Psychopharmacology (2007) 193:457–465
DOI 10.1007/s00213-007-0799-7

ORIGINAL INVESTIGATION

Involvement of alpha1-adrenergic receptors in tranylcypromine enhancement of nicotine self-administration in rat

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Received: 15 August 2006 / Accepted: 7 April 2007 / Published online: 8 May 2007
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Abstract
Rationale The mechanisms mediating tobacco addiction remain elusive. Nicotine, the psychoactive component in tobacco, is generally believed to be the main cause of reward and addiction. However, tobacco smoke contains thousands of constituents, some of which may interact with nicotine to enhance reward. It has previously been shown that monoamine oxidase (MAO) inhibition, known to result from smoking, can enhance nicotine self-administration. The aim of the present study was to evaluate the role of noradrenergic systems in mediating this enhancement of nicotine reward.
Objective The objective of this study was to test the hypothesis that MAO inhibitor pretreatment enhances nicotine self-administration by activation of noradrenergic pathways that regulate dopamine release in the nucleus accumbens (NAc).
Methods The effect of prazosin (0.0625–0.5 mg/kg, i.p.), a specific α1-adrenergic receptor antagonist, was examined on male rats pretreated with tranylcypromine (3 mg/kg), an irreversible inhibitor of MAO A and B. Acquisition of nicotine (10 μg kg^{-1} inj^{-1}, i.v.) self-administration behavior was examined over a 5-day period. Nicotine (60 μg kg^{-1} inj^{-1}, i.v.)-induced increase in NAc extracellular dopamine levels was examined by in vivo microdialysis in non-self-administering animals.
Results We have shown that (1) tranylcypromine enhances nicotine self-administration, (2) prazosin pretreatment blocks both the acquisition and the expression of nicotine self-administration, and (3) prazosin pretreatment diminishes nicotine-induced dopamine release in the NAc.
Conclusion These data indicate that the stimulation of α1-adrenergic receptors is critical for tranylcypromine enhancement of nicotine reward and suggest a critical interplay between the noradrenergic and dopaminergic systems in tobacco addiction.

Keywords Rat · MAOI · Tobacco · Prazosin · Norepinephrine · Nicotine · Tranylcypromine · Self-administration · Reward · Dopamine

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Introduction

Tobacco is one of the most abused reinforcing agents in humans (Henningfield et al. 1998). Nicotine, the main psychoactive component in cigarettes, is generally believed to be the cause of tobacco addiction (Dani and Heinemann 1996; Balfour et al. 2000; Di Chiara 2000). However, when nicotine is tested in animal models of reward and addiction, it seems to be a weak reinforcer (Stolerman and Jarvis 1995). This paradox has led to the hypothesis that nicotine may interact with other constituents in tobacco smoke to produce its addictive effects (e.g., Belluzzi et al. 2005). There are more than 4,000 compounds in tobacco smoke (CDC 1989), some of which are known to irreversibly inhibit monoamine oxidase (MAO; Lewis et al. 2007). It is well established that both central and peripheral MAO

activities are irreversibly inhibited in smokers (Fowler et al. 1996b, 2003). Recent studies suggest that the inhibition of MAO activity by tobacco smoke may enhance the reinforcing effects of nicotine (Guillem et al. 2005; Villégier et al. 2003, 2005, 2006). Indeed, pretreatment with tranylcypromine, an irreversible and nonselective MAO inhibitor (MAOI), increases the rewarding properties of nicotine (Guillem et al. 2005; Villégier et al. 2005, 2006). Therefore, animal models of tobacco addiction may be improved by combining MAOI with nicotine to assess underlying mechanisms. Moreover, the use of MAOI has provided promising clinical data to help smokers quit smoking (Berlin et al. 1995; George et al. 2003; Biberman et al. 2003).

Until now, mechanistic studies of tobacco addiction have examined nicotine alone. Such studies suggest that nicotine exerts its reinforcing properties through the activation of mesolimbic dopaminergic transmission (Di Chiara et al. 1999). Nicotine stimulates the nicotinic acetylcholine receptors (nAChR) located on the mesolimbic system (Champtiaux et al. 2003) and increases the firing activity of the mesolimbic dopaminergic neurons, thus increasing extracellular concentration of dopamine (DA) in the nucleus accumbens (NAc; Nisell et al. 1996). However, because of the large representation of nAChRs through the brain, other systems may be of importance for nicotine-induced reward. Interestingly, nAChRs are localized on noradrenergic cell bodies and terminals (Lena et al. 1999), and nAChR-evoked release of norepinephrine (NE) has been demonstrated in several brain regions that receive noradrenergic innervation from the locus coeruleus (LC; Mitchell et al. 1989; Engberg and Hajos 1994; Leslie et al. 2002; O'Leary and Leslie 2003). Although a few reports have analyzed the involvement of NE in the rewarding properties of nicotine, its release by nicotine seems to deserve more attention. In fact, a physiological coupling between noradrenergic and dopaminergic neurons has been shown to occur through the stimulation of α1-adrenergic receptors (Antelman and Caggiula 1977; Kokkinidis and Anisman 1978; Lategan et al. 1990). Moreover, noradrenergic transmission has been shown to exert a permissive effect on both biochemical and behavioral effects related to addictive processes. Amphetamine-induced locomotor hyperactivity and DA release are both inhibited by prazosin, an α1-adrenergic antagonist, or by the genetic elimination of the α1 receptor (Blanc et al. 1994; Darracq et al. 1998; Drouin et al. 2002a, b; Auclair et al. 2004).

In the present study, we have evaluated the possible involvement of noradrenergic transmission in nicotine-induced reward. To do so, we have tested the effect of prazosin on the acquisition and maintenance of nicotine self-administration in tranylcypromine pretreated-rats and on nicotine-induced DA release in the NAc in non-self-administering animals.

Materials and methods

Animals

Male Sprague–Dawley rats were obtained at postnatal day (P)16 and P80. Juvenile animals remained with their dam until weaning (P21) when they were housed in groups of four until surgery. After surgery, all animals were single-housed and maintained on a 12-h light/dark cycle (lights on at 07:00 A.M.) with food and water available ad libitum. Rats were allowed at least 3 days of postoperative recovery before any treatments began. All tests were performed during the light part of the light–dark cycle to reproduce conditions used in studies showing the enhancement of nicotine self-administration by tranylcypromine (Villégier et al. 2007).

Drugs

Nicotine [(−) nicotine hydrogen tartrate], tranylcypromine hydrochloride, and prazosin were purchased from Sigma-Aldrich. Doses are expressed as salts for all compounds except for nicotine, which is expressed as free base. Tranylcypromine and nicotine were dissolved in saline (NaCl, 0.9%) and the pH adjusted to 7.4 with NaOH. Prazosin was dissolved in water. Tranylcypromine, prazosin, and vehicles were injected intraperitoneally (i.p.; 0.5 ml per injection), while nicotine and vehicles were injected intravenously (i.v.; 20 μl per injection). DA stock solution was ordered as pre-made solutions from ESA with a concentration of 1 mg/ml. DA standard was stored at 2–8°C.

Self-administration

Surgical implantation of intravenous catheters Surgery commenced at ages P23–24 or P86–87, respectively. Animals were anesthetized with Equithesin (0.3 ml/100 g, i.p. for P86, 0.25 ml/100 g, i.p. for P23), and a chronic catheter was surgically implanted into the right external jugular vein (Belluzzi et al. 2005; Caine et al. 1993). Catheters were flushed daily with 0.2 ml sterile heparinized saline solution (0.6 or 0.3 ml of 1,000 U/ml heparin in 30 ml saline for P86 or P23, respectively) to maintain catheter patency. On test days, heparinized saline was injected before and after the self-administration session. Before testing and after the final daily test session, Propofol (0.2 ml P86; 0.1 ml P23) was injected through the catheter to test the patency of the i.v. catheter as indicated by rapid

(5–10 s) anesthesia. Data were discarded from all animals not demonstrating rapid anesthesia.

Drug self-administration The drug self-administration procedure was modified from that used previously (Belluzzi et al. 2005). Three to 4 days after surgery, rats were tested in self-administration chambers with two nose-poke holes side-by-side in the side of the chamber. A syringe, mounted in an infusion pump outside the test chamber, was filled with enough solution to provide a maximum of 200 injections. During each 1.1-s 20-μl infusion, the signal light over the hole associated with drug injection went on for a 1.1-s period after which the house light went off and all responses were counted but had no effect for a 60-s period. Nose pokes at the reinforced and non-reinforced holes were followed by a 1-min timeout during which responses were not counted. The delivery of all experimental parameters and the collection of all data were controlled by a multi-channel computer system (MED Associates, St. Albans, VT, USA).

Acquisition of nicotine self-administration after pretreatment with tranylcypromine Initial acquisition of drug self-administration was measured during two postnatal periods that have previously been defined as early adolescence and adulthood (Spear 2000): P27–31 and P90–94. A nose-poke response was used, which relies on the animals' natural olfactory exploration to provide adequate initial levels of responding. Priming at the start of each session was not employed because of possible aversive effects of non-contingent injections in naive animals. A total of 163 rats were used in the experiment. Tests for acquisition of self-administration commenced without prior response training and consisted of five consecutive daily 3-h sessions with a fixed-ratio one (FR 1) reinforcement schedule. As we previously reported, tranylcypromine pretreatment shifts the dose–effect curve for nicotine self-administration to the left (Villégier et al. 2005, 2007). Animals show strong self-administration at nicotine doses as low as 2.5–10 μg kg^{-1} inj^{-1} and no significant self-administration at the standard dose of 30 μg kg^{-1} inj^{-1}. For this reason, we chose to test the effect of prazosin on self-administration of nicotine 10 μg kg^{-1} inj^{-1} in tranylcypromine pretreated rats. One hour before each session, rats received a pretreatment of tranylcypromine (3 mg/kg, i.p.) or vehicle (1.5 and 3 ml/kg, i.p. for adults and adolescents, respectively). Each nose-poke at the reinforced hole delivered nicotine (10 μg/kg in 20 μl) or saline vehicle (20 μl). To control for nonspecific activating effects of drugs, non-reinforced nose-pokes (NR) at a second adjacent hole were counted but had no programmed consequences.

The involvement of the α1-adrenergic receptor was tested by injecting a specific antagonist, prazosin (0.0625–0.5 mg/kg, i.p.) or vehicle (1.5 and 3 ml/kg, i.p. for adults and adolescents, respectively), 30 min before the beginning of the self-administration session.

Microdialysis

Stereotaxic surgery Immediately after implantation of their intravenous catheter, animals for microdialysis were implanted with a cranial guide cannula (20 gauge; CMA/Microdialysis AB, Stockholm, Sweden) 2.0 mm above the target area, which was fixed to the skull with acrylic dental cement and sealed with a dummy cannula. Anatomical coordinates for NAc shell were established from the atlas of Paxinos and Watson (1986): antero-posterior, +2.0 mm relative to bregma; medio-lateral, ±1.2 mm; and dorso-ventral, 5.8 mm from dura.

Microdialysis procedure Animals were given 2 days to recover with daily handling after surgeries. Catheters were flushed daily, and animals were habituated to the microdialysis chambers for 5 min per day. Intravenous catheter patency was tested by propofol 1 day before the experiment. In vivo microdialysis studies were performed after 2 days of tranylcypromine pretreatment to mimic nicotine self-administration studies that illustrated an increase in reinforcing hole responding in tranylcypromine pretreated animals.

On the experimental day, the dummy cannula was replaced with a 2-mm microdialysis probe (CMA/12). The quality of probes was tested in vitro before the experiment with an average recovery of 10.8±1.7%, n=16. Microdialysis was carried out under a free-moving condition, with the probe continuously perfused with artificial cerebrospinal fluid (CSF; CMA Microdialysis N. Chelmsford, MA, USA) at a constant flow rate of 1.1 μl/min delivered by a microinfusion pump (CMA/100 microdialysis). After a 3-h equilibration period, samples were collected every 20 min during 1 h. At the 60-min time point, a third tranylcypromine (3 mg/kg, i.p.) injection was given. Samples were collected for another 90 min, until DA levels returned to baseline. Prazosin (0.5 mg/kg, i.p.) was injected 30 min before the nicotine injections (two 30 μg kg^{-1} inj^{-1} i.v., 1 min apart). Nicotine was not self-administered but injected by the experimenter. Samples were collected for a final 80 min. The drug treatment and data collection times were chosen to parallel behavioral experiments such that DA collection occurred in the time period analogous to peak self-administration. DA levels were quantified by high performance liquid chromatography with electrochemical detection (HPLC-ED). At the end of the experiment, rats were killed. Brains were put into a formaldehyde solution and cut on a microtome in serial coronal slices according to the atlas of Paxinos and Watson (1986). Histological

examination of microdialysis probes placement was subsequently made on coronal sections (Fig. 4).

HPLC-ED detection Microdialysate samples (20 μl) were automatically injected by an ESA 542 refrigerated autosampler onto a 150×3.2 mm ODS C^{18} column (ESA, Chelmsford MA, USA) connected to an ESA 580 HPLC pump. The column was kept at 35°C and perfused by MD-TM mobile phase (ESA) at a rate of 0.6 ml/min. DA levels were determined by an electrochemical ESA 5600 detector connected to a 5014B Microdialysis Cell (ESA) with the dominant potential set to 320 mV. The sensitivity of the detector is 500 fg. Measurements were analyzed using CoulArray for Windows[32] Software 2.0 (ESA). Standard curves were generated with DA (ESA) and DOPAC (Sigma-Aldrich, St. Louis, MO, USA) standards, and levels in experimental samples were determined from the curve and expressed as pg/20 μl, unadjusted for recovery. For the effects of tranylcypromine, baseline levels of DA were determined by averaging the three samples before tranylcypromine injection (T20–T60). For nicotine's effects, the period before the nicotine injection (T180) was used as the baseline.

Statistics

Data were analyzed using two-way analysis of variance (ANOVA; for reinforced/nonreinforced responding×day with repeated measures on reinforced/nonreinforced responding and on days and for treatment dose×time with repeated measures on time) and one-way ANOVA (for treatment and reinforced/nonreinforced effects). Significant main effects or interactions were tested separately with ANOVAs and Bonferroni- or Dunnett's-corrected post hoc comparisons. Day and reinforced/nonreinforced responding were considered as within-subject factors and pharmacological treatments corresponded to independent groups of animals and were considered as between-subject factors. All data analyses were performed using SYSTAT 10 statistical software. Statistical significance was set at $p<0.05$.

Results

Prazosin blockade of acquisition of nicotine self-administration

As has been reported previously, in our acquisition paradigm in which there is no prior training or food deprivation, nicotine alone is not reliably self-administered (Belluzzi et al. 2005; Villégier et al. 2005, 2006). However, as shown in Fig. 1a, rats pretreated with tranylcypromine (3 mg/kg, i.p.) quickly learned to self-administer nicotine and displayed sustained self-administration at rates substantially higher than those for saline (significant nicotine dose effect, $F_{1,24}=5.275$, $p=0.031$ tranyl + nico R vs tranyl + saline R; and significant nicotine dose × day interaction, $F_{4,96}=5.013$, $p=0.035$). Moreover, nicotine-reinforced responding was significantly higher than nonreinforced responding (Fig. 1a, $F_{1,13}=15.515$, $p=0.002$) with a significant rein-

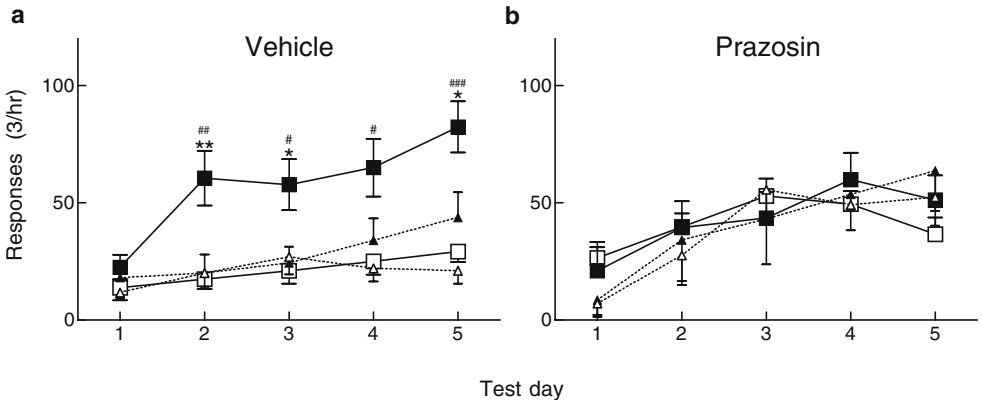

Fig. 1 Prazosin blocks tranylcypromine-enhanced acquisition of nicotine self-administration. Before the beginning of each self-administration session, rats received an injection of tranylcypromine (3 mg/kg, i.p.) 1 h prior and an injection of either prazosin (0.5 mg/kg, i.p.) or vehicle (2 ml/kg, i.p.) 30 min prior. Rats were offered either nicotine (10 μg kg^{-1} inj^{-1} in 20 μl, i.v.; *filled squares* for reinforced responding and *open squares* for nonreinforced responding) or saline (20 μl, i.v.; *filled triangles* for reinforced responding and *open triangles* for nonreinforced responding). The mean (±SEM) total responses are plotted daily for each treatment group. **a** Acquisition of nicotine self-administration in tranylcypromine-pretreated rats. Rats pretreated with tranylcypromine self-injected significantly more nicotine than saline; $^{*}p<0.05$, $^{**}p<0.01$. Moreover, reinforced responding for nicotine was significantly greater than responding at the non-reinforced hole, $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$; $n=12$–14/group. **b** Effect of prazosin on the acquisition of nicotine self-administration. In tranylcypromine/prazosin-treated rats, nicotine and saline reinforced responding were not significantly different ($p>0.05$), and nicotine reinforced responding was not significantly different from responding at the nonreinforced hole ($p>0.05$); $n=6$–17/group

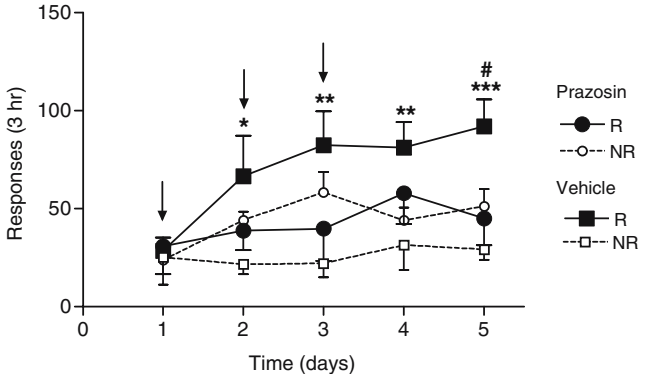

Fig. 2 Prolonged blockade of tranylcypromine-enhanced nicotine self-administration acquisition by prazosin. Prazosin (0.5 mg/kg, i.p.) or vehicle (3 ml/kg, i.p.) was administered 30 min prior to testing on days 1–3 (*arrows*). On days 4 and 5, all rats received a vehicle pretreatment. The mean (±SEM) total responses obtained on reinforced and nonreinforced holes are plotted for daily 3-h periods. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs vehicle nonreinforced (*NR*); $^{\#}p<0.05$ vs prazosin reinforced (*R*); n=8–9/group

forced/nonreinforced × day interaction ($F_{4,52}$=4.902, p=0.002). As shown in Fig. 1b, nicotine self-administration did not occur when prazosin (0.5 mg/kg, i.p.) was injected before each test session, as shown by the absence of difference between responding for nicotine and saline. Moreover, we did not obtain any significant difference between reinforced and nonreinforced responding and any reinforced/nonreinforced × time interaction.

Prazosin significantly increased nonreinforced responding (significant prazosin effect in nicotine i.v. group: $F_{1,26}$= 10.288, p=0.004; and in saline i.v. group: $F_{1,16}$=6.659, p= 0.02 and significant time × prazosin interaction in saline i.v. group: $F_{4,64}$=3.517, p=0.012).

Prazosin administration before early test sessions produced a prolonged blockade of nicotine self-administration. Figure 2 shows the time course of acquisition of nicotine self-administration in rats receiving either vehicle or prazosin on days 1–3, with all animals receiving vehicle on days 4 and 5. When nicotine self-administration was analyzed on days 4 and 5, a significant difference was found in reinforced vs nonreinforced responding ($F_{1,15}$= 8.153, p=0.012) with a prazosin×reinforced/nonreinforced responding interaction ($F_{1,15}$=9.498, p=0.008). Reinforced responding was significantly higher than responding at the nonreinforced hole on days 4 and 5 in rats pretreated with saline on days 1 to 3 ($F_{1,7}$=64.571, $p<0.001$) but not in rats receiving a prazosin pretreatment. Moreover, rats receiving prazosin treatment on days 1–3 had significantly lower responding at the reinforced hole on day 5 than vehicle-treated rats ($F_{1,15}$=7.822, p=0.014).

Prazosin blockade of established nicotine self-administration

Figure 3a shows the effect of prazosin on tranylcypromine-pretreated rats once nicotine self-administration has been established. Analysis across the first three daily sessions showed a significant difference between nicotine reinforced and nonreinforced responding ($F_{1,6}$=88.803, $p<0.001$). However, the administration of prazosin (0.5 mg/kg, i.p.) 30 min before self-administration testing on days 4 and 5 abolished the difference between reinforced and nonreinforced responding. Moreover, prazosin significantly reduced nicotine intake on days 4 and 5 when compared with day 3 ($p<0.001$ and p=0.023, respectively). Figure 3b shows that this inhibitory effect of prazosin was dose

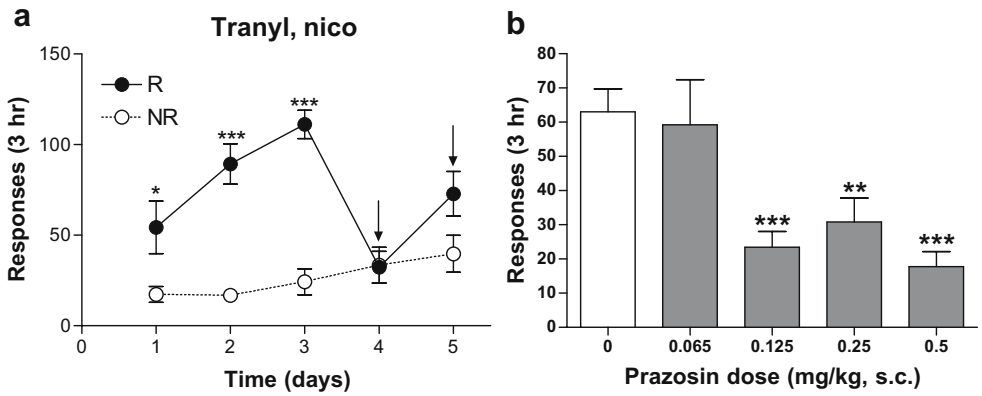

Fig. 3 Acute reduction of tranylcypromine-enhanced nicotine intake by prazosin. **a** Rats were pretreated with tranylcypromine (3 mg/kg, i.p.) 1 h prior to nicotine self-administration sessions. The mean (±SEM) total responses obtained on reinforced and nonreinforced holes are plotted daily. On days 4 and 5, rats received a pretreatment with prazosin (0.5 mg/kg, i.p.; *arrows*); *$p<0.05$, ***$p<0.001$ reinforced vs nonreinforced responding; n=7/group. **b** On day 4, rats received prazosin 0.065, 0.125, 0.25, or 0.5 mg/kg (i.p.) or vehicle (3 ml/kg, i.p.). The mean (±SEM) total responses obtained at the reinforced hole during the 3-h period of the test day are plotted for each treatment group. **$p<0.01$, ***$p<0.001$ vs vehicle treated rats; n=4–9/group

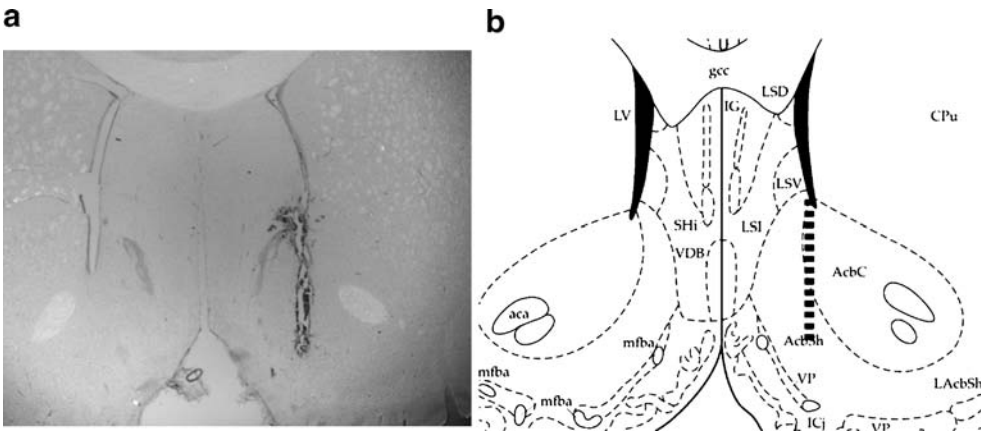

Fig. 4 Photomicrograph localization of microdialysis probes in the NAc shell as shown by a representative **a** cresyl violet stained brain section and **b** schematic brain section from the Paxinos and Watson atlas. The *dashed lines* in **b** illustrate the positioning of the 2-mm membrane of the microdialysis probe

dependent. Analysis of the effect of prazosin on responding for nicotine self-administration after tranylcypromine showed a significant effect of dose ($F_{4,25}$= 8.916, p<0.001). Nicotine self-administration was significantly decreased after prazosin treatment at doses of 0.125 mg/kg.

Effect of prazosin on tranylcypromine enhancement of nicotine-induced DA release

Tranylcypromine (3 mg/kg, i.p.) produced a significant ($F_{5,70}$=13.366, p<0.0001) increase in extracellular DA levels, but there were no statistical differences between prazosin or nicotine treatment groups, and the data were collapsed across treatments (Fig. 5a). After injections at 180 min (Fig. 5b), there were significant effects of nicotine ($F_{1,14}$=31.778, p=0.0001) and prazosin ($F_{1,14}$=8.230, p= 0.0124). Nicotine (60 μg/kg, i.v.) significantly increased extracellular DA levels in the NAc shell of tranylcypromine pretreated rats for 40 min after administration, whereas prazosin significantly reduced the nicotine-induced DA levels (Fig. 5b).

Discussion

In the present study, we have shown the involvement of noradrenergic systems in mediating tranylcypromine enhancement of nicotine self-administration. Prazosin, a specific α1-adrenergic receptor antagonist, inhibits both

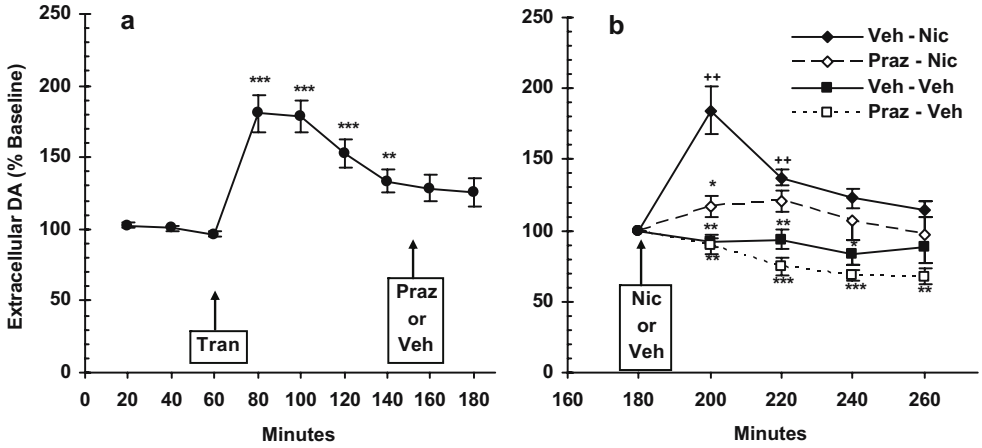

Fig. 5 Prazosin reduces tranylcypromine-enhanced nicotine stimulated increase of extracellular DA levels in NAc. **a** After a 60-min baseline period, tranylcypromine (3 mg/ml, i.p.) was injected and DA levels in NAc were recorded for 120 min. Tranylcypromine significantly increased DA levels over baseline for 80 min after injection. Extracellular DA levels are expressed as mean percentage baseline, where the baseline is the mean pg/20 μl for T20–T60 min. ***p< 0.001 vs baseline; **p<0.01; n=18. **b** Ninety minutes after the injection of tranylcypromine (3 mg/kg, i.p.), rats received an injection of either prazosin (0.5 mg/kg, i.p.) or vehicle (3 ml/kg, i.p.). Nicotine (30 μg/kg, i.v. ×2) or saline (20 μl, i.v. ×2) was injected 30 min later. Extracellular DA levels are expressed as mean percentage baseline, where the baseline is the pg/20 μl for T180 min. Nicotine significantly increased extracellular DA levels in tranylcypromine pretreated rats but not after administration of prazosin. ***p<0.001 vs Veh–Nic; **p<0.01; *p<0.05; $^{++}$p≤0.01 vs T180; n=3–7/group

the acquisition and expression of nicotine self-administration in tranylcypromine pretreated rats. Furthermore, prazosin pretreatment reduced nicotine-induced DA release in the NAc. Taken together, these results suggest a critical interplay between noradrenergic and dopaminergic systems in tobacco reward.

Nicotine and MAOI as a model of smoking

We have replicated previous findings that MAOI pretreatment enhances nicotine self-administration in a rigorous acquisition paradigm in which untreated rats do not respond (Villégier et al. 2005, 2006). As there are natural MAOIs contained in tobacco, we have proposed that the combined use of nicotine and MAOIs may be a better model than the use of nicotine alone to assess the rewarding effects of tobacco. Although it is well established that smoking reduces MAO-A and MAO-B activities in smoker's brains and peripheral tissues (Fowler et al. 1996a, b), the irreversible MAOI in tobacco has not yet been identified (Lewis et al. 2007). We have therefore used tranylcypromine, an irreversible MAOI, which inhibits both MAO-A and MAO-B within 1 h of administration (Baker et al. 1992; Griebel et al. 1998). The tranyclypromine dose used in our study is consistent with previous investigations on the MAO-inhibiting effects of this drug (McManus and Greenshaw 1991a, b; Todd and Baker 1995). Moreover, we have confirmed that tranylcypromine-pretreatment inhibits MAO-A and MAO-B by 80 and 90%, respectively (data not shown).

NE in nicotine-induced reward

Although nicotine-induced release of NE has been demonstrated in several brain regions (Mitchell et al. 1989; Summers and Giacobini 1995; Fu et al. 1999a, b; Lena et al. 1999; Li and Eisenach 2002), it has rarely been correlated with nicotine-induced reward. In the present study, the blockade of α1-adrenergic receptors reduced both nicotine self-administration of tranylcypromine-treated rats and extracellular DA levels in the NAc measured in non-self-administering, tranylcypromine-pretreated rats. This finding suggests that in animals pretreated with a MAOI, nicotine exerts its rewarding properties through an activation of NAc DA terminals that are under the permissive control of NE transmission. This observation is in accordance with previous reports describing the modulatory action of noradrenergic transmission on dopaminergic functions (Darracq et al. 1998; Linner et al. 2001; Auclair et al. 2002). Studies have provided evidence that the burst firing of the dopaminergic neurons are inhibited by an α1-adrenergic receptor antagonist, prazosin (Grenhoff et al. 1993; Grenhoff and Svensson 1993; Andersson et al. 1994), and increased by a blocker of NE reuptake, reboxetine, or nisoxetine (Shi et al. 2000; Linner et al. 2001).

Although it has recently been suggested that prazosin at a dose of 0.5 mg/kg can block motivational processes in general, including food-seeking behavior, a lower dose of 0.3 ml/kg has been shown to selectively inhibit both cocaine-induced reinstatement of drug-seeking behavior (Zhang and Kosten 2005) and cocaine priming of subsequent self-administration (Zhang and Kosten 2006). Our present finding of significant effects of prazosin at doses as low as 0.125 mg/kg suggests a specific role of α1-adrenergic receptors in tranylcypromine-enhanced nicotine reinforcement.

Noradrenergic stimulation may be mediated by α1b-adrenergic receptors, as KO mice for these receptors show a deficit in locomotor hyperactivity, conditioned place preference, and oral preference induced by psychostimulants and opiates, and elimination of D-amphetamine-induced increases in extracellular DA levels in the NAc (Drouin et al. 2002a, b; Auclair et al. 2004). Ventral tegmental area (VTA)-dopaminergic cell bursting activities may be controlled by α1-adrenergic transmission located in the prefrontal cortex (PFC), as intra-PFC prazosin administration reduces both D-amphetamine-induced DA release in the NAc and locomotor hyperactivity in rat (Blanc et al. 1994; Darracq et al. 1998). Anatomically, glutamatergic afferents originating from several areas, including the PFC (Gariano and Groves 1988; Sesack and Pickel 1992; Chergui et al. 1993; Darracq et al. 1998), could be at the origin of this control, as they are regulated by α1-adrenergic receptors and provide an excitatory input to the mesolimbic dopaminergic cells on cell bodies and terminals (Trovero et al. 1994; Lu et al. 1997; Li et al. 1999; Carr and Sesack 2000; Darracq et al. 2001). Alpha1b-adrenergic receptors located in the VTA may also be important to control DA mesolimbic cells, which have been implicated in behavioral sensitization to psychostimulants (Vezina 1993).

Conclusion

This study provides support for the view that α1-adrenergic transmission plays an important role in tobacco addiction and may, therefore, be a useful therapeutic target. According to some theories, vulnerability and individual sensitivity to drugs of abuse, such as psychostimulants and opiates, may be explained by genetic or epigenetic variations in the reactivity of noradrenergic neurons to environmental cues affecting the activation of VTA dopaminergic neurons (O'Brien et al. 1986; Piazza et al. 1989; Shiffman 1991; Hooks et al. 1991; Tassin 1998). The present study tends to generalize this theory to tobacco addiction. Alpha1-adrenergic transmission may then be a

common mechanism driving addiction regardless of the addictive substance class.

Acknowledgments This work was supported by PHS Grant DA21267 and a fellowship from TRDRP 13DT-0033 (S.L). Experiments comply with the current laws of the country in which they were performed. All experimental procedures were performed in compliance with NIH Guide for Care and Use of Laboratory Animals (NIH No 85-23, rev. 1985) and approved by the UCI Institutional Animal Care and Use Committee.

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