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The frequency-dependence of the nicotine-induced inhibition of dopamine is controlled by the $\alpha 7$ nicotinic receptor

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Abstract

Voltammetric analyses show that low (100–500 nM) doses of nicotine regulate striatal dopamine by inhibiting release evoked by a single stimulation to a greater extent than release evoked by high frequency stimulations. This frequency-dependent inhibition is because of nicotine desensitizing heteromeric $\beta 2$ subunit-containing nicotinic acetylcholine receptor (nAChR) subtypes. Surprisingly, a high dose of nicotine (2 μ M; capable of interacting with additional nAChR subtypes) produced an inhibition of dopamine evoked by high frequency stimulation, an effect that was not seen with the low dose of nicotine or the $\beta 2$ antagonist, dihydro- β -erythroidine hydrobromide. This inhibition was replicated by application of $\alpha 7$ nAChR antagonists methyllycaconitine citrate or α -bungarotoxin in conjunction with the low dose of nicotine or dihydro- β -erythroidine hydrobromide. Blocking $\alpha 7$ receptor function alone produced a modest increase in dopamine evoked by single pulse stimulation while not affecting dopamine evoked by high frequency stimulation. The antagonist results were mimicked using selective $\alpha 7$ agonists PHA 543613 and PNU 282987. The frequency dependence of the low dose nicotine inhibition therefore requires functional $\alpha 7$ nAChRs, and may arise from differing levels of endogenous acetylcholine evoked by the stimulation.

Keywords

$\alpha 7$ receptor; $\beta 2$ receptor; dopamine; nicotine; striatum; voltammetry

Tobacco use is one of the leading causes of preventable death in the United States. Nicotine is highly addictive and accordingly tobacco use continues to pose a substantial public health risk decades after its harmful effects were first reported. However, nicotine may have beneficial aspects as well, such as reducing the incidence of Parkinson's Disease in humans (Morens *et al.* 1995; Gorell *et al.* 1999). Increased knowledge about how the nicotinic acetylcholine receptor (nAChR) system interacts with dopamine signaling may lead to improved nicotine cessation treatments, as well as aiding in the struggle against neurodegenerative conditions like Parkinson's Disease.

The nAChRs are expressed throughout the rodent nucleus accumbens and striatum, with expression of the relatively high nicotine affinity $\beta 2$ -containing heteromeric nAChRs, and the low affinity homomeric $\alpha 7$ nAChRs (Zoli *et al.* 2002; Champtiaux *et al.* 2003; Grady *et al.* 2007; Gotti *et al.* 2010). There is no evidence to date of $\alpha 7$ receptor function directly on dopamine terminals.

Active dopamine neurons fire at approximately 5 Hz under normal conditions, but exhibit sub-second duration bursts of high frequency firing at approximately 20 Hz (Clark and Chiodo 1988), which correlates with reward-based events (Schultz and Romo 1990). The application of doses of nicotine approximating the levels seen in human smokers (~100 nM) produces an inhibition of dopamine release evoked by single pulse stimulations that is mimicked by $\beta 2$ receptor-selective antagonists, and is not observed in mice with the $\beta 2$ subunit knocked out (Zhou *et al.* 2001). The nicotine-induced inhibition (through desensitization of $\beta 2$ -containing nAChRs) was also found to decrease in strength as the frequency of stimulation increased (Rice and Cragg 2004; Zhang and Sulzer 2004). Thus, dopamine evoked under low frequency, basal firing conditions is inhibited by nicotine to a greater extent than during high frequency burst conditions. This increases the signal to noise level of dopamine produced by the burst potentials. This frequency-dependence magnifies the apparent concentration of dopamine produced by high frequency bursts, thereby acting as a filter of dopamine signals and potentially playing a role in nicotine's rewarding effects (Exley and Cragg 2008).

Although some evidence for $\alpha 7$ receptor modulation of dopamine levels exists (Kaiser and Wonnacott 2000; Livingstone *et al.* 2009), the impact of these receptors has not been thoroughly tested. The ability of $\alpha 7$ receptors to modulate evoked dopamine alone or in conjunction with nicotine was examined. We found $\alpha 7$ nAChR involvement in the nicotine action upon evoked dopamine.

Methods

Animals

Sprague–Dawley rats between 14 and 21 days old were anesthetized with halothane and killed. Coronal slices 250- μ m thick were obtained and allowed to equilibrate in buffer solution for 1 h prior to placement in the recording chamber. Recordings were performed in striatal tissue in the dorsal lateral striatum from slices cut posterior 1.5–2 mm from bregma. All procedures were approved and performed in compliance with NIEHS/NIH Humane Care and Use of Animals in Research protocols.

Voltammetry

Electrodes were fabricated by aspirating T-650 carbon fibers (courtesy of Cytec Industries, Woodland Park, NJ, USA) into glass capillaries which were then pulled in a pipette puller and cut to a length of less than 20 μ m. A triangle waveform from -0.4 V to 1.0 V and then back to -0.4 V was applied at 250 V/s, and reapplied at a rate of 10 Hz. Data were recorded using the TH-1 voltammetry software (ESA Bioscience, Chelmsford, MA, USA). Local electrical stimulations were produced in the dorsal lateral striatum with a bipolar stimulating electrode placed approximately 100 μ m from the recording site. Stimulations (600 μ A, 1 pulse at 100 Hz) were applied every 5 min until stable dopamine release was obtained, after which alternating stimulations (1 pulse or 4 pulses at 100 Hz) were applied every 5 min for the duration of the experiment. After 30 min of pre-drug control data collection, pharmacological agents (or buffer for control) were added to the bath solution for 1 h. Statistical significance was calculated using 1 way ANOVA with Dunet's post-test. AUTHOR: Please check the word 'Dunet's' here in text.. Significance was assigned with 95% confidence interval ($p < 0.05$).

Materials

The buffer solution used during slicing and recording consisted of 125 mM sodium chloride, 3.5 mM potassium chloride, 1.2 mM sodium phosphate, 25 mM sodium bicarbonate, 2 mM calcium chloride, 1.3 mM magnesium chloride and 11 mM glucose. Nicotine hydrogen

tartrate salt (500 nM, 2 μ M), dihydro- β -erythroidine hydrobromide (DH β E; 100 nM), α -bungarotoxin (10 nM dissolved in phosphate-buffered saline; Tocris Bioscience, Ellisville, MO, USA), PHA 543613 hydrochloride (50 nM; Tocris Bioscience), PNU 282987 (100 nM in 1 eq HCl; Tocris Bioscience), 6-cyano-7-nitroquinoxaline-2,3-dione disodium (25 μ M), D-(–)-2-amino-5-phosphonopentanoic acid (25 μ M) and methyllycaconitine citrate (MLA; 10 nM) were used for the experiments. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in water unless otherwise noted.

Results

We investigated whether $\alpha 7$ -containing nAChRs, which previously were believed to play a minor role, modulate evoked dopamine. Two stimulation patterns were used; a single 600 μ A electrical stimulation that previously displayed the strongest inhibition by nicotine, and a high frequency stimulation with a 4 pulse burst of 600 μ A electrical pulses given at 100 Hz (Zhou *et al.* 2001; Rice and Cragg 2004; Zhang *et al.* 2009). The latter stimulation was used to model the short bursts dopamine neurons exhibit while the former models low frequency basal firing.

Evoked dopamine is a complex signal controlled by diffusion from release sites, uptake by the dopamine transporter, and dopamine release (Wu *et al.* 2001; Cragg and Rice 2004; Rice and Cragg 2008). The pharmacological manipulations described in this work are believed to modulate evoked dopamine signals primarily through altering dopamine release.

Single pulse stimulation

Regulation of evoked dopamine by nicotine during single pulse stimulations

—The ability of nicotine at both low (500 nM) and high (2 μ M) doses to modulate evoked dopamine in the striatum was examined (Fig. 1). Nicotine at the low dose of 500 nM inhibited evoked dopamine to only $39 \pm 5\%$ ($n = 9$, $p < 0.05$) of pre-drug evoked dopamine. As observed previously, this effect was mimicked by the $\beta 2$ receptor antagonist, DH β E, which inhibited evoked dopamine to $35 \pm 6\%$ ($n = 6$, $p < 0.05$) of pre-drug evoked dopamine (Fig. 1b). A high concentration of nicotine (2 μ M), a dose which is capable of interacting with $\alpha 7$ nAChRs (Papke *et al.* 2007), produced a similar inhibition ($25 \pm 4\%$, $n = 5$, $p < 0.05$).

Regulation of evoked dopamine by $\alpha 7$ receptors during single pulse stimulations

—The application of 10 nM of the $\alpha 7$ -selective antagonist MLA alone increased evoked dopamine ($123 \pm 7\%$, $n = 8$; $p < 0.05$) (Fig. 2). However, when either 500 nM nicotine or 100 nM DH β E was combined with 10 nM MLA, a significant inhibition similar to 500 nM nicotine alone was elicited ($29 \pm 5\%$, $n = 7$, $p < 0.05$; $37 \pm 7\%$, $n = 8$, $p < 0.05$, respectively). Therefore, MLA did not appear to have an effect on either the nicotine- (low dose) or DH β E-induced decrease in evoked dopamine.

The effects of selective $\alpha 7$ agonists upon evoked dopamine were also tested. The agonists, 50 nM PHA 543613 and 100 nM PNU 282987 (Bodnar *et al.* 2005; Wishka *et al.* 2006; Acker *et al.* 2008) did not affect observed dopamine ($86 \pm 9\%$, $n = 4$; $81 \pm 2\%$, $n = 3$, respectively) (Fig. 3). The combination of PHA 543613 with 500 nM nicotine reduced evoked dopamine to $47 \pm 10\%$ ($n = 4$, $p < 0.05$) of pre-drug evoked dopamine and PNU 282987 with nicotine reduced evoked dopamine to $28 \pm 6\%$ ($n = 3$, $p < 0.05$) of pre-drug evoked dopamine. The $\alpha 7$ agonists did not appear to have an effect upon the nicotine-induced decrease in evoked dopamine, which is consistent with previous findings that nicotine decreases dopamine evoked by single pulse stimulation through $\beta 2$ desensitization.

High frequency stimulation

Modulation of evoked dopamine by nicotine during high frequency stimulation

In contrast to the effect on single pulse stimulations, dopamine evoked by high frequency stimulations was not significantly decreased by 500 nM nicotine ($73 \pm 6\%$, $n = 9$, $p > 0.05$) or 100 nM DH β E ($75 \pm 12\%$, $n = 6$, $p > 0.05$) (Fig. 4a and b). Unexpectedly, however, evoked dopamine was significantly reduced by the high dose (2 μ M) of nicotine ($38 \pm 6\%$, $n = 5$, $p < 0.05$), which suggests that non- $\beta 2$ subunit-containing nAChRs, such as the $\alpha 7$ nAChR, may also modulate dopamine evoked by high frequency stimulation.

Manipulation of $\alpha 7$ nAChRs along with nicotine inhibits evoked dopamine during high frequency stimulation

The application of 10 nM MLA had no impact upon observed dopamine ($106 \pm 10\%$, $n = 8$) when given alone. However, when 500 nM nicotine was combined with 10 nM MLA, evoked dopamine was significantly reduced to $48 \pm 6\%$ of pre-drug evoked dopamine under high frequency stimulation ($n = 7$, $p < 0.05$) (Fig. 5a and b), which, unlike the effect of nicotine alone under these conditions, is significantly lower than control. The substitution of nicotine with 100 nM DH β E produced a similar inhibition (59 ± 9 , $n = 8$, $p < 0.05$). These data suggest that when the $\alpha 7$ nAChR is blocked, the low (500 nM) nicotine dose will inhibit dopamine evoked by high frequency stimulation, indicating that $\alpha 7$ nAChR function is regulating evoked dopamine under these conditions.

Dose response data was obtained for MLA with and without 500 nM nicotine (Fig. 6). MLA alone produced a significant change in evoked dopamine for 1 pulse stimulations at 10 nM ($123 \pm 7\%$, $n = 8$, $p < 0.05$) while not producing significant changes in dopamine at 1, 5 or 50 nM (not shown). When combined with nicotine, all tested MLA doses displayed significant reductions in evoked dopamine versus control [%Pre-drug evoked dopamine (1 nM MLA: $48 \pm 6\%$, $n = 3$; 5 nM MLA: $33 \pm 12\%$, $n = 3$; 10 nM: $29 \pm 5\%$, $n = 7$; 50 nM: $30 \pm 6\%$, $n = 3$, $p < 0.05$)]. MLA alone did not affect dopamine evoked by 4 pulse, 100 Hz stimulations at any concentration. For high frequency stimulations, the combination of nicotine with 5, 10, and 50 nM (not shown) MLA produced significant results [%Pre-drug evoked dopamine (5 nM: $56 \pm 1\%$, $n = 3$; 10 nM: $48 \pm 6\%$, $n = 7$; 50 nM: $52 \pm 23\%$, $n = 3$, $p < 0.05$)] unlike the combination of nicotine with 1 nM MLA.

The selective $\alpha 7$ agonists, PHA 543613 and PNU 282987, both displayed similar results to 10 nM MLA alone, and when paired with 500 nM nicotine (Fig. 7). Both 50 nM PHA and 100 nM PNU doses failed to modulate evoked dopamine ($96 \pm 10\%$, $n = 4$; $107 \pm 2\%$, $n = 3$, respectively). When combined with 500 nM nicotine, PHA 543613 reduced evoked dopamine to $52 \pm 10\%$ ($n = 4$, $p < 0.05$) of pre-drug evoked dopamine. Nicotine combined with PNU 282987 decreased evoked dopamine to $64 \pm 3\%$ ($n = 5$, $p < 0.05$) of pre-drug evoked dopamine. These results indicate that the agonists are desensitizing $\alpha 7$ nAChRs as they are producing a reduction in dopamine evoked by high frequency stimulation when combined with nicotine, similar to the effect of direct antagonism of $\alpha 7$ nAChRs with MLA.

α -Bungarotoxin application

Inhibition of $\alpha 7$ nAChRs with α -bungarotoxin along with nicotine inhibits evoked dopamine

The selective $\alpha 7$ nAChR antagonist, α -bungarotoxin (10 nM) had no impact upon dopamine evoked by single pulse ($103 \pm 7\%$, $n = 4$) or high frequency ($95 \pm 4\%$, $n = 4$) stimulation (Fig. 8). When combined with the low 500 nM dose of nicotine, α -bungarotoxin significantly inhibited dopamine evoked by single pulse ($51 \pm 4\%$, $n = 4$, $p < 0.05$) and high frequency ($53 \pm 7\%$, $n = 4$, $p < 0.05$), an effect similar to that seen with MLA. These data provide further evidence that the inhibition of dopamine evoked by high frequency stimulation involves the $\alpha 7$ nAChR.

Glutamate antagonist application

Antagonism of α -amino-3-hydroxy-5-methylisoxazole-4-propionate and NMDA receptors does not modulate nicotine's inhibition of evoked dopamine—Slices were pre-treated with 25 μ M 6-cyano-7-nitroquinoxaline-2,3-dione disodium and 25 μ M D-(–)-2-amino-5-phosphonopentanoic acid for 1 h prior to addition of 500 nM nicotine. Dopamine evoked by 1 pulse stimulations was significantly decreased to $60 \pm 9\%$ ($n = 4$, $p < 0.05$) of pre-nicotine level. With 4 pulse, 100 Hz stimulations, evoked dopamine was not significantly altered from pre-nicotine evoked dopamine ($79 \pm 13\%$, $n = 4$). The block of α -amino-3-hydroxy-5-methylisoxazole-4-propionate and NMDA signaling was unable to modulate the effect of 500 nM nicotine administration upon evoked dopamine release.

Discussion

The previously reported findings that nicotine produces a frequency-dependent inhibition of evoked dopamine are critical to understanding how nAChRs modulate dopamine neurotransmission in the striatum. Nanomolar doses of nicotine strongly inhibit dopamine evoked by single pulse stimulation, and the inhibition diminishes as the number of stimulus pulses and stimulus frequency are increased. Though much of the current debate has focused upon characterizing the mechanisms behind the inhibition, no investigation of the mechanisms behind the frequency-dependence has been reported. Here, we have shown that the $\alpha 7$ nAChR is required for the frequency-dependence of the inhibition (i.e. the lack of effect seen under high frequency stimulations).

The $\alpha 7$ receptor mitigates the effect of $\beta 2$ receptor desensitization with high frequency stimulation

Unlike dopamine evoked by single pulse stimulation, dopamine evoked by high frequency stimulation is not significantly inhibited by $\beta 2$ nAChR desensitization or antagonism. However, when applied at a high dose (2 μ M), nicotine significantly inhibits dopamine release under both single and 4 pulse, 100 Hz stimulus paradigms. This high dose of nicotine is capable of interacting with and desensitizing $\beta 2$ and $\alpha 7$ nAChRs, and the inhibition it produced suggests that the frequency-dependent inhibition of evoked dopamine by nicotine is more complex than simply $\beta 2$ nAChR desensitization.

Antagonism of $\alpha 7$ nAChRs alone did not impact evoked dopamine, however the combination of $\alpha 7$ antagonists with 500 nM nicotine or DH β E produced an inhibition of dopamine evoked by high frequency stimulation. Agonism of the $\alpha 7$ nAChR with PHA 543613 or PNU 282987 mimicked the results observed with the antagonists, MLA and α -bungarotoxin, which suggests that the agonist effects upon evoked dopamine are because of desensitization of the $\alpha 7$ receptors. From this observation, we conclude that the loss of $\beta 2$ nAChR function through either antagonism or desensitization inhibits evoked dopamine release regardless of stimulus paradigm. Instead, the $\alpha 7$ nAChR blocks or compensates for the inhibition of dopamine during high frequency stimulation, and is responsible for the frequency-dependent aspect of the inhibition of evoked dopamine by nanomolar nicotine doses.

The $\alpha 7$ receptor does not modify the nicotine effect with single pulse stimulation

The loss of $\beta 2$ receptor function via either desensitization induced by a low nicotine dose, or use of the $\beta 2$ antagonist DH β E, significantly reduces dopamine evoked by single pulse stimulation, an effect similar to that observed with a high nicotine dose. Loss of $\alpha 7$ receptor function alone using the specific antagonist MLA produces a modest yet significant increase in dopamine evoked by single pulse stimulation. The application of α -bungarotoxin alone did not affect evoked dopamine, nor did either of the selective $\alpha 7$ agonists, PHA 543613 or

PNU 282987. Therefore, the $\alpha 7$ receptor has little to no discernable effect upon dopamine evoked by single pulse stimulation, alone or in the presence of nicotine.

The local stimulations are generating endogenous acetylcholine release

The inhibition of evoked dopamine by nAChR antagonists ($\beta 2$ and $\beta 2/\alpha 7$ combination) for high frequency but not single pulse stimulations suggests that the stimulations are evoking the release of significant levels of endogenous acetylcholine (ACh). The striatum contains the terminals of cholinergic interneurons that will be affected along with dopamine terminals by the local electrical stimulation. The two stimulation paradigms are potentially producing two distinctly different neurochemical environments with unique levels of $\alpha 7$ nAChR activity. For single pulse stimulations, we suggest that the $\alpha 7$ receptors are not saturated and nanomolar concentrations of nicotine strongly inhibit release, while the $\alpha 7$ receptors may be saturated during high frequency stimulations and nanomolar concentrations of nicotine appear to have no effect. By removing the influence of $\alpha 7$ receptors with selective antagonists or desensitization, nicotine produced similar inhibitions for both stimulus paradigms. The frequency dependence of the nicotine inhibition may therefore occur because the high frequency stimulation produces an ACh concentration capable of activating $\alpha 7$ receptors, while the single pulse stimulation evokes an ACh level insufficient to saturate the $\alpha 7$ receptors.

The actions of the $\alpha 7$ antagonists are not because of non-selective binding

At higher concentrations than those used here, MLA has been shown to lose selectivity for $\alpha 7$ nAChRs and also bind to $\beta 2$ receptors with the highest affinity for the $\alpha 6\beta 2$ receptor (Klink *et al.* 2001). Though still a matter of debate, the $\alpha 6\beta 2$ receptor has been specifically implicated as the primary $\beta 2$ nAChR responsible for inhibition of dopamine evoked by low frequency stimulation by nanomolar nicotine concentrations and DH β E (Exley *et al.* 2008). Non-specific antagonism of the $\alpha 6\beta 2$ nAChR is an unlikely explanation for the observed inhibitions of dopamine evoked by high frequency stimulation for multiple reasons. First, the $\alpha 6\beta 2$ receptor is likely desensitized or antagonized by both 500 nM nicotine and 100 nM DH β E administration (respectively), conditions under which dopamine evoked by the high frequency stimulation is not significantly affected. Second, MLA did not inhibit dopamine evoked by single pulse stimulation as would be expected if $\beta 2$ nAChRs were also blocked. Lastly, the ability of the $\alpha 7$ nAChR to modulate evoked dopamine was confirmed by substitution of α -bungarotoxin and two selective agonists for the $\alpha 7$ nAChR. Therefore, we conclude that the inhibition of dopamine evoked by high frequency stimulation that we have observed is because of antagonism of $\alpha 7$ (and not $\alpha 6\beta 2$) nAChRs.

Potential implications

Direct block or desensitization of striatal $\alpha 7$ receptors would remove the frequency-dependence of the nicotine inhibition and greatly diminish dopamine evoked by both burst and basal firing patterns, potentially reducing the rewarding effect of nicotine. Though the receptors are expressed in the midbrain in a subset of dopamine neurons (Klink *et al.* 2001; Zoli *et al.* 2002), there is currently a lack of evidence for functional $\alpha 7$ nAChR expression directly on dopamine terminals. It is thought that pre-synaptic $\alpha 7$ nAChRs in the striatum are primarily located on cholinergic and glutamatergic terminals, and may modulate dopamine indirectly (Kaiser and Wonnacott 2000; Quik *et al.* 2005; Livingstone *et al.* 2009).

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Abbreviations used

ACh	acetylcholine
DHβE	dihydro- β -erythroidine hydrobromide
MLA	methyllocaconitine citrate
nAChR	nicotinic acetylcholine receptor

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Fig. 1. Manipulation of β_2 receptors modulates dopamine evoked from single pulse stimulations. (a) Representative traces of dopamine evoked by single pulse stimulations are shown with pre-drug (–) and post-drug (+) values from the same experiment for bath application of 500 nM (top) and 2 μ M nicotine (bottom) are shown. Tick marks depict the onset of the electrical stimulation. Voltammograms with solid lines are from the corresponding pre-drug trace and dashed lines are from the post-drug trace. (b) The averaged data for 500 nM nicotine, 100 nM DH β E, and 2 μ M nicotine are shown as percent of pre-drug evoked dopamine. Error bars display SEM and * denotes $p < 0.05$ versus control.

Fig. 2.

Block of $\alpha 7$ receptors has modest effects upon dopamine evoked from single pulse stimulations. (a) Representative traces of dopamine evoked by single pulse stimulations are shown with pre-drug (-) and post-drug (+) values from the same experiment for bath application of 10 nM MLA (top) and 10 nM MLA with 500 nM nicotine (bottom) are shown. Tick marks depict the onset of the electrical stimulation. Voltammograms with solid lines are from the corresponding pre-drug trace and dashed lines are from the post-drug trace. (b) The averaged data for 10 nM MLA, 10 nM MLA with 500 nM nicotine, and 10 nM MLA with 100 nM DH β E are shown as percent of pre-drug evoked dopamine. Error bars display SEM and * denotes $p < 0.05$ versus control.

Fig. 3.

Selective $\alpha 7$ agonists do not modulate evoked dopamine or the nicotine inhibition of evoked dopamine. (a) Representative traces of dopamine evoked by single pulse stimulations are shown with pre-drug (-) and post-drug (+) values from the same experiment for bath application of 50 nM PHA 543613 with 500 nM nicotine (top) and 100 nM PNU 282987 with 500 nM nicotine (bottom) are shown. Tick marks depict the onset of the electrical stimulation. Voltammograms with solid lines are from the corresponding pre-drug trace and dashed lines are from the post-drug trace. (b) The averaged data for 50 nM PHA 543613, 50 nM PHA 543613 with 500 nM nicotine, 100 nM PNU 282987, and 100 nM PNU 282987 with 500 nM nicotine are shown as percent of pre-drug evoked dopamine release. Error bars display SEM and * denotes $p < 0.05$ versus control.

Fig. 4.

Manipulation of β_2 receptors has no significant effect upon dopamine evoked by high frequency stimulations. (a) Representative traces of dopamine evoked by 4 pulse, 100 Hz stimulations are shown with pre-drug (-) and post-drug (+) values from the same experiment for bath application of 500 nM (top) and 2 μ M nicotine (bottom) are shown. The box depicts the onset of the 4 pulse, 100 Hz electrical stimulation. Voltammograms with solid lines are from the corresponding pre-drug trace and dashed lines are from the post-drug trace. (b) The averaged data for 500 nM nicotine, 100 nM DH β E, and 2 μ M nicotine are shown as percent of pre-drug evoked dopamine. Error bars display SEM and * denotes $p < 0.05$ versus control.

Fig. 5.

The block of $\alpha 7$ receptors in conjunction with nicotine inhibits dopamine evoked by high frequency stimulation. (a) Representative traces of dopamine evoked by 4 pulse, 100 Hz stimulations are shown with pre-drug (–) and post-drug (+) values from the same experiment for bath application of 10 nM MLA (top) and 10 nM MLA with 500 nM nicotine (bottom) are shown. The box depicts the onset of the 4 pulse, 100 Hz electrical stimulation. Voltammograms with solid lines are from the corresponding pre-drug trace and dashed lines are from the post-drug trace. (b) The averaged data for 10 nM MLA, 10 nM MLA with 500 nM nicotine, and 10 nM MLA with 100 nM DH β E are shown as percent of pre-drug evoked dopamine. Error bars display SEM and * denotes $p < 0.05$ versus control.

Fig. 6.

Dose response for MLA and MLA with nicotine for single pulse and high frequency stimulation. (Top) Averaged data for dopamine evoked by single pulse stimulation for 1, 5, and 10 nM MLA (square) and 1, 5, and 10 nM MLA with 500 nM nicotine (circle). (Bottom) Averaged data for dopamine evoked by 4 pulse, 100 Hz stimulation for 1, 5, and 10 nM MLA (square) and 1, 5, and 10 nM MLA with 500 nM nicotine (circle). Error bars represent SEM and * denotes $p < 0.05$ versus control.

Fig. 7.

Desensitization of $\alpha 7$ receptors with selective agonists in conjunction with nicotine inhibits dopamine evoked by high frequency stimulation. (a) Representative traces of dopamine evoked by 4 pulse, 100 Hz stimulations are shown with pre-drug (-) and post-drug (+) values from the same experiment for bath application of 50 nM PHA 543613 with 500 nM nicotine (top) and 100 nM PNU 282987 with 500 nM nicotine (bottom) are shown. The box depicts the onset of the 4 pulse, 100 Hz electrical stimulation. Voltammograms with solid lines are from the corresponding pre-drug trace and dashed lines are from the post-drug trace. (b) The averaged data for 50 nM PHA 543613, 50 nM PHA 543613 with 500 nM nicotine, 100 nM PNU 282987, and 100 nM PNU 282987 with 500 nM nicotine are shown as percent of pre-drug evoked dopamine. Error bars display SEM and * denotes $p < 0.05$ versus control.

Fig. 8. Nicotine with α -bungarotoxin inhibits dopamine evoked by high frequency stimulation. (a) Representative traces of dopamine evoked by single pulse stimulations (top) and 4 pulse, 100 Hz stimulations (bottom) are shown with pre-drug (–) and post-drug (+) values from the same experiment with the bath application of 500 nM nicotine with 10 nM α -bungarotoxin. Tick marks depict the application of the single electrical stimulation while the box depicts the onset of the 4 pulse, 100 Hz electrical stimulation. Voltammograms with solid lines are from the corresponding pre-drug trace and dashed lines are from the post-drug trace. (b) The averaged data for relative dopamine evoked by 1 pulse (gray bars) and 4 pulse, 100 Hz (black bars) electrical stimulations for 10 nM α -bungarotoxin and 10 nM α -bungarotoxin with 500 nM nicotine are shown. Error bars display SEM and * denotes $p < 0.05$ versus control.