhibition of (-)-nicotine-stimulated [3 H]dopamine release from striatal synaptosomes. A possible explanation of the "surmountable" antagonism of nicotinic agonist-evoked [3 H]dopamine release from synaptosomes is that MLA may also interact with high affinity with combinations of nAChR subunits that have not yet been examined but that are activated by lower agonist concentrations than $\alpha 7^*$ nAChRs. This observation empha-

sizes that antagonists presumed to be subtype-selective may not be definitive when used in heterogeneous systems.

However, the ability of three structurally unrelated $\alpha 7^*$ nAChR-selective antagonists to partially inhibit (to the same extent) the release of [3 H]dopamine in slices stimulated with 25 μ M AnTx-a provides compelling evidence for the involvement of $\alpha 7^*$ nAChRs. Indeed, the apparent EC₅₀ value of 5.1 μ M determined for the low-affinity component of the AnTx-a concentration-response curve in slices (Fig. 2) is strikingly similar to the EC₅₀ value of 3.9 μ M reported for α -Bgt- and MLA-sensitive type IA currents in hippocampal neurons (Alkondon and Albuquerque, 1993). The most parsimonious interpretation of the data is that $\alpha 7^*$ nAChRs reside on striatal glutamatergic nerve terminals and directly modulate glutamate release (see Fig. 5). There is precedent for such an arrangement from some elegant electrophysiological studies in other brain regions in the rat (Alkondon et al., 1996; Radcliffe and Dani, 1998). Furthermore, the stimulatory effect of nicotine on dopamine output in the nucleus accumbens, monitored by microdialysis, has been attributed to enhanced glutamate transmission via presynaptic $\alpha 7^*$ nAChRs in the ventral tegmentum (Schilstrom et al., 1998). Reports to date suggest that presynaptic nAChRs modulating glutamate release may be exclusively of the $\alpha 7$ subtype. There is good rationale for this association: the relatively low affinity for ACh, rapid desensitization, and modulation by choline (Alkondon et al., 1999) of these receptors render them most suitable for the regulation of the release of the major excitatory transmitter whose levels must be rigorously controlled and limited to avoid the risk of overexcitation and Ca²⁺-associated neurotoxicity.

Although it would be desirable to demonstrate directly that nicotinic agonists elevate glutamate release from in vitro preparations, attempts to do this have been unsuccessful (Wonnacott et al., 1995). In addition to practical difficulties in measuring transmitter glutamate release, the rapid desensitization of $\alpha 7^*$ nAChRs may confound the ability to observe release evoked by nicotinic agonists using a superfusion protocol with relatively poor temporal resolution (Kaiser and Wonnacott, 1999).

Physiological Implications of Transmitter Cross-Talk. This study used pharmacological tools to dissect a relationship among glutamate, dopamine, and presynaptic nAChRs in the rat striatum. Although axo-axonic synapses between dopaminergic and cortical afferents have not been demonstrated in the striatum, these inputs form synapses in close proximity to each other on the dendrites and dendritic spines of the GABAergic medium spiny neurons (Smith and Bolam, 1990; see Fig. 5). Thus, glutamate would have to diffuse only a relatively short distance to influence neighboring dopaminergic terminals. The source of ACh (or choline) for activation of nAChRs is currently less clear. The medium spiny neurons are the major synaptic target for cholinergic interneurons (Izzo and Bolam, 1988), but the spatial relationship between these synapses and those using dopamine and glutamate as transmitters has not yet been established. However, less than 10% of cholinergic varicosities in the rat striatum appeared to be synaptic (Contant et al., 1996), consistent with volume transmission, a concept that is gaining currency. This interplay between transmitters at the level of the nerve terminal (illustrated in the model in Fig. 5) will constitute a subtle means of local coordination and modula-

TABLE 1

Effect of antagonists on mecamylamine-sensitive [3 H]dopamine release from rat striatal slices stimulated with 25 μ M AnTx-a

Antagonist Treatment	[3 H]Dopamine Release % 25 μ M AnTx-a control ^a
None	100.0
α -Bgt (40 nM)	60.2 \pm 15.6 (n = 8)
MLA (50 nM)	68.7 \pm 9.0 (n = 7)
α Ctx-MII (112 nM)	70.9 \pm 4.8 (n = 4)
α -Bgt + DNQX (100 μ M)	61.1 \pm 9.6 (n = 5)
MLA + DNQX (100 μ M)	67.3 \pm 11.9 (n = 3)
α -Bgt + α Ctx-MII	27.4 \pm 18.0 (n = 5)*
MLA + α Ctx-MII	28.7 \pm 2.5 (n = 3)**

^a Mecamylamine-sensitive [3 H]dopamine release.

* $P < .01$, significantly different from response in the presence of α -Bgt alone.

** $P < .01$, significantly different from response in the presence of MLA alone (one-way ANOVA, Bonferroni's t test).

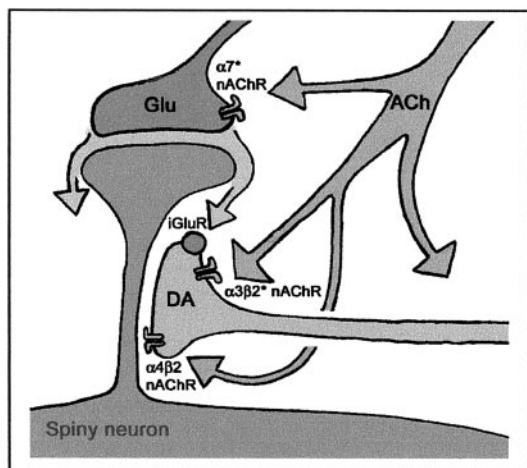


Fig. 5. Model of putative relationship between nAChRs and presynaptic boutons in the rat striatum. The dendritic spine of a medium spiny GABAergic projection neuron is illustrated. A glutamatergic input from cortex or thalamus makes an asymmetric synapse with the head of the spine; $\alpha 7^*$ nAChRs are located presynaptically and can modulate glutamate (Glu) release. A nigrostriatal dopaminergic input forms a symmetric synapse with the neck of the spine. The dopaminergic terminal contains ionotropic glutamate receptors and both $\alpha 3\beta 2^*$ and $\alpha 4\beta 2$ nAChRs, all of which can modulate dopamine release. The source of ACh, the endogenous agonist of nAChRs, is suggested to diffuse from local cholinergic interneurons.

tion. This may be reflected in altered levels of basal (tonic) transmitter release, as recorded in this study, but may also govern other regulatory mechanisms (Radcliffe and Dani, 1998), perhaps by interfacing with second messenger cascades via the Ca^{2+} permeabilities of neuronal nAChRs.

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