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The novel nicotinic receptor antagonist *N,N′*-dodecane-1,12-diyl-*bis*-3-picolinium dibromide decreases nicotine-induced dopamine metabolism in rat nucleus accumbens

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Abstract

The current study examined the effect of the novel nicotinic acetylcholine receptor antagonist, *N,N′*-dodecane-1,12-diyl-*bis*-3-picolinium dibromide (bPiDDB), on nicotine-induced dopamine metabolism in rat nucleus accumbens, striatum and medial prefrontal cortex. Acute nicotine (0.5 mg/kg, s.c.) produced an increase in the content of dihydroxyphenylacetic acid (DOPAC) in nucleus accumbens, but not in striatum or medial prefrontal cortex. Pretreatment with bPiDDB (1 or 3 mg/kg, s.c.) dose-dependently inhibited the nicotine-induced increase in DOPAC content in nucleus accumbens. These results indicate that bPiDDB inhibits the nicotine-induced increase in DOPAC in reward-relevant brain region targeting nicotinic acetylcholine receptors.

Keywords

bPiDDB; nicotinic receptor; dopamine metabolism; DOPAC; nucleus accumbens; HPLC

1. Introduction

The midbrain dopamine system is a target for the reinforcing actions of many addictive drugs, including nicotine (Balfour 2004; Di Chiara et al., 2004; Picciotto and Corrigall, 2002). In the mammalian brain, nicotine exerts its effects by stimulating a variety of nicotinic acetylcholine receptors, which are distributed throughout the brain dopamine system (Klink *et al*., 2001; Gotti et al., 2006). Given the abundant number of nicotinic acetylcholine receptors in the brain, it is likely that the development of nicotinic antagonists that specifically block nicotinic

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acetylcholine receptors involved in modulating midbrain dopamine function may offer novel pharmacotherapeutic approaches to treat nicotine dependence.

Recently, our laboratory has synthesized a number of bis-azaaromatic quaternary ammonium analogs as nicotinic acetylcholine receptors antagonists (Ayers et al., 2002). The central inhibitory effects of the novel *bis*-analog, *N*,*N*′-dodecane-1,12-diyl-*bis*-3-picolinium dibromide (bPiDDB), are realized following its entry into brain via the blood brain barrier choline transporter (Geldenhuys et al., 2005; Lockman et al., 2008; Albayati et al., 2008). bPiDDB inhibits nicotine-evoked striatal dopamine release *in vitro* (Dwoskin et al., 2004, 2008) and reduces the acute nicotine-induced increase in extracellular dopamine in rat nucleus accumbens (Rahman et al., 2007, 2008), which likely explains, at least in part, the ability of bPiDDB to decrease intravenous nicotine self-administration in rats (Neugebauer et al., 2006). bPiDDB does not inhibit dopamine uptake by the striatal dopamine transporter (Dwoskin et al., 2008), and thus it does not appear to interact directly with this plasmalemma protein. However, it remains to be determined if bPiDDB alters dopamine metabolism to dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase. The present study examined the effects of bPiDDB on the acute nicotine-induced increase in tissue content of DOPAC in rat nucleus accumbens, striatum and medial prefrontal cortex using an *ex-vivo* assay and high performance liquid chromatography with electrochemical detection. (HPLC-EC)

2. Materials and methods

2.1. Animal

Male Sprague-Dawley rats (250–275 g) were used. Rats had ad lib access to food and water and were maintained on a 12:12 light/dark cycle (lights on at 0700 hr). Experimental protocols were in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

2.2. Drugs and dosage regimen

S(−)-Nicotine ditartrate was prepared in saline solution. Doses of nicotine represent the free base weight. Both bPiDDB and mecamylamine HCl were prepared in saline and doses represent the salt weights. All drugs were administered subcutaneously (sc).

2.3. Brain tissue preparation and HPLC analysis of DOPAC content

Rats were randomly assigned to one of six treatment groups: (1) saline; (2) nicotine (0.5 mg/kg) alone; (3) bPiDDB 1 mg/kg alone; (4) bPiDDB 3 mg/kg alone; (5) bPiDDB 1 mg/kg plus nicotine (0.5 mg/kg) and (6) bPiDDB 3 mg/kg plus nicotine (0.5 mg/kg). In a separate study, the effect of the nonselective nicotinic acetylcholine receptors antagonist mecamylamine (5 mg/kg) on nicotine-induced changes in DOPAC was determined. Each group consisted of 8–10 rats. Doses of antagonist were given 15 min before nicotine (0.5 mg/kg) administration. Doses of nicotine, bPiDDB and mecamylamine were selected based on previous reports (Brazell et al., 1990; Janhunen et al., 2005; Neugebauer et al., 2006; Rahman et al., 2007). Thirty min after nicotine or saline injection, rats were killed by rapid decapitation, brains were obtained, and nucleus accumbens, medial prefrontal cortex and striatum dissected on an ice-cold dissection plate as described previously (Zhu et al., 2004). Brain regions from individual rats were stored at −70°C in 10 volumes (medial prefrontal cortex) or 20 volumes (nucleus accumbens and striatum) of 0.1 N perchloric acid until assay. Upon assay, samples were thawed on ice, sonicated and centrifuged at 30,000 × g for 15 min at 4°C. For each sample, 20 μl of the resulting supernatant was injected onto a high performance liquid chromatography system coupled with electrochemical detection unit (HPLC-ECD, ESA Inc., Chelmsford, MA) to determine DOPAC content. DOPAC was measured because it represents the major dopamine metabolite in rat ventral striatum (see Wilk and Stanley, 1978). The HPLC system consisted

of a solvent delivery unit, a Coulochem III electrochemical detector equipped with a 5011 analytical cell and a 5020 guard cell. The guard cell was set at +350 mV, electrode 1 at −150 mV, and electrode 2 at +200 mV. The mobile phase consisted of 75 mM NaH_2PO_4, 1.7 mM 1-octanesulfonic acid, 25uM EDTA, 100 μl/L triethylamine and 10% acetonitrile (adjusted to pH 3.0 with phosphoric acid), and the flow rate was 0.5 ml/min. Samples were loaded into a 20 μl sample loop and injected onto a reverse phase analytical column (BetaBasic-18 column, 150mm × 3mm, Thermo Hypersil-Keystone, PA). Chromatograms were integrated, compared with the standards and analyzed using an ESA Chromatography Data System (EZChrom Elite, Chelmsford, MA). Peak heights and calibration factors based on standards were used to calculate the amount of DOPAC.

2.4. Data analysis

The amount of tissue DOPAC level were expressed as μg/g tissue wet weight and analyzed for significant differences by one way analysis of variance (ANOVA) followed by *post hoc* tests for multiple comparison. The significance level was set at $P < 0.05$.

3. Results

A one way ANOVA revealed a significant main effect of treatment, $F(5, 41) = 2.83$, $P < 0.05$, in nucleus accumbens (Fig 1), but not in either striatum or medial prefrontal cortex (Fig 2). *Post-hoc* tests revealed that nicotine (0.5 mg/kg, s.c.) produced a significant increase in DOPAC content in nucleus accumbens. There was also a significant difference between the bPiDDB (3 mg/kg) plus nicotine and the nicotine alone groups in nucleus accumbens DOPAC. Pretreatment with bPiDDB (1 or 3 mg/kg) dose-dependently inhibited the nicotine-induced increase in DOPAC content in nucleus accumbens. Importantly, rats treated with bPiDDB (3 mg/kg) and nicotine did not differ significantly from saline control, indicating that the inhibition of nicotine-induced dopamine metabolism was complete. Similarly, pretreatment with mecamylamine (5 mg/kg, s.c.) blocked completely the nicotine-induced increase in DOPAC content in nucleus accumbens (245% vs 123% of saline control; results not shown).

4. Discussion

Acute nicotine administration preferentially increased tissue DOPAC content in nucleus accumbens, but not in striatum or medial prefrontal cortex. Pretreatment with mecamylamine, a non-selective nicotinic acetylcholine receptor antagonist, blocked the nicotine-induced enhancement of DOPAC content, demonstrating the involvement of a nicotinic acetylcholine receptor-mediated mechanism. Like mecamylamine, bPiDDB dose-dependently and completely inhibited the nicotine-induced increase in DOPAC content in nucleus accumbens. The present results showing that acute nicotine preferentially elevated DOPAC content in nucleus accumbens is consistent with previous neurochemical studies using brain tissue assays and microdialysis procedures (Benwell et al., 1992; Brazell et al., 1990; Di Chiara and Imperato, 1988; Janhunen et al., 2005; Mitchell et al., 1989; Nisell et al., 1994). Moreover, the blockade of the nicotine-induced increase in DOPAC content produced by pretreatment with mecamylamine is also in accord with previous tissue dopamine metabolism studies (Brazell et al., 1990; Mitchell et al., 1989; Nisell et al., 1994). Overall, these results indicate that acute nicotine produces a specific effect on tissue DOPAC content in rat nucleus accumbens and that this effect mediated by nicotinic acetylcholine receptors.

Pretreatment with bPiDDB dose-dependently inhibited the nicotine-induced increase in DOPAC content in nucleus accumbens, suggesting that bPiDDB acts similarly to mecamylamine in regulating dopamine metabolism in nucleus accumbens. However, evidence suggests that bPiDDB may be more selective than mecamylamine in blocking a subset of mecamylamine-sensitive nicotinic acetylcholine receptors (i.e., α6β2-containing) in brain

(Dwoskin et al., 2008). In any case, the ability of bPiDDB to inhibit the effect of nicotine on dopamine metabolism observed in this report extends our previous microdialysis studies showing that bPiDDB also inhibits nicotine-induced dopamine release in nucleus accumbens (Rahman et al., 2007, 2008). The observation that acute nicotine did not have an effect on DOPAC content in the striatum or medial prefrontal cortex in this study remains to be explained. Regional variations in nicotine-induced effects on dopamine metabolism and function have been reported previously in both *in vivo* and *in vitro* studies (Brazell et al., 1990; Janhunen et al., 2005; Mitchell et al., 1989). Several factors may account for the region-specific effects of acute nicotine, including differential distribution of pre-synaptic nicotinic acetylcholine receptors and/or dopamine transporter function in specific brain areas. For example, acute nicotine has been shown to increase dopamine transporter function in rat striatum and prefrontal cortex through a trafficking-independent mechanism and trafficking-dependent mechanisms, respectively (Middleton et al., 2007; Zhu et al., 2007). Whether regional differences in dopamine transporter function and regulation are contributing to the differential effects of nicotine on DOPAC content in the current report remain to be determined. Nevertheless, the ability of mecamylamine and bPiDDB to block the nicotine-induced increase in DOPAC in nucleus accumbens indicates that dopamine utilization in this critical reward-relevant region is regulated by nicotinic acetylcholine receptors.

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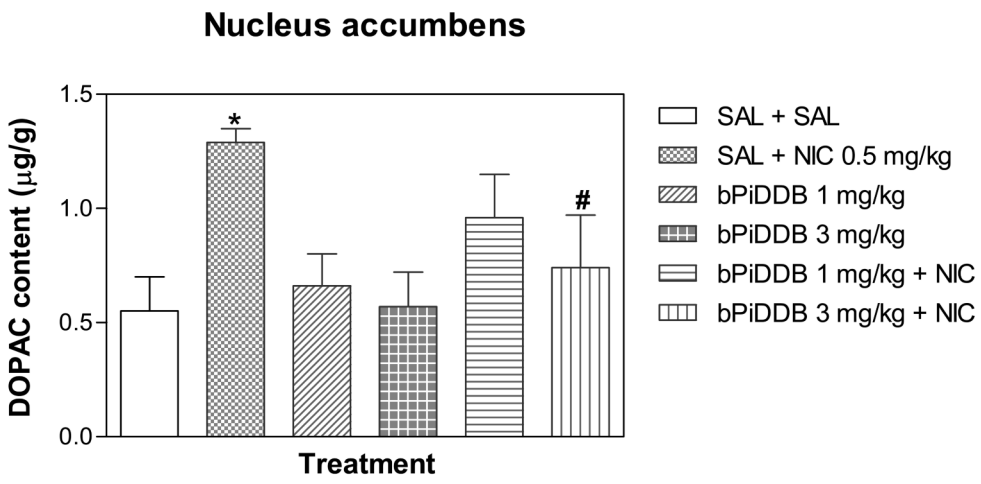

Fig. 1.

Effects of systemic bPiDDB (1 or 3 mg/kg) on the acute nicotine (NIC; 0.5 mg/kg)-induced increase in DOPAC content in nucleus accumbens. bPiDDB was administered 15 min prior NIC and rats were killed 30 min after NIC administration. Data are expressed as µg/g tissue (mean ± S.E.M, n=7–8 rats/group). * $P < 0.05$, indicates significant difference from saline control (SAL+SAL), # $P < 0.05$, indicates significant difference from NIC alone (SAL + NIC 0.5 mg/kg).

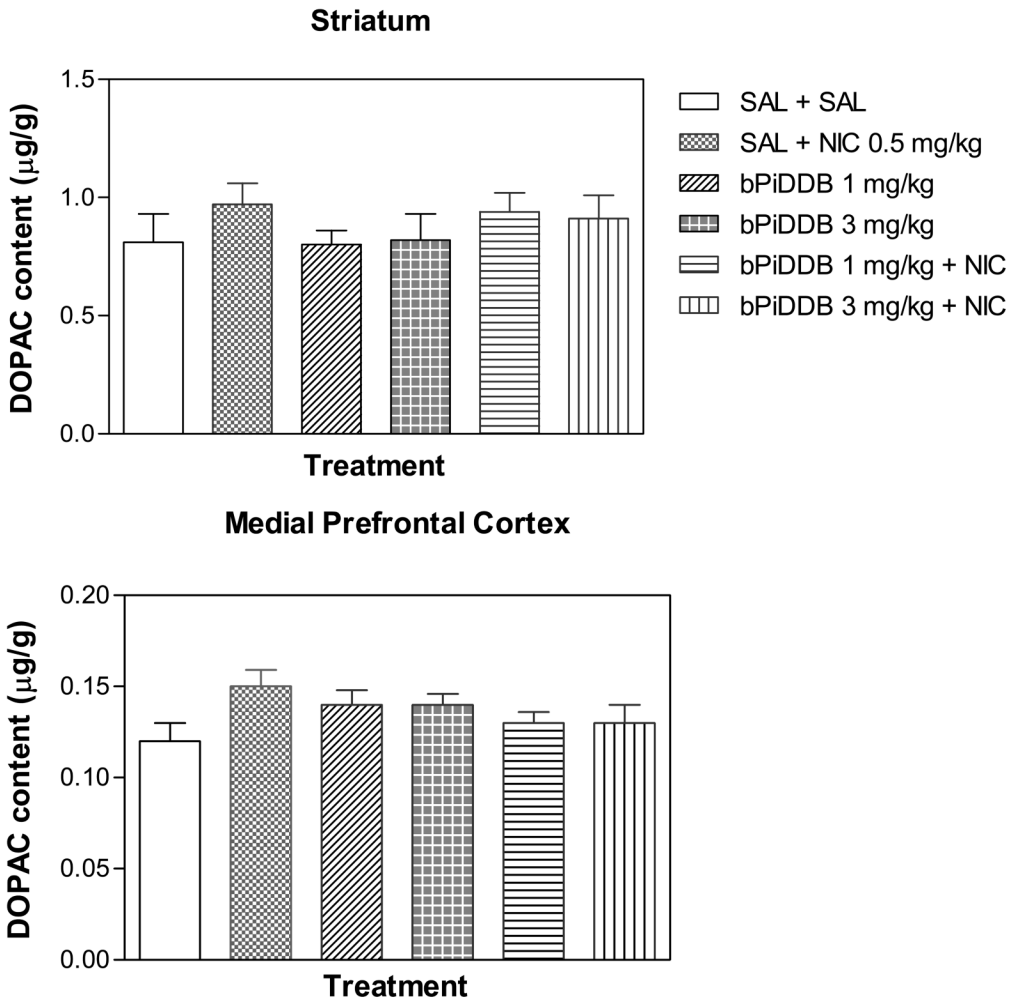

Fig. 2.

Effects of systemic bPiDDB (1 or 3 mg/kg) on the acute nicotine (NIC; 0.5 mg/kg)-induced changes in DOPAC content in rat striatum (upper panel) and medial prefrontal cortex (lower panel). bPiDDB was administered 15 min prior NIC and rats were killed 30 min after NIC administration. Data are expressed as µg/g tissue (mean ± S.E.M, n=7–8 rats/group).