Biochemical Pharmacology 80 (2010) 402–409

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Repeated nicotine administration robustly increases bPiDDB inhibitory potency at α6β2-containing nicotinic receptors mediating nicotine-evoked dopamine release

Andrew M. Smith [a], Marharyta Pivavarchyk [a], Thomas E. Wooters [b], Zhenfa Zhang [a], Guangrong Zheng [a], J. Michael McIntosh [d], Peter A. Crooks [a], Michael T. Bardo [b,c], Linda P. Dwoskin [a,c,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536, USA
[b] Department of Psychology, College of Arts and Sciences, University of Kentucky, Lexington, KY 40506, USA
[c] Center for Drug Abuse Research Translation, University of Kentucky, Lexington, KY 40506, USA
[d] Departments of Biology and Psychiatry, University of Utah, School of Medicine, Salt Lake City, UT 84132, USA

ARTICLE INFO

Article history:
Received 1 January 2010
Accepted 18 March 2010

Keywords:
Smoking cessation
Nicotine
Nicotinic acetylcholine receptor
Dopamine release
Nicotinic receptor antagonist

ABSTRACT

The novel nicotinic receptor (nAChR) antagonist, *N,N′*-dodecane-1,12-diyl-*bis*-3-picolinium dibromide (bPiDDB), and its chemically reduced analog, r-bPiDDB, potently inhibit nicotine-evoked dopamine (DA) release from rat striatal slices. Since tobacco smokers self-administer nicotine repeatedly, animal models incorporating repeated nicotine treatment allow for mechanistic evaluation of therapeutic candidates following neuroadaptive changes. The current study determined the ability of bPiDDB, r-bPiDDB and α-conotoxin MII (α-CtxMII), a peptide antagonist selective for α6β2-containing nAChRs, to inhibit nicotine-evoked [^{3}H]DA release from striatal slices from rats repeatedly administered nicotine (0.4 mg/kg for 10 days) or saline (control). Concomitant exposure to maximally effective concentrations of r-bPiDDB (1 nM) and α-CtxMII (1 nM) resulted in inhibition of nicotine-evoked [^{3}H]DA release no greater than that produced by either antagonist alone, suggesting that r-bPiDDB inhibits α6β2-containing nAChRs. Repeated nicotine treatment increased locomotor activity, demonstrating behavioral sensitization. Concentration–response curves for nicotine-evoked [^{3}H]DA release were not different between nicotine-treated and control groups. Maximal inhibition for α-CtxMII was greater following repeated nicotine compared to control (I_{max} = 90% vs. 62%), with no change in potency. bPiDDB was 3-orders of magnitude more potent in inhibiting nicotine-evoked [^{3}H]DA release in nicotine-treated rats compared to control rats (IC_{50} = 5 pM vs. 6 nM), with no change in maximal inhibition. Neither a shift to the left in the concentration response nor a change in maximal inhibition was observed for r-bPiDDB following repeated nicotine. Thus, repeated nicotine treatment may differentially regulate the stoichiometry, conformation and/or composition of α6β2-containing nAChRs mediating nicotine-evoked striatal DA release. Therefore, bPiDDB and r-bPiDDB appear to target different α6β2-containing nAChR subtypes.

1. Introduction

Tobacco smoking is a chronic, relapsing disorder [1,2], and nicotine plays a major role in continued tobacco use through its intrinsic reinforcing properties [3,4]. Nicotine is self-administered intravenously in animal models [5–7] and by smokers that have been tobacco deprived [8]. Nicotine self-administration is decreased (>60%) by the nonselective nAChR antagonist, mecamylamine [9,10], indicating mediation by nAChRs. Nicotine activation of nAChRs leads to increased extracellular dopamine (DA) concentrations, which produces reward and ultimately tobacco addiction [11,12]. Although the exact subunit composition and stoichiometry of native nicotinic receptors has not been elucidated conclusively, based on research using transgenic mice and on α-conotoxin MII (α-CtxMII)-sensitivity, six different nAChR subtypes appear to contribute to nicotine-evoked DA release from mouse striatal synaptosomes, including: α6β2-, α6β2β3-, α6α4β2- and

Abbreviations: nAChR, nicotinic acetylcholine receptor; DA, dopamine; NIC, nicotine; SAL, saline; MEC, mecamylamine; DHβE, dihydro-β-erythroidine; α-CtxMII, α-conotoxin MII; bPiDDB, *N,N′*-dodecane-1,12-diyl-*bis*-3-picolinium dibromide; r-bPiDDB, 1,12-*bis*(3-methyl-1,2,5,6-tetrahydropyridinyl)dodecane; ANOVA, analysis of variance.

* Corresponding author at: Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 725 Rose Street, Lexington, KY 40536-0082, USA. Tel.: +1 859 257 4743; fax: +1 859 323 3575.
E-mail address: ldwoskin@email.uky.edu (L.P. Dwoskin).

0006-2952/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2010.03.018

α6α4β2β3-containing receptors, which are α-CtxMII-sensitive, and α4β2- and α4α5β2-containing receptors, which are α-CtxMII-insensitive [13,14]. Furthermore, the α4α6β2β3-containing receptor subtype, which constitutes ~50% of α6-containing nAChRs on DA terminals in striatum from wild-type mice, has the highest sensitivity to nicotine [14,15], strongly implicating this subtype in nicotine-evoked DA release and reward.

Neuroadaptations in response to repeated nicotine treatment are thought to play an important role in the maintenance of nicotine addiction [2]. In recombinant receptor systems, incubation with nicotine (1 μM–1 mM for 24 h) alters nAChR subtype stoichiometry, function, and post-translational maturation, i.e., the process of subunits leaving the endoplasmic reticulum and the subsequent incorporation into functional nAChRs [16–18]. Incubation with nicotine (10 μM for 24 h) also influences nAChR conformation (i.e., subunit folding) in cell expression systems [19]. Recombinant nAChR systems allow the study of single subtypes in isolation from other subtypes and from cellular factors that could complicate or obscure receptor responses. However, this approach is not capable of capturing the anatomical and multi-transmitter complexity inherent in brain tissue, and thus, translation of findings in these studies to smokers is limited inherently.

Early studies using animal models report increases in [^{3}H]nicotine and [^{3}H]cytisine binding in brain in both rats and mice repeatedly treated with nicotine, indicating an increased number of nAChR binding sites [20,21]. Subsequent studies using fluorescently tagged α4 subunits have demonstrated that chronic nicotine increases expression of nAChRs containing α4 subunits in the ventral tegmental area and substantia nigra pars compacta [22]. Deletion of the β2 subunit in mice eliminates increases in [^{125}I]epibatidine binding observed after continuous nicotine administration (1–4 mg/kg/h for 10 days) [23]. Collectively, these studies indicate that chronic nicotine administration results in up-regulation of α4- and β2-containing nAChR subtypes. Recently, binding studies using [^{125}I]α-CtxMII to selectively label α6-containing nAChRs showed a decrease in receptor number in rat and mouse striatum following chronic nicotine treatment [24–26]. Immunoprecipitation studies have demonstrated that the presence of α5 and β3 subunits in α4α5β2- and α6β2β3-containing nAChR subtypes, respectively, prevents the alterations (both up and down regulation, respectively) in subtype expression following chronic nicotine treatment [24,27]. Thus, expression of specific subunits in a given subtype can prevent observed neuroadaptations in response to repeated nicotine treatment. Therefore, pharmacological history is a critical consideration when investigating the role of different nAChR subtypes mediating the CNS response to nicotine.

The novel nAChR antagonist, *N*,*N*′-dodecane-1,12-diyl-*bis*-3-picolinium dibromide (bPiDDB; Fig. 1), potently (IC_{50} = 2 nM) inhibits nicotine-evoked striatal [^{3}H]DA release through an interaction with α6β2-containing nAChRs [28,29] and inhibits nicotine-evoked DA release from nucleus accumbens in *in vivo* microdialysis studies [30]. Further, peripherally administered bPiDDB decreases intravenous nicotine self-administration in rats [31]. In addition, as part of the optimization to improve drug-like properties of bPiDDB, a structural analog was designed in which the two 3-picolinium moieties were reduced chemically to tertiary amino moieties (3-methyl-1,2,5,6-tetrahydropyridyl), thus affording r-bPiDDB (Fig. 1). r-bPiDDB is 10-fold more potent (IC_{50} = 0.3 nM) at inhibiting nicotine-evoked [^{3}H]DA release than bPiDDB, and also decreases nicotine self-administration [32]. The purpose of the current study was to determine if bPiDDB and/or r-bPiDDB inhibits nicotine-evoked [^{3}H]DA release from striatal slices from rats repeatedly administered nicotine. Also, studies were performed to determine whether r-bPiDDB inhibits α6-containing nAChR subtypes in rats administered nicotine or saline repeatedly.

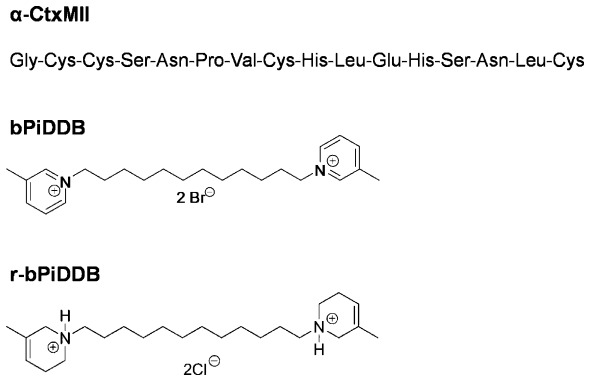

Fig. 1. The amino acid sequence for α-conotoxin MII (α-CtxMII; top) and the structures of *N*,*N*-dodecane-1,12-diyl-*bis*-picolinium dibromide (bPiDDB; middle) and reduced-bPiDDB (r-bPiDDB; bottom).

2. Methods

2.1. Materials

[^{3}H]DA (dihydroxyphenylethylamine, 3,4-[ring-2,5,6-3H]-; specific activity 28.0 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences, Inc. (Boston, MA). S(−)nicotine ditartrate (Nicotine), nomifensine maleate, dihydro-beta-erythroidine, mecamylamine and pargyline hydrochloride were obtained from Sigma–Aldrich (St. Louis, MO). TS-2 tissue solubilizer and scintillation cocktail were purchased from Research Products International Corp. (Mt. Prospect, IL). All other chemicals used in the *in vitro* assay buffers were purchased from Thermo Fisher Scientific (Waltham, MA). α-CtxMII and bPiDDB were synthesized as described previously [33,34]. r-bPiDDB was prepared by chemical reduction of the parent *bis*-quaternary ammonium analog, bPiDDB [35] and was used as the dihydrochloride salt. Chemical structures of bPiDDB and r-bPiDDB (Fig. 1) were verified by ^{1}H- and ^{13}C-NMR spectroscopy, mass spectrometry and X-ray crystallography.

2.2. Animals

Adult male Sprague–Dawley rats (200–225 g) were obtained from Harlan Laboratories, Inc. (Indianapolis, IN) and housed two per cage with *ad libitum* access to food and water in the Division of Laboratory Animal Resources (University of Kentucky, Lexington, KY). All experimental animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Kentucky. Groups of rats were administered nicotine (0.4 mg/kg; free base, sc) or saline once daily for 10 consecutive days. Immediately following each injection, locomotor activity was measured for 60 min. All injections were administered in a volume of 1 ml/kg body weight. Striatal slices were obtained from non-, saline-, and nicotine-injected rats 24 h after the last injection.

2.3. [^{3}H]DA overflow assay

Nicotine-evoked [^{3}H]DA overflow was determined using superfused rat striatal slices preloaded with [^{3}H]DA [36]. Briefly, coronal slices of striata (500 μm, 5–7 mg) were incubated for 30 min in Krebs' buffer (in mM; 118 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 1 NaH_2PO_4, 1.3 $CaCl_2$, 11.1 α-D-glucose, 25 $NaHCO_3$, 0.11 L-ascorbic acid and 0.004 disodium EDTA, pH 7.4, saturated with 95% O_2/5% CO_2) at 34 °C, and then incubated with 0.1 μM [^{3}H]DA (final concentration) for 30 min. After rinsing in fresh buffer, slices were transferred to a Brandel 2500 Suprafusion system (Biomedical Research and Development Laboratories, Inc., Gaithersburg, MD)

and superfused (0.6 ml/min) for 60 min with Krebs' buffer at 34 °C. Superfusion buffer contained nomifensine (10 μM), a DA transporter inhibitor, and pargyline (10 μM), a monoamine oxidase inhibitor, to prevent reuptake and metabolism of [^{3}H]DA, respectively, and to assure that the [^{3}H] collected in superfusate primarily represented parent neurotransmitter. Following an initial 60 min period of superfusion, two 4-min samples (2.4 ml/sample) were collected to determine basal [^{3}H]DA outflow.

To determine the concentration-dependent effect of nicotine to evoke [^{3}H]DA release from striatal slices obtained from rats injected with nicotine or saline repeatedly, a series of experiments was conducted in which each striatal slice from an individual rat was superfused for 36 min in the absence (buffer control) or presence of a single concentration of nicotine (0.1–100 μM). Nicotine remained in the buffer throughout the experiment and samples were collected every 4 min until the end of the experiment. Based on the results from the concentration response, 10 μM nicotine was chosen as appropriate for assessing antagonist-induced inhibition.

The inhibitory potency of bPiDDB, r-bPiDDB and α-CtxMII was determined in rats administered nicotine or saline repeatedly. To determine if these inhibitors evoked [^{3}H]DA overflow (intrinsic activity), each striatal slice from an individual rat was superfused for 36 min in either the absence or presence of a single concentration of bPiDDB (1 nM–10 μM), r-bPiDDB (10 pM–1 μM) or α-CtxMII (1 pM–10 nM); each antagonist remained in the buffer throughout the experiment. Concentration ranges were chosen from previous studies [29,32]. Subsequently, nicotine (10 μM) was added to the buffer of each superfusion chamber for 36 min. Antagonist-induced inhibition of nicotine-evoked [^{3}H]DA overflow was determined. At the end of each experiment, slices were solubilized, and the [^{3}H]-content of the tissue and superfusate samples was determined using a Tri-Carb 2900 TR liquid scintillation counter (Perkin Elmer, Inc., Waltham MA).

To determine if r-bPiDDB interacts with α-CtxMII-sensitive nAChRs, maximal inhibitory concentrations (1 nM) of α-CtxMII, r-bPiDDB, and α-CtxMII plus r-bPiDDB concurrently were superfused for 36 min in duplicate slices from either repeated nicotine-injected or non-injected rats. Then, nicotine (10 μM) was added to the buffer of all chambers and superfusion continued for an additional 36 min. Duplicate slices in each experiment were superfused for 36 min in the absence of antagonist, followed by superfusion with 10 μM nicotine (nicotine control). Duplicate slices also were superfused with maximally inhibitory concentrations of mecamylamine (10 μM), a nAChR antagonist at all known receptor subtypes, or dihydro-β-erythroidine (DHβE; 10 μM), an α4β2 antagonist [37,38], as positive controls.

2.4. Data analysis

To determine if repeated nicotine resulted in behavioral sensitization, a two-way repeated measures analysis of variance (ANOVA) was used to assess the effect of repeated nicotine on total distance travelled (cm), with nicotine treatment as a between-group factor and time as a within-subject factor. Significant interactions were assessed using one-way ANOVAs, and Tukey's *post hoc* analyses were used to make pairwise comparisons.

Fractional release was calculated by dividing the amount of [^{3}H] in each 4-min sample by the total tissue-[^{3}H] at the time of sample collection. Basal [^{3}H]DA outflow was calculated as the average fractional release in the two samples just before addition of antagonist to the superfusion buffer. Antagonist- or nicotine-evoked total [^{3}H]DA overflow was calculated by summing the increases in fractional release above basal for an equivalent period of exposure. Nicotine and antagonist concentration response data were fit by nonlinear least-squares regression using a variable slope, sigmoidal function. IC_{50} and percent maximal inhibition (I_{max}) values were determined using Prism 5.0 (GraphPad Software Inc., San Diego, CA). Statistical analyses were conducted using SPSS (version 17.0; SPSS Inc., Chicago, IL). A two-way repeated measures ANOVA analyzed the effect of repeated nicotine on the *in vitro* nicotine concentration response in the [^{3}H]DA overflow assay, with repeated nicotine treatment as a between-group factor and *in vitro* nicotine concentration as a within-subject factor. Two-way repeated measures ANOVAs were also used to analyze the effect of repeated nicotine on the concentration response curves for bPiDDB-, r-bPiDDB- and α-CtxMII-induced inhibition of nicotine-evoked [^{3}H]DA overflow, with repeated nicotine as a between-group factor and antagonist concentration as a within-subject factor. When appropriate, Tukey's *post hoc* analysis determined significant differences between the repeated nicotine and control groups across each antagonist concentration. To determine if nicotine treatment altered the parameters of antagonist-induced inhibition, Student's *t*-tests compared either log IC_{50} values or arithmetic I_{max} values between the nicotine treatment group and the control group.

To determine if r-bPiDDB acts at α-CtxMII-sensitive nAChR subtypes, the inhibitory effect of concomitant r-bPiDDB and α-CtxMII exposure was compared to r-bPiDDB alone and to α-CtxMII alone using a one-way ANOVA with antagonist as a within-subject factor. When appropriate, Tukey's *post hoc* analyses were used to make pairwise comparisons. Statistical significance was declared at $p < 0.05$.

3. Results

3.1. Inhibition produced by r-bPiDDB and α-CtxMII is not additive

Concomitant exposure to two antagonists acting at the same receptor would be expected to produce inhibition not different from either antagonist alone when maximally inhibitory concentrations are evaluated. To determine if r-bPiDDB interacts with α-CtxMII-sensitive nAChRs, inhibition produced by concomitant exposure to maximally inhibitory concentrations (1 nM) of r-bPiDDB and α-CtxMII were compared to inhibition produced by each antagonist alone. A one-way ANOVA revealed a significant effect of antagonist ($F_{4,17} = 23.8$, $p < 0.01$; Fig. 2). *Post hoc* analysis revealed that the nicotine-evoked [^{3}H]DA overflow in the presence of each antagonist was different from nicotine-evoked [^{3}H]DA overflow in the absence of antagonist. Mecamylamine

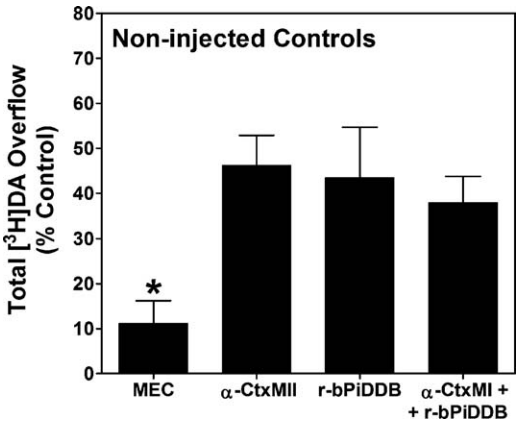

Fig. 2. Concomitant exposure to r-bPiDDB and α-CtxMII inhibits nicotine-evoked [^{3}H]DA overflow compared to control, but is not different from either antagonist alone. Control nicotine-evoked [^{3}H]DA overflow (in the absence of antagonist) was 1.06 ± 0.29 total [^{3}H]DA overflow (mean ± SEM). $^*p < 0.05$, indicates mecamylamine (MEC; 10 μM) inhibition was different from inhibition produced by all antagonists; $n = 4$.

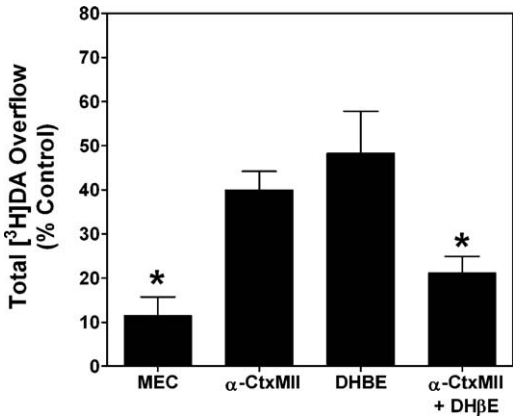

Fig. 3. Inhibition of nicotine (10 μM)-evoked [^{3}H]DA release produced by concomitant exposure to α-CtxMII (1 nM) and DHβE (10 μM) was greater than that produced by either drug alone. Control nicotine-evoked [^{3}H]DA overflow (in the absence of antagonist) was 1.69 ± 0.31 total [^{3}H]DA overflow (mean ± SEM). Mecamylamine (MEC; 10 μM) was included as a positive control and inhibited 93 ± 3% of total [^{3}H]DA overflow. $^*p < 0.05$, indicates different from inhibition produced by α-CtxMII and DHβE alone; $n = 6$.

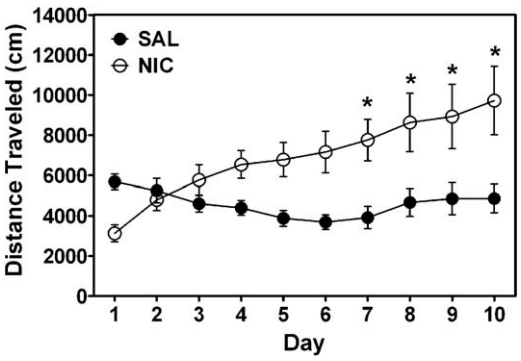

Fig. 4. Repeated nicotine treatment produces behavioral sensitization. Rats were injected with nicotine (0.4 mg/kg/day; sc) or saline for 10 days. Activity was measured as total distance traveled (cm) measured during a 60-min session immediately following each injection. Data are mean ± SEM total distance traveled (cm). $^*p < 0.05$, indicates different from activity on the first day of testing, and different from the control group on the indicated day; $n = 12$/group.

inhibited almost completely (93 ± 3%) nicotine-evoked [^{3}H]DA overflow and produced greater inhibition than r-bPiDDB, α-CtxMII or the combination. In separate experiments, striatal slices were exposed concomitantly to maximally inhibitory concentrations of α-CtxMII (1 nM) plus DHβE (10 μM), an α4β2 antagonist, both alone and in combination (Fig. 3). One-way ANOVA revealed a significant effect of antagonist ($F_{4,25} = 11.9$, $p < 0.0001$). *Post hoc* analysis revealed that inhibition (~80%) produced by concomitant exposure to α-CtxMII and DHβE was greater than that produced by either antagonist alone. Further, concomitant exposure to α-CtxMII and DHβE was not different from inhibition produced by the nonselective antagonist mecamylamine. Thus, the nearly complete inhibition of nicotine-evoked [^{3}H]DA overflow produced by mecamylamine and by co-exposure to DHβE and α-CtxMII demonstrate that a floor effect is not responsible for the observation with r-bPiDDB and α-CtxMII. Therefore, r-bPiDDB interacts with α-CtxMII-sensitive nAChRs.

3.2. Repeated nicotine administration produces locomotor sensitization, but does not alter the concentration response for nicotine to evoke [^{3}H]DA overflow from superfused rat striatal slices

A two-way repeated measures ANOVA revealed main effects of repeated nicotine treatment ($F_{1,198} = 5.80$, $p < 0.05$; Fig. 4) and time ($F_{1,198} = 5.44$, $p < 0.0001$) and a significant treatment × time interaction ($F_{9,198} = 8.86$, $p < 0.0001$) on locomotor activity. Simple effect analyses revealed that repeated nicotine-treated rats ($F_{9,110} = 3.34$, $p < 0.01$), but not repeated saline-treated rats, exhibited greater locomotor activity on days 7–10 compared to the first day of testing, and that rats administered nicotine repeatedly exhibited greater locomotor activity on days 7–10 compared to the saline control group. Thus, behavioral sensitization to nicotine was observed.

Striatal slices from rats repeatedly treated with nicotine were superfused with nicotine (0.1–100 μM) and evoked [^{3}H]DA overflow determined to assess the effects of repeated nicotine treatment on the *in vitro* nicotine concentration–response curve. Two-way repeated measures ANOVA revealed no main effect of treatment and no treatment × time interaction, but a main effect of nicotine concentration ($F_{6,78} = 26.0$, $p < 0.0001$; Fig. 5) was observed. Thus, repeated nicotine treatment did not alter the *in vitro* concentration response to nicotine. A concentration of 10 μM nicotine was chosen for subsequent experiments determining antagonist-induced inhibition of nicotine-evoked [^{3}H]DA overflow in rats repeatedly treated with nicotine.

3.3. r-bPiDDB interacts with α-CtxMII-sensitive nAChRs in nicotine-sensitized rats

To determine if repeated nicotine treatment alters the interaction of r-bPiDDB with α6β2-containing nAChRs, inhibition produced by concomitant exposure to maximally inhibitory concentrations (1 nM) of r-bPiDDB and α-CtxMII were compared to inhibition produced by each antagonist alone using striatal slices from nicotine-sensitized rats. Similar to the findings in non-injected control rats, one-way ANOVA revealed a significant effect of antagonist ($F_{4,23} = 86.9$, $p < 0.0001$; Fig. 6), and *post hoc* analysis revealed that the nicotine-evoked [^{3}H]DA overflow in the presence of antagonist was different from nicotine-evoked [^{3}H]DA overflow in the absence of either antagonist. Mecamylamine inhibited nicotine-evoked [^{3}H]DA overflow almost completely (90 ± 1%) and produced greater inhibition than either r-bPiDDB, α-CtxMII or the combination. However, concomitant exposure to r-bPiDDB and α-CtxMII resulted in inhibition of nicotine-evoked [^{3}H]DA overflow not different from either antagonist alone. Thus, r-bPiDDB acts at the same receptors subtypes as α-CtxMII (i.e., α6β2-containing), including in animals repeatedly treated with nicotine.

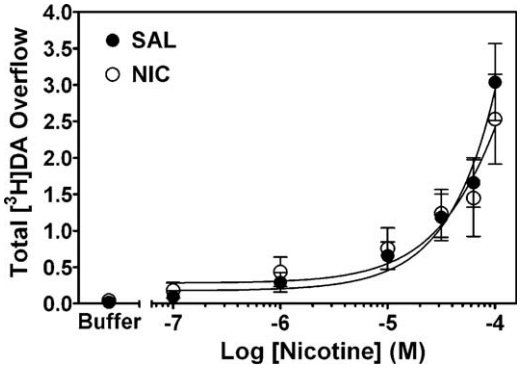

Fig. 5. Concentration response for nicotine-evoked [^{3}H]DA overflow from striatal slices taken from rats treated repeatedly with nicotine or saline. Rats were injected (sc) with 0.4 mg/kg/day of nicotine or saline for 10 days. Twenty-four hours after the last injection, striata were obtained and nicotine concentration response was determined. Buffer represents [^{3}H]DA overflow in the absence of nicotine. Data are mean ± SEM total [^{3}H]DA overflow; $n = 8$ and 7 rats for repeated nicotine-treated and control groups, respectively. Concentration–response curves were generated using nonlinear regression.

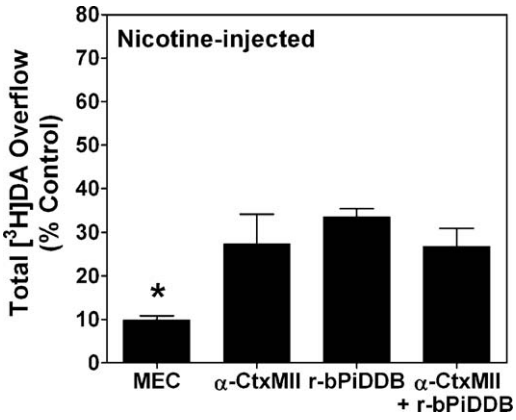

Fig. 6. In repeated nicotine-treated rats, concomitant exposure of r-bPiDDB and α-CtxMII inhibits nicotine-evoked [^{3}H]DA overflow compared to control, but is not different from either antagonist alone. Rats were injected with nicotine (0.4 mg/kg/day, sc) or saline for 10 days and striata obtained 24 h after the last injection. Control nicotine-evoked [^{3}H]DA overflow (in the absence of antagonist) was 0.73 ± 0.16 (mean ± SEM). $^*p < 0.05$, indicates mecamylamine (MEC; 10 μM) inhibition was different from inhibition produced by all antagonists; $n = 5$.

3.4. Repeated nicotine administration increases the potency for bPiDDB, but not for r-bPiDDB or α-CtxMII, to inhibit nicotine-evoked [^{3}H]DA overflow from striatal slices

α-CtxMII, bPiDDB and r-bPiDDB inhibition of nicotine-evoked [^{3}H]DA overflow from striatal slices obtained from rats treated repeatedly with nicotine or saline was analyzed using separate two-way repeated measures ANOVA. Initially, the α6-containing nAChR subtype-selective antagonist α-CtxMII was evaluated. A main effect of α-CtxMII concentration ($F_{6,60} = 14.59$, $p < 0.0001$; Fig. 7, top) was found; however, no main effect of nicotine treatment or treatment × concentration interaction was observed. Using nonlinear regression, a sigmoidal function for α-CtxMII was revealed with IC_{50} values for nicotine-treated and control groups of 31.4 ± 15.1 and 26.1 ± 10.1 pM, respectively ($p > 0.05$). However, repeated nicotine significantly increased ($t_8 = 2.70$, $p < 0.05$) α-CtxMII-induced maximal inhibition (90 ± 7%) compared to control (62 ± 7%). Thus, while repeated nicotine treatment did not alter α-CtxMII potency, a greater percent of the response to nicotine *in vitro* was mediated by α-CtxMII-sensitive nAChRs.

For the *bis*-azaaromatic quaternary ammonium compound bPiDDB, a main effect of both nicotine treatment ($F_{1,60} = 5.44$, $p < 0.05$; Fig. 7, middle) and bPiDDB concentration ($F_{6,60} = 21.0$, $p < 0.0001$) was observed; however, the interaction was not significant. Tukey's *post hoc* analysis revealed significant differences between the repeated nicotine-treated and control groups at the 0.1 and 1 nM bPiDDB concentrations. Nonlinear regression revealed a sigmoidal function for bPiDDB in both nicotine-treated and control groups; the IC_{50} value in the nicotine-treated group was 3-orders of magnitude lower than that in the saline control group ($IC_{50} = 5.27 \pm 1.51$ pM and 6.17 ± 2.24 nM, respectively; $t_6 = 2.75$, $p < 0.05$). No differences in I_{max} values (76 ± 3% and 76 ± 8%) were observed. Thus, repeated nicotine treatment robustly increased the potency of bPiDDB to inhibit nicotine-evoked striatal [^{3}H]DA release from striatum.

For the tertiary amine analog of bPiDDB, r-bPiDDB, a main effect of concentration ($F_{6,60} = 15.2$, $p < 0.0001$; Fig. 7, bottom) was observed, but neither the main effect of nicotine treatment, nor the treatment x concentration interaction were significant. Nonlinear regression revealed a sigmoidal function for r-bPiDDB with IC_{50} values for nicotine-treated and control groups of 0.08 ± 0.03 and 0.15 ± 0.07 nM, respectively ($p > 0.05$). I_{max} values (64 ± 10% and 67 ± 13%) were not different between the groups. Thus, repeated nicotine treatment did not alter the concentration response for r-bPiDDB to inhibit nicotine-evoked [^{3}H]DA overflow.

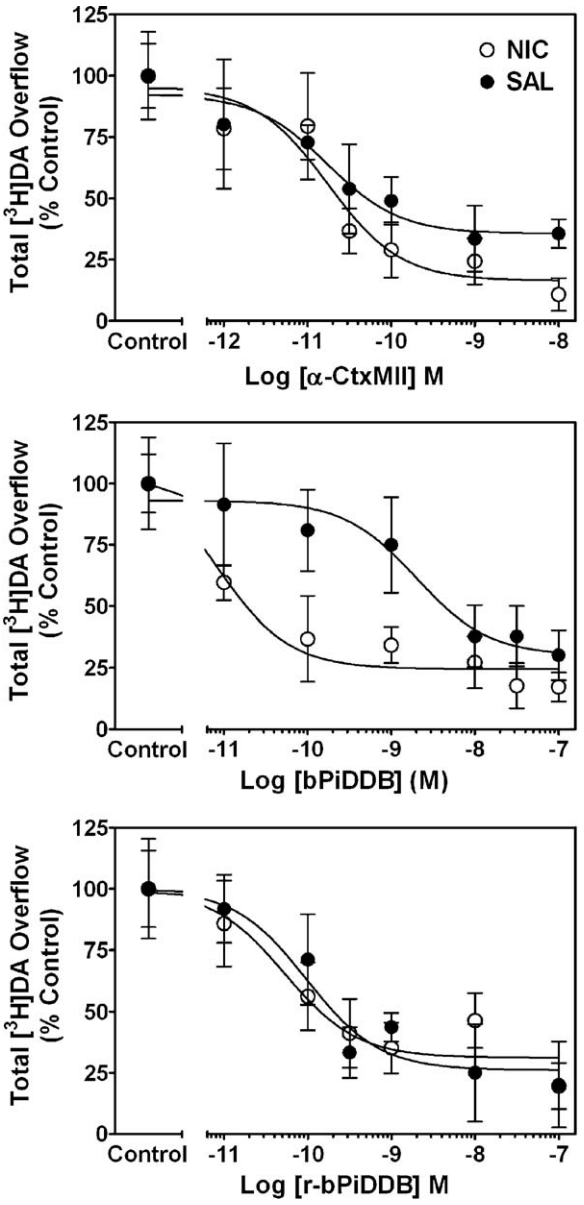

Fig. 7. Concentration response for α-CtxMII (top), bPiDDB (middle) and r-bPiDDB (bottom) to inhibit nicotine-evoked [^{3}H]DA overflow from striatal slices obtained from rats repeatedly treated with nicotine or saline. Rats were injected (sc) with nicotine (0.4 mg/kg/day) or saline for 10 days and striata obtained 24 h after the last injection. Control represents [^{3}H]DA overflow in response to 10 μM nicotine (total [^{3}H]DA overflow as a percentage of tissue [^{3}H]-content, mean ± SEM). Data are expressed as percentage of control; $n = 6$/group. For nicotine- and saline-treated groups, nicotine control response *in vitro* (in the absence of antagonist) was 0.71 ± 0.09 and 0.66 ± 0.12 total [^{3}H]DA overflow in α-CtxMII experiments, 1.16 ± 0.14 and 1.08 ± 0.20 total [^{3}H]DA overflow, respectively in bPiDDB experiments, and 0.61 ± 0.12 and 0.68 ± 0.16 total [^{3}H]DA overflow in r-bPiDDB experiments. No significant differences between control responses within or between experiments were observed. Concentration–response curves were generated by nonlinear regression.

4. Discussion

Our previous studies showed that bPiDDB, a novel *bis*-azaaromatic quaternary ammonium analog, potently ($IC_{50} = 2$ nM) inhibits nicotine-evoked [^{3}H]DA release from rat striatal slices via an interaction with α6β2-containing nAChRs [29]. Peripheral

administration of bPiDDB to rats was shown to inhibit nicotine-evoked DA release in nucleus accumbens using *in vivo* microdialysis [30] and to decrease intravenous nicotine self-administration [31]. bPiDDB is brain bioavailable following peripheral administration and is transported actively by the blood–brain barrier choline transporter into the central compartment [39]. After subcutaneous administration of behaviorally relevant doses of bPiDDB to rats, bPiDDB has been detected in brain as shown by both mass spectrometry and HPLC with radiometric analysis [40]. Nevertheless, bPiDDB is a polar, cationic compound with limited oral bioavailability; thus, the drug-like properties of bPiDDB as defined in the literature [41] remain an issue with respect to its therapeutic utility.

To improve the drug-like properties of bPiDDB, a simple chemical reduction was performed to convert the two quaternary ammonium moieties (i.e., 3-picolinium headgroups) into tertiary amino moieties (i.e., 3-methyl-1,2,5,6-tetrahydropyridines), yielding r-bPiDDB. r-bPiDDB is a highly lipophilic molecule with predicted physicochemical properties of a log *P* value of 5, pK_a of 9 and a MW of 434 Da, suggesting good oral bioavailability [42]. Similar to bPiDDB, r-bPiDDB inhibits nicotine-evoked [^{3}H]DA release from superfused rat striatal slices, but with a 10-fold higher potency (IC_{50} = 0.3 nM; [32]). The current results demonstrate that inhibition of nicotine-evoked [^{3}H]DA release produced by a maximally inhibitory concentration of r-bPiDDB is not different from that produced by concomitant exposure to maximally inhibitory concentrations of r-bPiDDB and α-CtxMII, indicating that r-bPiDDB interacts with α6β2-containing nAChRs. Thus, r-bPiDDB appears to retain the pharmacological properties of the parent compound, bPiDDB.

Importantly, the nonselective, noncompetitive nAChR antagonist mecamylamine inhibited nicotine-evoked DA release by ~90%. Thus, the incomplete inhibition produced by r-bPiDDB plus α-CtxMII was not the result of a "floor" effect. Additionally, the ~80% inhibition produced by co-exposure to maximally inhibitory concentrations of DHβE and α-CtxMII provides evidence that nearly complete inhibition of the effect of nicotine is obtained when an α4β2 antagonist and an α6β2 antagonist act in concert. Thus, the current results support the suggestion that r-bPiDDB is selective for α-CtxMII-sensitive, α6β2-containing nAChR subtypes.

Individuals undergoing treatment for smoking cessation will have been exposed to nicotine repeatedly prior to the initiation of cessation treatment. Taking this into account, the current study determined both the ability of nicotine to evoke [^{3}H]DA release and the inhibitory effects of the nAChR antagonists using striatum from rats repeatedly administered nicotine (0.4 mg/kg daily for 10 days). The concentration response for nicotine to evoke [^{3}H]DA release from striatal slices obtained from rats injected with nicotine repeatedly was not different from that generated following repeated saline. Thus, the *in vitro* response to nicotine was not altered in striatum despite the dynamic neuronal adaptation that occurred following repeated nicotine treatment, as evidenced by the expression of behavioral sensitization. These findings are in agreement with earlier studies demonstrating that nicotine-evoked striatal DA release, either *in vitro* or *in vivo*, is not altered following chronic nicotine (0.125–1.0 mg/kg/h) administered via osmotic minipump for 10 or 14 days [43,44]. However, these results are not consistent with studies showing that nicotine-evoked DA release is either increased [25,45] or decreased [23,25,46] following chronic nicotine administration. These discrepant findings are likely due to differences in experimental design and the parameters used (e.g., length of nicotine administration, animal models employed, tissue preparation utilized, brain region assessed). These discrepancies could be resolved through parametric analyses of the effect of repeated nicotine administration on subsequent nicotine-evoked DA release. For example, parametric analyses would require the evaluation of the same repeated nicotine dose and route of administration, employment of the same brain region and tissue preparation in several species, followed by determination of the *in vitro* concentration–response for nicotine-evoked DA release.

In the current study, maximum inhibition produced by α-CtxMII was significantly greater in repeated nicotine-treated rats compared to control rats (I_{max} = 90% and 62%, respectively). This increase in I_{max} for α-CtxMII between the repeated-nicotine group and the control group was observed in different series of experiments using nicotine-sensitized and non-injected control rats evaluated at different times, as well as when striata from nicotine-sensitized and saline-control rats were evaluated contemporaneously. In contrast, maximum inhibition produced by either r-bPiDDB or by the concurrent administration of r-bPiDDB and α-CtxMII was not different between the repeated-nicotine group and the non-injected controls. The increase in α-CtxMII I_{max} may be attributed to an up-regulation of nAChRs. In contrast to this interpretation, binding studies using [^{125}I]α-CtxMII to label α6-containing nAChRs consistently report decreased (36–50%) receptor number in striatum following repeated nicotine administration [24,25,47]. However, [^{125}I]α-CtxMII probes multiple subtypes of α6-containing nAChRs, i.e., α6β2-, α6β2β3-, α6α4β2- and α6α4β2β3-containing subtypes [13,14]. To determine if specific α6-containing subtypes are regulated by repeated nicotine administration, the α-CtxMII analog E11A, which is an α6α4β2 subtype-selective antagonist, was used to differentiate between subtypes containing both α6 and α4 subunits (i.e., α6α4β2 and α6α4β2β3) and those α6-containing subtypes which do not contain α4 subunits (i.e., α6β2 and α6β2β3) [25]. Repeated nicotine was reported to down regulate α6α4β2-containing nAChRs by 50% and up-regulate α6(non-α4)β2-containing nAChRs by ~25%. Thus, the relative increase (~25%) in α6(non-α4)β2-containing nAChRs [25] is consistent with the observed increase (28%) in α-CtxMII inhibition of nicotine-evoked [^{3}H]DA release from striatum from nicotine-sensitized rats. The observed increase in I_{max} occurred without a change in IC_{50}, suggesting that the affinity of α-CtxMII for these α6(non-α4)β2 receptors is not changed.

In contrast to the results with α-CtxMII, the concentration response for bPiDDB to inhibit nicotine-evoked [^{3}H]DA release was shifted to the left by 3-orders of magnitude (IC_{50} = 5.27 pM) in the nicotine-sensitized rats compared to control (IC_{50} = 6.17 nM), with no change in I_{max}. With r-bPiDDB, neither the IC_{50} nor the I_{max} were altered following repeated nicotine treatment. Of note, r-bPiDDB has high potency in both the nicotine-sensitized and control conditions. Thus, unlike α-CtxMII, neither bPiDDB nor r-bPiDDB showed an increase in I_{max} following repeated nicotine treatment. One potential interpretation is that bPiDDB and r-bPiDDB do not act at α6(non-α4)β2-containing nAChRs, since I_{max} was not increased after repeated nicotine. Furthermore, previous studies have shown that subtypes incorporating β3 subunits are resistant to regulation of receptor number by chronic nicotine treatment [24]. Since the concentration response for r-bPiDDB was not altered following repeated nicotine, this antagonist may act at the α6α4β2β3-containing subtype, which has been suggested to comprise ~50% of nAChRs on DA terminals in striatum and to be the most sensitive of the nAChR subtypes to nicotine [14,15].

With respect to bPiDDB and the observed 3-orders of magnitude shift in the concentration response following repeated nicotine treatment, several interpretations of these results are possible. Repeated nicotine treatment alters nAChR stoichiometry, conformation and composition [19], and long-term exposure to nicotine alters nAChR maturation by increasing nAChR subunit oligomerization and folding [17,48]. In addition, nicotine alters

protein kinase A- or C-induced phosphorylation of specific amino acid residues on the nAChR complex, an effect that modulates the sensitivity of nAChRs to ligands [49,50]. Thus, one interpretation is that bPiDDB acts more potently at the particular stoichiometry, conformation, composition and/or phosphorylated state of nAChR(s) that mediate nicotine-evoked DA release and that predominate following repeated nicotine administration. Alternatively, repeated nicotine treatment is thought to initiate the transformation of nAChRs through different receptor states, and large shifts in ligand affinity can accompany these changes in receptor state [49–52]. Thus, rather than interacting with an alternate subtype composition following repeated nicotine, another interpretation is that bPiDDB may stabilize or "lock" the specific receptor subtype(s) into a high affinity state of the receptor that remains "activatable". Although not clearly defined, such a mechanism would be consistent with the large magnitude of the shift in potency for bPiDDB when evaluated following repeated nicotine.

In summary, bPiDDB and r-bPiDDB represent novel small molecules that potently inhibit nicotine-evoked DA release and appear to discriminate between subpopulations of α6β2-containing nAChR subtypes. Importantly, both compounds have been shown to decrease nicotine self-administration in an animal model, suggesting that these compounds are not only leads in the search for novel smoking cessation agents, but may represent novel pharmacological tools for unraveling the complexity and diversity of nAChR effects.

Acknowledgments

The research reported was supported by NIH grants U19 DA17548, R01 MH53631, T32 DA16176 and F31 DA023853. The University of Kentucky holds patents on bPiDDB and r-bPiDDB. A potential royalty stream to L.P.D. and P.A.C. may occur consistent with University of Kentucky policy.

References

[1] George TP, O'Malley SS. Current pharmacological treatments for nicotine dependence. Trends Pharmacol Sci 2004;25:42–8.
[2] Le Foll B, Goldberg SR. Effects of nicotine in experimental animals and humans: an update on addictive properties. Handbook Exp Pharmacol 2009;192:335–67.
[3] Balfour DJ. The neurobiology of tobacco dependence: a commentary. Respiration 2002;69:7–11.
[4] Mathieu-Kia AM, Kellogg SH, Butelman ER, Kreek MJ. Nicotine addiction: insights from recent animal studies. Psychopharmacology 2002;162:102–18.
[5] Goldberg SR, Spealman RD, Goldberg DM. Persistent behavior at high rates maintained by intravenous self-administration of nicotine. Science (New York NY) 1981;214:573–5.
[6] Corrigall WA, Coen KM. Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology 1989;99:473–8.
[7] Markou A, Paterson NE. The nicotinic antagonist methyllycaconitine has differential effects on nicotine self-administration and nicotine withdrawal in the rat. Nicotine Tob Res 2001;3:361–73.
[8] Harvey DM, Yasar S, Heishman SJ, Panlilio LV, Henningfield JE, Goldberg SR. Nicotine serves as an effective reinforcer of intravenous drug-taking behavior in human cigarette smokers. Psychopharmacology 2004;175:134–42.
[9] Shoaib M, Schindler CW, Goldberg SR. Nicotine self-administration in rats: strain and nicotine pre-exposure effects on acquisition. Psychopharmacology 1997;129:35–43.
[10] Rauhut AS, Mullins SN, Dwoskin LP, Bardo MT. Reboxetine: attenuation of intravenous nicotine self-administration in rats. J Pharmacol Exp Ther 2002;303:664–72.
[11] Corrigall WA, Franklin KB, Coen KM, Clarke PB. The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. Psychopharmacology 1992;107:285–9.
[12] Rahman S, Zhang Z, Papke RL, Crooks PA, Dwoskin LP, Bardo MT. Region-specific effects of *N*,*N*′-dodecane-1,12-diyl-bis-3-picolinium dibromide on nicotine-induced increase in extracellular dopamine in vivo. Br J Pharmacol 2008;153:792–804.
[13] Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, et al. Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. Mol Pharmacol 2004;65:1526–35.
[14] Salminen O, Drapeau JA, McIntosh JM, Collins AC, Marks MJ, Grady SR. Pharmacology of α-conotoxin MII-sensitive subtypes of nicotinic acetylcholine receptors isolated by breeding of null mutant mice. Mol Pharmacol 2007;71:1563–71.
[15] Grady SR, Salminen O, Laverty DC, Whiteaker P, McIntosh JM, Collins AC, et al. The subtypes of nicotinic acetylcholine receptors on dopaminergic terminals of mouse striatum. Biochem Pharmacol 2007;74:1235–46.
[16] Lopez-Hernandez GY, Sanchez-Padilla J, Ortiz-Acevedo A, Lizardi-Ortiz J, Salas-Vincenty J, Rojas LV, et al. Nicotine-induced up-regulation and desensitization of α4β2 neuronal nicotinic receptors depend on subunit ratio. J Biol Chem 2004;279:38007–15.
[17] Sallette J, Pons S, Devillers-Thiery A, Soudant M, Prado de Carvalho L, Changeux JP, et al. Nicotine upregulates its own receptors through enhanced intracellular maturation. Neuron 2005;46:595–607.
[18] Corringer PJ, Sallette J, Changeux JP. Nicotine enhances intracellular nicotinic receptor maturation: a novel mechanism of neural plasticity? J Physiol Paris 2006;99:162–71.
[19] Harkness PC, Millar NS. Changes in conformation and subcellular distribution of α4β2 nicotinic acetylcholine receptors revealed by chronic nicotine treatment and expression of subunit chimeras. J Neurosci 2002;22:10172–81.
[20] Marks MJ, Burch JB, Collins AC. Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. J Pharmacol Exp Ther 1983;226:817–25.
[21] Flores CM, Rogers SW, Pabreza LA, Wolfe BB, Kellar KJ. A subtype of nicotinic cholinergic receptor in rat brain is composed of alpha 4 and beta 2 subunits and is up-regulated by chronic nicotine treatment. Mol Pharmacol 1992;41:31–7.
[22] Nashmi R, Xiao C, Deshpande P, McKinney S, Grady SR, Whiteaker P, et al. Chronic nicotine cell specifically upregulates functional α4* nicotinic receptors: basis for both tolerance in midbrain and enhanced long-term potentiation in perforant path. J Neurosci 2007;27:8202–18.
[23] McCallum SE, Collins AC, Paylor R, Marks MJ. Deletion of the β2 nicotinic acetylcholine receptor subunit alters development of tolerance to nicotine and eliminates receptor upregulation. Psychopharmacology 2006;184:314–27.
[24] Perry DC, Mao D, Gold AB, McIntosh JM, Pezzullo JC, Kellar KJ. Chronic nicotine differentially regulates α6- and β3-containing nicotinic cholinergic receptors in rat brain. J Pharmacol Exp Ther 2007;322:306–15.
[25] Perez XA, Bordia T, McIntosh JM, Grady SR, Quik M. Long-term nicotine treatment differentially regulates striatal α6α4β2* and α6(non-α4)β2* nAChR expression and function. Mol Pharmacol 2008;74:844–53.
[26] Perez XA, O'Leary KT, Parameswaran N, McIntosh JM, Quik M. Prominent role of alpha3/alpha6beta2* nAChRs in regulating evoked dopamine release in primate putamen: effect of long-term nicotine treatment. Mol Pharmacol 2009;75:938–46.
[27] Mao D, Perry DC, Yasuda RP, Wolfe BB, Kellar KJ. The α4β2α5 nicotinic cholinergic receptor in rat brain is resistant to up-regulation by nicotine in vivo. J Neurochem 2008;104:446–56.
[28] Dwoskin LP, Sumithran SP, Zhu J, Deaciuc AG, Ayers JT, Crooks PA. Subtype-selective nicotinic receptor antagonists: potential as tobacco use cessation agents. Bioorg Med Chem Lett 2004;14:1863–7.
[29] Dwoskin LP, Wooters TE, Sumithran SP, Siripurapu KB, Joyce BM, Lockman PR, et al. *N*,*N*′-Alkane-diyl-*bis*-3-picoliniums as nicotinic receptor antagonists: inhibition of nicotine-induced dopamine release and hyperactivity. J Pharmacol Exp Ther 2008;326:563–76.
[30] Rahman S, Neugebauer NM, Zhang Z, Crooks PA, Dwoskin LP, Bardo MT. The effects of a novel nicotinic receptor antagonist *N*,*N*′-dodecane-1,12-diyl-*bis*-3-picolinium dibromide (bPiDDB) on acute and repeated nicotine-induced increases in extracellular dopamine in rat nucleus accumbens. Neuropharmacology 2007;52:755–63.
[31] Neugebauer NM, Zhang Z, Crooks PA, Dwoskin LP, Bardo MT. Effect of a novel nicotinic receptor antagonist, *N*,*N*′-dodecane-1,12-diyl-*bis*-3-picolinium dibromide, on nicotine self-administration and hyperactivity in rats. Psychopharmacology 2006;184:426–34.
[32] Dwoskin LP, Smith AM, Wooters TE, Zhang Z, Crooks PA, Bardo MT. Nicotinic receptor antagonist-based therapies and candidates for smoking cessation. Biochem Pharmacol 2009;78:732–43.
[33] Cartier GE, Yoshikami D, Gray WR, Luo S, Olivera BM, McIntosh JM. A new α-conotoxin which targets α3β2 nicotinic acetylcholine receptors. J Biol Chem 1996;271:7522–8.
[34] Ayers JT, Dwoskin LP, Grinevich VP, Deaciuc AG, Crooks PA. *bis*-Azaaromatic quaternary ammonium analogs: ligands for α4β2* and α7* subtypes of neuronal nicotinic receptors. Bioorg Med Chem Lett 2002;12:3067–71.
[35] Zhang Z, Zheng G, Pivavarchyk M, Deaciuc AG, Dwoskin LP, Crooks PA. Novel bis-, tris-, and tetrakis-tertiary amine antagonists at neuronal nicotinic receptors that mediate nicotine-evoked dopamine release; unpublished results.
[36] Grinevich VP, Crooks PA, Sumithran SP, Haubner AJ, Ayers JT, Dwoskin LP. *N*-*n*-Alkylpyridinium analogs, a novel class of nicotinic receptor antagonists: selective inhibition of nicotine-evoked [^{3}H] dopamine overflow from superfused rat striatal slices. J Pharmacol Exp Ther 2003;306:1011–20.
[37] Teng L, Crooks PA, Buxton ST, Dwoskin LP. Nicotinic-receptor mediation of S(−)nornicotine-evoked -^{3}H-overflow from rat striatal slices preloaded with -^{3}H-dopamine. J Pharmacol Exp Ther 1997;283:778–87.
[38] Papke RL, Dwoskin LP, Crooks PA, Zheng G, Zhang Z, McIntosh JM, et al. Extending the analysis of nicotinic receptor antagonists with the study of α6 nicotinic receptor subunit chimeras. Neuropharmacology 2008;54:1189–200.

[39] Lockman PR, Manda VK, Geldenhuys WJ, Mittapalli RK, Thomas F, Albayati ZF, et al. Carrier-mediated transport of the quaternary ammonium neuronal nicotinic receptor antagonist *N*,*N*′-dodecane-1,12-diyl-*bis*-3-picolinium dibromide at the blood–brain barrier. J Pharmacol Exp Ther 2008;324:244–50.
[40] Albayati ZA, Dwoskin LP, Crooks PA. Pharmacokinetics of the novel nicotinic receptor antagonist *N*,*N*′-dodecane-1,12-diyl-*bis*-3-picolinium dibromide in the rat. Drug Metab Dispos 2008;36:2024–9.
[41] Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods 2000;44:235–49.
[42] Pharma Algorithms, http://www.pharma-algorithms.com/webboxes/.
[43] Janson AM, Meana JJ, Goiny M, Herrera-Marschitz M. Chronic nicotine treatment counteracts the decrease in extracellular neostriatal dopamine induced by a unilateral transection at the mesodiencephalic junction in rats: a microdialysis study. Neurosci Lett 1991;134:88–92.
[44] Grilli M, Parodi M, Raiteri M, Marchi M. Chronic nicotine differentially affects the function of nicotinic receptor subtypes regulating neurotransmitter release. J Neurochem 2005;93:1353–60.
[45] Visanji NP, Mitchell SN, O'Neill MJ, Duty S. Chronic pre-treatment with nicotine enhances nicotine-evoked striatal dopamine release and α6 and β3 nicotinic acetylcholine receptor subunit mRNA in the substantia nigra pars compacta of the rat. Neuropharmacology 2006;50:36–46.
[46] Lai A, Parameswaran N, Khwaja M, Whiteaker P, Lindstrom JM, Fan H, et al. Long-term nicotine treatment decreases striatal α6* nicotinic acetylcholine receptor sites and function in mice. Mol Pharmacol 2005;67:1639–47.
[47] Mugnaini M, Garzotti M, Sartori I, Pilla M, Repeto P, Heidbreder CA, et al. Selective down-regulation of [^{125}I]Y0-α-conotoxin MII binding in rat mesostriatal dopamine pathway following continuous infusion of nicotine. Neuroscience 2006;137:565–72.
[48] Lester HA, Xiao C, Srinivasan R, Son CD, Miwa J, Pantoja R, et al. Nicotine is a selective pharmacological chaperone of acetylcholine receptor number and stoichiometry. Implications for drug discovery. AAPS J 2009;11:167–77.
[49] Giniatullin R, Nistri A, Yakel JL. Desensitization of nicotinic ACh receptors: shaping cholinergic signaling. Trends Neurosci 2005;28:371–8.
[50] Picciotto MR, Addy NA, Mineur YS, Brunzell DH. It is not "either/or": activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. Prog Neurobiol 2008;84:329–42.
[51] Dani JA, Heinemann S. Molecular and cellular aspects of nicotine abuse. Neuron 1996;16:905–8.
[52] Marks MJ, Rowell PP, Cao JZ, Grady SR, McCallum SE, Collins AC. Subsets of acetylcholine-stimulated 86Rb+ efflux and [^{125}I]-epibatidine binding sites in C57BL/6 mouse brain are differentially affected by chronic nicotine treatment. Neuropharmacology 2004;46:1141–57.