Pharmacology, Biochemistry and Behavior 98 (2011) 552–558

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

L-DOPA attenuates nicotine withdrawal-induced behaviors in rats

Yu Ohmura [a,b], Emily M. Jutkiewicz [a], Edward F. Domino [a,*]

[a] Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109-5632, USA
[b] Department of Pharmacology, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060-8638, Japan

ARTICLE INFO

Article history:
Received 17 November 2010
Received in revised form 27 January 2011
Accepted 7 February 2011
Available online 24 February 2011

Keywords:
Levodopa
Tobacco
Smoking
Stop smoking aids
Quit smoking aids

ABSTRACT

There is some evidence that during nicotine abstinence brain dopamine levels are reduced. The hypothesis for the present study was that the precursor amino acid L-DOPA would relieve nicotine withdrawal-induced behaviors. Separate groups of adult male Sprague–Dawley rats were used. (−)-Nicotine bitartrate (9 mg/kg/day, salt content) or equimolar sod ium tartrate was infused into each rat via a subcutaneous osmotic minipump for 7 days. To assess nicotine withdrawal behaviors, locomotor activity was measured for 24 h in their home cage. Somatic signs were also counted approximately 22 h after pump removal. Moreover, depressive-like behaviors were evaluated in the forced swimming test approximately 48 h after pump removal. One day after removal of pumps, locomotor activity was suppressed in nicotine-infused rats compared to the tartrate-infused controls. Somatic signs of nicotine withdrawal were increased in nicotine-infused rats compared to the controls. Two days after removal of pumps, increased immobility in the forced swimming test was observed in abstinent nicotine-infused rats as compared with controls. The administration of L-DOPA methyl ester (equivalent to 50 mg/kg L-DOPA, s.c.) and benserazide (10 mg/kg, s.c.) attenuated somatic signs of withdrawal and reversed nicotine withdrawal-induced depressive-like behaviors in the forced swimming test. It did not mitigate nicotine withdrawal-induced locomotor suppression in the animals' home cages. These results indicate that L-DOPA could be a useful agent to alleviate some nicotine withdrawal symptoms.

1. Background

Abrupt tobacco cessation in chronic smokers causes withdrawal symptoms including anxiety, craving for tobacco, difficulty in concentrating, depressed mood, increased appetite, insomnia, fatigue, decreased arousal, and irritability (Hughes, 2007, 2008; Malin and Goyarzu, 2009; American Psychiatric Association, 2000; World Heatlh Organization, 1993). Alleviating these symptoms may help smokers quit smoking. Depressed mood and craving for tobacco predict relapse while other symptoms are relatively weak predictors of relapse (review, Hughes, 2007). However, irritability is the most frequently reported symptom(s) during smoking cessation (Hughes, 2007).

Abrupt withdrawal from repeated nicotine decreases basal levels of dopamine (DA) release in rat nucleus accumbens (Takahashi et al., 1998; Rahman et al., 2004). Mecamylamine precipitates nicotine withdrawal and significantly decreases extracellular DA in nucleus accumbens though it is not clear whether the decreased DA release reflects decreased basal DA levels or attenuated nicotine-induced DA release (Hildebrand et al., 1998; Rada et al., 2001). Domino and Tsukada (2009) also found that basal levels of DA release in the monkey dorsal striatum were decreased after overnight abstinence from daily nicotine. However, after overnight abstinence, nicotine induced DA release was enhanced over controls, indicating sensitization and not attenuation. In humans, smokers abstinent from tobacco for 11 to 17 h have only 54% of the cerebrospinal fluid concentration of the DA metabolic homovanillic acid (HVA) as compared with nonsmokers (Geracioti et al., 1999).

Based on the above findings, we examined whether L-DOPA, a precursor of DA, is effective for relieving nicotine withdrawal-induced behaviors. If decreased DA levels after nicotine abstinence were a cause of nicotine withdrawal, a precursor of DA should be effective for relieving withdrawal signs. As a measure of nicotine withdrawal, we used nicotine withdrawal-induced rat locomotor depression (Gäddnäs et al., 2000; Catania et al., 2003) and somatic signs of withdrawal (Malin et al., 1992). Somatic signs of nicotine withdrawal are considered to reflect mainly irritability (Malin and Goyarzu, 2009). The rat forced swimming test was also employed as an animal model suggested to predict or evaluate mood changes in emotional state (Porsolt et al., 1977, 1978). The acute nicotine abstinence syndrome in rats does not exactly duplicate tobacco smoking withdrawal in humans. Nevertheless, it does provide quantitative measures that do replicate some of the predominant mood changes of smoker tobacco abstinence and how much they are modified by L-DOPA in an animal model.

* Corresponding author. Tel.: +1 734 764 9115; fax: +1 734 763 4450.
E-mail address: efdabcde@umich.edu (E.F. Domino).

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doi:10.1016/j.pbb.2011.02.007

2. Methods

2.1. Animals

Male Sprague Dawley rats (Harlan, Indianapolis, IN), weighing 260–320 g in the beginning of the experiment were housed 2–3 per cage at a constant temperature of 20–21 °C. Animals were maintained on a 12 h light:dark cycle (lights on at 7:00, lights off at 19:00). Each animal had free access to rodent chow and water. Animal treatment complied with the NIH Animal Care Guidelines, and all procedures were performed according to a protocol approved by the University of Michigan Committee for Use and Care of Animals. Fig. 1 delineates the timeline that was used for the overall experimental design across days as described below.

2.2. Drugs

(−)-Nicotine bitartrate salt, sodium tartrate, benserazide, and L-DOPA methyl ester were purchased from Sigma-Aldrich, St. Louis, MO, USA. Each compound was dissolved in saline. The pH of the solution was adjusted using NaOH to approximately 7.0. Equimolar sodium tartrate dissolved in saline was used as the control solution for nicotine solution. All drugs for acute injection were administered s.c. in a volume of 1 ml/kg.

2.3. Surgical procedure

2.3.1. Osmotic minipump implantations and removals

For chronic nicotine administration, osmotic minipumps (Model 2ML2, Durect Corporation, Cupertino, CA, USA) for drug infusion surgically were implanted s.c. between the scapulae under ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia. The pumps were filled with either control solution or nicotine. The nicotine concentration was adjusted to deliver a dose of 9 mg/kg/day of nicotine salt (3.16 mg/kg/day nicotine base). Nicotine or the control solution was infused via implanted pumps at 5 μl/h for 1 week. This dose and duration has been used in previous studies of nicotine withdrawal (Malin et al., 1992; Hamilton et al., 2009, 2010). Moreover, the blood concentrations resulting from this dose in rats are almost same concentrations as those measured in heavy smokers (Benowitz et al., 1982; LeSage et al., 2002). One week after implantation of osmotic minipumps, minipumps were surgically removed under ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.) anesthesia.

2.3.2. Telemetry device implantations for locomotor activity

Telemetry devices (model ER-4000 E-Mitter; Mini Mitter Co., Bend, OR) were placed into the abdomen at the same time as osmotic pumps implant. The radiotransmitters were implanted inside the peritoneal cavity sending radio signals to a receiver (Model ER-4000 Receiver; Mini Mitter Co.) placed under the home cage of each rat. Data were collected and processed simultaneously by the Vital View data acquisition system (Mini Mitter Co.). This method was used previously to measure the effects of drugs on locomotor activity (e.g., Chen et al., 2007; Jutkiewicz et al., 2008).

2.4. Spontaneous somatic signs of nicotine withdrawal

Behavioral observations were performed from 9 to 12 am in a clear plastic observation chamber (48 × 23 × 20 cm). We observed behaviors 20–24 h after pump removal, based on previous studies (Malin et al., 1992; Rylkova et al., 2008). Rats' behaviors were counted for 30 min by observers who were blind to the experimental condition. Teeth-chattering/chews, stretches/gasps, shakes, ptosis, and miscellaneous other less frequent signs (e.g. diarrhea and yawns) were counted.

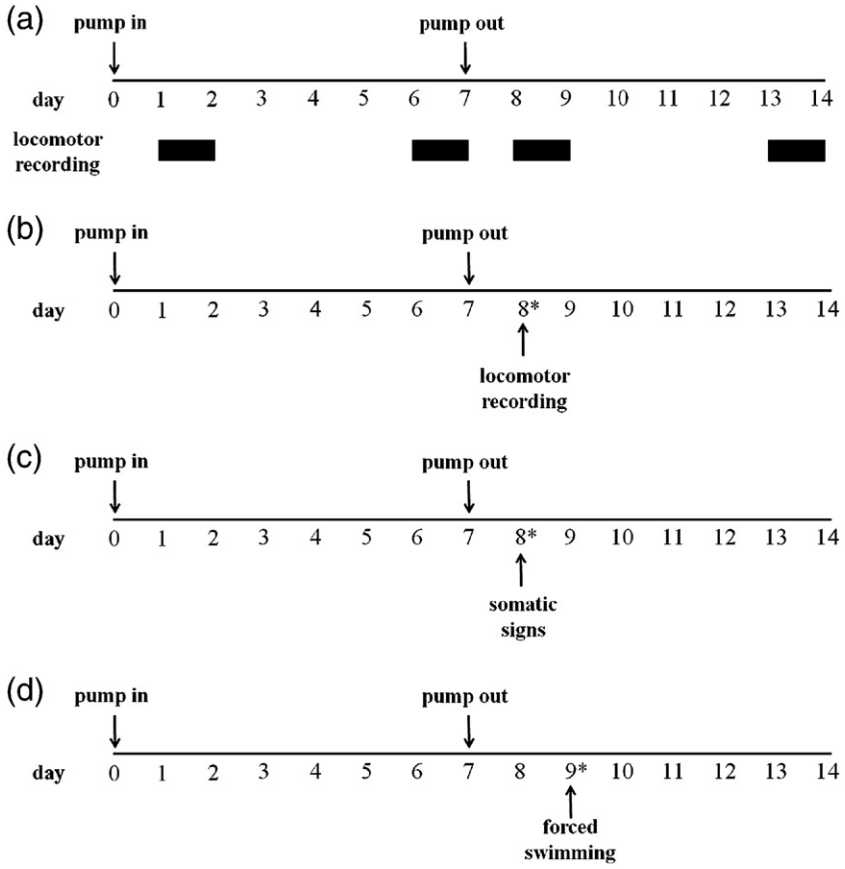

Fig. 1. Timeline of experimental designs for experiments 1 (a), 2 (b), 3 (c) and 4 (d).

Although the counting method is based on previous studies (Malin et al., 1992, 2006), the term "stretches" was used instead of "writhes" because apparent writhes could not be observed. Tremors were not included because none were observed.

2.5. Forced swimming test

To measure antidepressant-like activity after nicotine abstinence, rats were subjected to a modified forced swim test as previously described (Jutkiewicz et al., 2006). Briefly, each rat was placed in an opaque cylindrical acrylic container (55 cm tall × 20 cm in diameter) filled with 45 cm of 25 °C (± 1 °C) water. Rats experienced one 15 min swim session. Cylinder water was changed after every rat. Following the swim test, the rats were removed from the water, towel-dried, and placed in a warm heated cage for 15 min.

Videotaped 15 min test swims were scored for immobility, swimming, and climbing behaviors (Detke et al., 1995). The individual scoring the videotapes was blind to the drug treatments received by each rat. Every 5 s, the scorer rated the subject's behavior as one of the three behaviors, immobility, swimming, or climbing. The total counts of each behavior during the 15 min test swim were averaged within treatment groups. These behaviors were defined as: immobility — floating in the water without struggling and using only small movements to keep the head above water; swimming — moving limbs in an active manner (more than required to keep head above water) causing movement around the circumference; climbing — making active movements with the forepaws in and out of the water, often directed at the wall of the swim tank.

2.5.1. Experiment 1: effects of continuous infusion of nicotine and nicotine withdrawal on locomotor activity

To find the best time to detect the effects of nicotine withdrawal on locomotor activity, individual 24 h locomotor activity in the home cage of each rat was assessed. (−)-Nicotine bitartrate (9 mg/kg/day, salt) or equimolar sodium tartrate was infused into each rat via a subcutaneous osmotic minipump for 7 days. Locomotor activity was assessed at 1 and 6 days after implantation of an osmotic pump, and at 1 and 6 days after removal of the osmotic pump (see timeline in Fig. 1). Baseline locomotor activity prior to osmotic pump implantation could not be measured because both the telemetry transmitter and osmotic pump had to be implanted at the same time due to restrictions dictated by the approved animal protocols.

2.5.2. Experiment 2: effects of L-DOPA on nicotine withdrawal-induced locomotor depression

To examine whether L-DOPA was effective for relieving nicotine withdrawal-induced locomotor depression, we injected 62.8 mg/kg of L-DOPA methyl ester (equivalent to 50 mg/kg L-DOPA, s.c.) or saline to each rat just before the start of dark period (i.e. 19:00) one day after removal of the osmotic pump. The locomotor activity during 20:00–21:00 h was used to test the effects of L-DOPA because nicotine withdrawal-induced locomotor depression was clearly observed during this time period in Experiment 1. Moreover, previous studies have shown that L-DOPA methyl ester administration-induced DA release in the brain starts about 60 min after the administration (Kannari et al., 2006; Arai et al., 2008). Thirty minutes before the injection of L-DOPA methyl ester, benserazide (10 mg/kg, s.c.) or saline was injected to inhibit peripheral L-amino acid decarboxylase. In this and all subsequent experiments benserazide plus L-DOPA administration was given simultaneously and will be referred to as L-DOPA in the text that follows. Larger doses of L-DOPA were not given in this study because too much L-DOPA would mask all other signs of nicotine withdrawal.

2.5.3. Experiment 3: effects of acute L-DOPA administration on nicotine withdrawal signs

One day after removal of the osmotic pump, behavioral signs were counted. Rats received s.c. injection of 62.8 mg/kg L-DOPA methyl ester (equivalent to 50 mg/kg L-DOPA) 60 min prior to behavioral observation. In the control group, saline (1 ml/kg, s.c.) was injected twice 90 min and 60 min prior to behavioral observation.

2.5.4. Experiment 4: effects of L-DOPA on nicotine withdrawal-induced depressive-like behaviors in the forced swimming test

To examine whether L-DOPA was effective for relieving nicotine withdrawal-induced depressive-like behaviors, 62.8 mg/kg of L-DOPA methyl ester (equivalent to 50 mg/kg L-DOPA, s.c.) was injected into each rat 60 min before the start of forced swimming test. The forced swimming test was conducted during light period approximately 48 h after removal of the osmotic pump.

2.6. Data analysis

In Experiment 1, locomotor activity during the experimental period was analyzed by using three-way analysis of variance (ANOVA) for repeated measures with day post surgery (4 levels: 1 or 6 days after implantation of an osmotic pump, 1 or 6 days after removal of the osmotic pump) and time of day (2 levels: dark period or light period) as within-, and type of pump (2 levels: tartrate or nicotine pump) as between-subject factor. Two-way ANOVA was performed where significant interactions occurred and then one-way ANOVA was performed where significant interactions occurred again. In Experiments 2 and 4, two-way ANOVA was employed with the type of pump (2 levels: sodium tartrate or nicotine pump) and drug (2 levels: saline or L-DOPA) as between-subject factor, respectively. In Experiment 3, one-way ANOVA was used with the treatment conditions as between-subject factor (3 levels: sodium tartrate or nicotine pump with acute saline injection, and nicotine pump with acute L-DOPA). Multiple comparisons with Bonferroni's correction were also conducted following each ANOVA if necessary. The alpha level was set at 0.05 for all comparisons. All statistical procedures were conducted using SPSS (version 15.0 J).

3. Results

3.1. Experiment 1: effects of continuous infusion of nicotine and nicotine withdrawal on locomotor activity

Fig. 2 summarizes the effect of nicotine infusion and subsequent nicotine withdrawal on locomotor activity at 1 and 6 days after implantation of an osmotic pump, and at 1 and 6 days after removal of the osmotic pump in rats' home cages. Three-way ANOVA for repeated measures with day post surgery and time of day (i.e. dark period or light period) as within-, and type of pump as between-subject factor revealed a significant day post surgery × time of day × the type of pump interaction ($F_{3,51} = 3.81$, $P < 0.05$). Therefore we conducted two-way ANOVA with day post surgery as within-, and type of pump as between-subject factor at each level of time of day. The two-way ANOVA showed a significant day post surgery × the type of pump interaction regardless of time of day (dark period: $F_{3,51} = 9.58$, light period: $F_{3,51} = 2.82$, $Ps < 0.05$). Then we conducted one-way ANOVA with type of pump as between-subject factor at each level of time of day and day post surgery. Within light period, the one-way ANOVA revealed a significant main effect of type of pump in one day and 6 days after removal of the osmotic pump (one day: $F_{1,17} = 7.00$, 6 days: $F_{1,17} = 5.66$, $Ps < 0.05$). Within dark period, the one-way ANOVA revealed a significant main effect of type of pump only in one day after removal of the osmotic pump ($F_{1,17} = 6.87$, $P < 0.05$).

Because the effects of nicotine infusion on locomotor activity were found mainly in one day after removal of the pump (Fig. 2), we further analyzed locomotor activity every 2 h during the 24 h following pump removal (Fig. 3). Two-way ANOVA for repeated measures with time as within-, and type of pump as between-subject factor revealed a

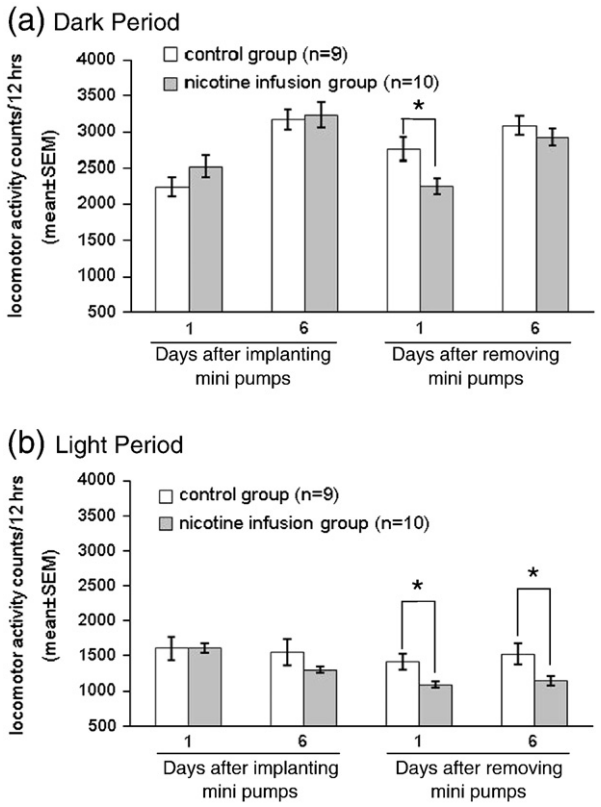

Fig. 2. Effects of continuous nicotine infusion via osmotic pump and subsequent withdrawal on locomotor activity in home cage during (a) dark period and (b) light period. *P value<.05.

significant time×type of pump interaction ($F_{11,187}=2.08$, $P<0.05$). Therefore we conducted one-way ANOVA with type of pump as between-subject factor at each level of time. Significant differences were observed in 17:00–19:00, 19:00–21:00, 21:00–23:00, and 23:00–1:00 ($F_{1,17}=5.44$, 8.78, 9.30, and 7.95, respectively, $Ps<0.05$). The suppressive effects of nicotine withdrawal on locomotor activity were due to locomotor depression during the late phase of light period and the early phase of dark period.

3.2. Experiment 2: effects of L-DOPA on nicotine withdrawal-induced locomotor depression

Data were analyzed between 2000 and 2100 h (Fig. 4) since locomotor activity was elevated to the greatest extent at that time (see Fig. 3). Two-way ANOVA with the type of pump and drug as between-subject factor showed a main effect of the type of pump ($F_{1,37}=6.16$, $P<0.05$) while it did not show a main effect of drug or an

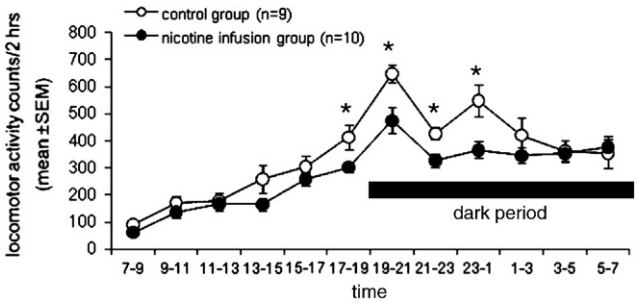

Fig. 3. Effects of nicotine withdrawal after continuous nicotine infusion for 7 days via osmotic pump on 24 h locomotor activity in home cage. Pumps were removed one day before. *$P<0.05$ compared to nicotine pump group.

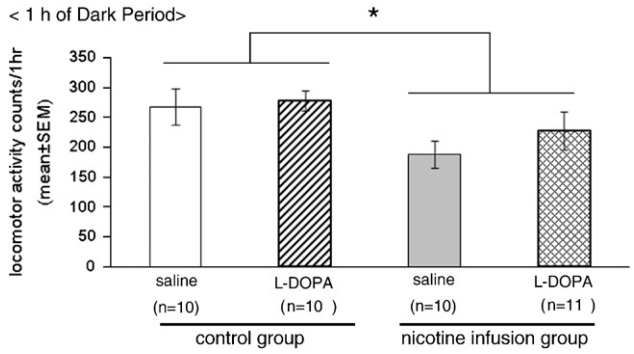

Fig. 4. Effects of L-DOPA on locomotor activity in home cage during 20–21 h (dark period) one day after removal of pump. Benserazide (10 mg/kg, s.c.) or saline was injected 90 min before the start of observation. L-DOPA methyl ester (equivalent to 50 mg/kg L-DOPA, s.c.) or saline was injected 60 min before. *$P<0.05$ (main effect of the type of pump).

interaction. Thus, nicotine withdrawal-induced locomotor depression was observed (Fig. 4), consistent with the results of Experiment 1 (Figs. 2 and 3). However, this dose of L-DOPA did not attenuate locomotor depression in nicotine treated groups or stimulate locomotor activity in control groups (Fig. 4).

3.3. Experiment 3: effects of acute L-DOPA administration on nicotine withdrawal signs

One-way ANOVA indicated a significant main effect of treatment conditions on overall withdrawal signs ($F_{2,25}=9.97$, $P<0.01$, see Fig. 5A). Post hoc comparisons showed that abstinence from 7-day nicotine infusion significantly increased withdrawal signs ($P<0.05$, Fig. 5A), and that 50 mg/kg L-DOPA significantly mitigated these withdrawal signs ($P<0.05$, Fig. 5A).

When the withdrawal signs were divided into each individual behavior, one-way ANOVA indicated a significant main effect of treatment conditions on teeth-chattering/chews ($F_{2,25}=6.08$, $P<0.01$, see Fig. 5B) and shakes ($F_{2,25}=21.37$, $P<0.01$, Fig. 5B), but not other signs. Post hoc comparisons showed that abstinence from 7-day nicotine infusion significantly increased teeth-chattering/chews and shakes ($Ps<0.05$, Fig. 5B), and that 50 mg/kg L-DOPA reversed them ($Ps<0.05$, Fig. 5B).

3.4. Experiment 4: effects of L-DOPA on nicotine withdrawal-induced depressive-like behaviors in the forced swimming test

Two-way ANOVA with the type of pump and drug as between-subject factors indicated a significant main effect for type of pump ($F_{1,16}=7.4$, $P=0.02$), significant trend for acute injection ($F_{1,16}=3.5$, $P=0.08$) and a significant interaction ($F_{1,16}=8.1$, $P=0.01$). Post hoc analyses demonstrated that 48 h withdrawal from nicotine infusion increased immobility ($P<0.01$) and decreased swimming ($P<0.01$) in the forced swim test. L-DOPA attenuated nicotine withdrawal-induced increases in immobility such that immobility levels in the L-DOPA-treated rats were not significantly different from control ($P>0.05$) (see Fig. 6).

4. Discussion

In the present study, withdrawal from 7 days of continuous nicotine infusion induced locomotor depression (Figs. 2 and 3) consistent with previous studies (Gäddnäs et al., 2000; Catania et al., 2003; Hamilton et al., 2009, 2010). Hamilton et al. (2010) reported that nicotine dependent male and female Sprague Dawley adolescent rats had significantly more withdrawal signs on days 1 and 2 than Long Evans rats. Female Long Evans rats showed no withdrawal signs after cessation

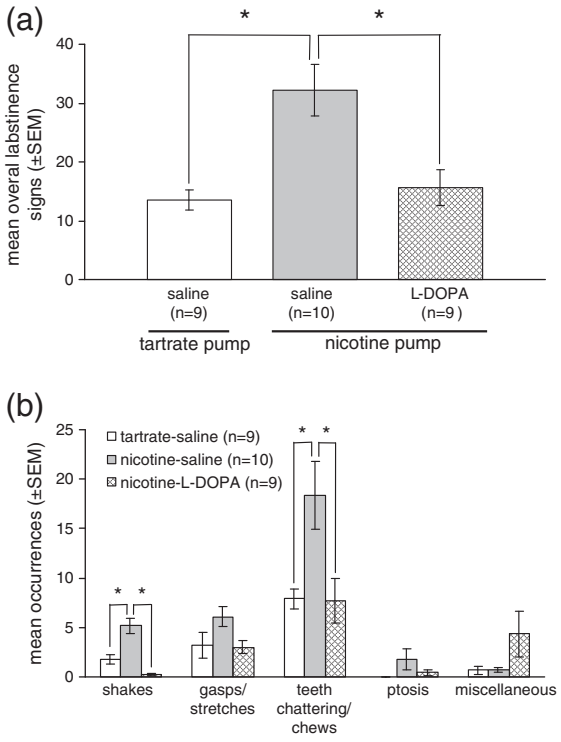

Fig. 5. Effects of L-DOPA on (a) overall somatic signs and (b) each category of nicotine withdrawal. Approximately 22 h after removal of the osmotic pump, behavioral signs were counted. Drugs were injected as described in Methods and Fig. 4. *$P<0.05$.

of nicotine. Their study results in Sprague Dawley rats are consistent with our nicotine withdrawal signs in male Sprague Dawley rats. It adds age, gender, and strain factors when studying nicotine withdrawal signs and supports our use of a similar strain of male rats on days 1 and 2 post-abstinence. However, L-DOPA administration did not significantly mitigate nicotine withdrawal-induced locomotor depression (Fig. 4). Somatic signs of withdrawal were increased in nicotine-infused rats compared to the controls (Fig. 5), consistent with previous studies (Malin et al., 1992; Rylkova et al., 2008). L-DOPA administration alleviated these withdrawal signs (Fig. 5). In the forced swimming test, increased immobility and decreased swimming behaviors were observed approximately 48 h after removal of nicotine pumps (Fig. 6), indicating that nicotine withdrawal induced depressive-like behaviors in rats. These effects were reversed by L-DOPA administration (Fig. 6).

Considering that locomotor depression was found only during the late phase of light period and the early phase of dark period, it is unlikely that locomotor depression was due to motor deficit. Moreover, handling and s.c. saline injection to nicotine-treated rats stimulated locomotor activity for 40 min after the injection to the same degree as tartrate-treated rats (data not shown). This also indicates that locomotor depression observed in this study was not due to a motor deficit.

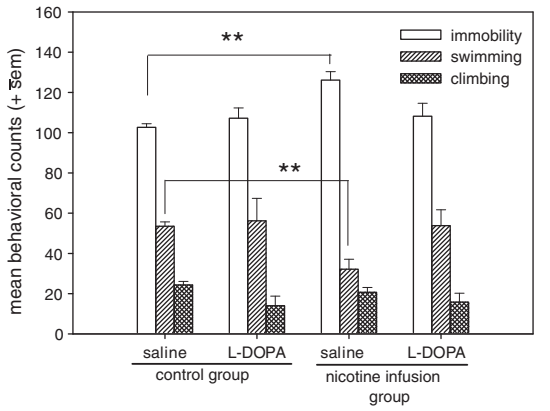

Fig. 6. Effects of L-DOPA on depressive-like state in the forced swimming test two days after removal of pump. Drugs were injected as described in Methods and Fig. 4. All tests were conducted during light period. *P* value *<.05, **<.01.

Although it has been suggested that locomotor depression partially reflects fatigue, decreased arousal, and depressed mood (Paulson et al., 1991; Davis et al., 2003; Takayasu et al., 2006), locomotor depression observed in the present study might reflect the disturbance of circadian rhythm because locomotor depression was found only at a specific time. It should also be noted that locomotor depression was significantly observed in the early phase of dark period when rats are most active. This may be consistent with reports from abstinent smokers experiencing daytime sleepiness (Colrain et al., 2004).

This time-dependent locomotor depression might reconcile prior contradictory studies. Some studies have failed to detect nicotine withdrawal-induced locomotor depression (Helton et al., 1993; Faraday et al., 2001). It could be because circadian rhythm of locomotor activity in rodents (Paulson et al., 1991) and time-dependent effects of nicotine withdrawal have not been considered in these studies.

Although the relationship between decreased DA levels in the brain and nicotine withdrawal has been reported (Takahashi et al., 1998; Hildebrand et al., 1998; Rada et al., 2001; Rahman et al., 2004; Domino and Tsukada, 2009), 50 mg/kg of L-DOPA administration did not significantly mitigate nicotine withdrawal-induced locomotor depression. However it is unlikely that the dose of L-DOPA used in the present study was too small because 50 mg/kg of L-DOPA administration significantly enhanced DA release in the rat striatum lesioned by 6-hydroxydopamine (Kannari et al., 2006). It should also be noted that 50 mg/kg of L-DOPA administration did not stimulate DA release in the striatum of intact rats (Wachtel and Abercrombie, 1994). Moreover, as shown in Figs. 5 and 6, the same dose of L-DOPA significantly attenuated other behaviors induced by nicotine withdrawal. Although higher doses of L-DOPA might reverse locomotor depression considering that higher doses increased locomotor activity even in intact rats (Schlosberg and Harvey, 1979), thus the stimulating effect might be observed independent of whether nicotine infusion was infused or not.

Somatic signs induced by nicotine withdrawal were almost completely reversed by L-DOPA administration (Fig. 5). These results suggest that L-DOPA administration could alleviate some signs of physical distress, discomfort, or irritability during smoking cessation. Although irritability is the most frequently reported symptoms during smoking cessation, alleviating these symptoms might not be enough to help smokers quit smoking because these symptoms are relatively weak predictors of relapse (Hughes, 2007).

Nicotine withdrawal increased immobility and decreased swimming in the forced swimming test (Fig. 6), consistent with a previous report using mice (Mannucci et al., 2006). It has been suggested that increases in immobility are consistent with pro-depressant-like behaviors considering a similar behavioral profile is observed with pharmacological treatments that induce depression in humans (e.g., reserpine, withdrawal from chronic amphetamine (Cryan et al., 2003) and exposure to chronic stress (Kompagne et al., 2008)). Therefore, these results indicate that nicotine withdrawal might induce depressive-like behaviors in rats. Given that clear locomotor depression was observed during the early phase of dark period and that the forced swimming test was conducted during light period, it is unlikely that increased immobility and decreased swimming in the forced swimming test simply reflected decreased spontaneous locomotor activity. Moreover, 50 mg/kg of L-DOPA administration significantly reversed the nicotine withdrawal-induced increase in immobility and decrease in swimming but did not alter behaviors in tartrate-treated rats (Fig. 6), indicating that L-DOPA administration could reverse

nicotine withdrawal-induced depressive-like behaviors. Because L-DOPA administration did not affect immobility or swimming in tartrate-treated rats (Fig. 6) and it did not significantly increase locomotor activity in home cages (Fig. 4), it is likely that L-DOPA administration attenuated depressive-like behaviors, and not merely stimulated locomotor activity.

To the best of our knowledge, this is the first study using an animal model to demonstrate that L-DOPA administration could alleviate nicotine withdrawal signs. Several studies have shown that abrupt nicotine cessation decreases DA levels in the brain (Takahashi et al., 1998; Geracioti et al., 1999; Rahman et al., 2004; Domino and Tsukada, 2009). Our results suggest that the decreased DA levels in the brain might be a cause of some nicotine withdrawal symptoms.

To alleviate the depressive state induced by nicotine withdrawal would help smokers who want to quit smoking because depressed mood predicts relapse (for a review, see Hughes, 2007). Our results suggest that L-DOPA could be a useful drug to aid smoking cessation. Quik et al. (2003) demonstrated in monkeys that repeated administration of L-DOPA decreased nAChR expression in the striatum where chronic nicotine treatment upregulates nAChR expression (Marks et al., 1992; Collins et al., 1994). It should be noted that a previous study using adequate doses of carbidopa/levodopa (25 mg/100 mg) for Parkinson's disease reported that carbidopa/levodopa significantly reduced mean the number of cigarettes per day (28.6→7.2 cigarettes per day) and CO levels from baseline (28.0→13.0 ppm; Hurt et al., 2000). Because carbidopa/levodopa treatment did not increase smoking abstinence rates despite the decreased number of cigarettes per day (Hurt et al., 2000), traditional usage of L-DOPA might not be better than existing treatments such as nicotine patch, bupropion, and varenicline. However, it deserves further research. It might be worthwhile to consider smaller doses and/or combination of L-DOPA and other drugs. For example, lower doses of L-DOPA (0.3–1.5 mg/kg/day) have therapeutic effects on laryngeal dystonia in xeroderma pigmentosum patients (Miyata et al., 2010). Nomura and Segawa (2003) showed that lower doses of L-DOPA (0.5–1 mg/kg/day) improved Tourette's syndrome. A low dose of L-DOPA (3 mg/kg) enhanced antipsychotic-like effects of raclopride in rats (Eltayb et al., 2005). The combination of L-DOPA (8 mg/kg) and rimonabant improved motor functions in rats with unilateral 6-hydroxydopamine lesions (Kelsey et al., 2009). Rimonabant slightly but significantly facilitates smoking cessation (Cahill and Ussher, 2007). Thus, it is likely that L-DOPA administration could be a useful stop smoking aid by combining it with other drugs and/or by devising the doses and frequency of administration.

Acknowledgments

This work was supported in part by the University of Michigan, Psychopharmacology Research Fund 361024. Dr. Yu Ohmura was a Seevers Michigan Fellow during the period of this research.

References

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington DC: American Psychiatric Association; 2000.
Arai A, Tomiyama M, Kannari K, Kimura T, Suzuki C, Watanabe M, et al. Reuptake of L-DOPA-derived extracellular DA in the striatum of a rodent model of Parkinson's disease via norepinephrine transporter. Synapse 2008;62(8):632–5.
Benowitz NL, Kuyt F, Jacob III P. Circadian blood nicotine concentrations during cigarette smoking. Clin Pharmacol Ther 1982;32:758–64.
Cahill K, Ussher M. Cannabinoid type 1 receptor antagonists (rimonabant) for smoking cessation. Cochrane Database Syst Rev 2007;4:CD005353 [Oct 17].
Catania MA, Firenzuoli F, Crupi A, Mannucci C, Caputi AP, Calapai G. Hypericum perforatum attenuates nicotine withdrawal signs in mice. Psychopharmacol 2003;169:186–9.
Chen R, Zhang M, Park S, Gnegy ME. C57BL/6J mice show greater amphetamine-induced locomotor activation and dopamine efflux in the striatum than 129S2/SvHsd mice. Pharmacol Biochem Behav 2007;87:158–63.
Collins AC, Luo Y, Selvaag S, Marks MJ. Sensitivity to nicotine and brain nicotinic receptors are altered by chronic nicotine and mecamylamine infusion. J Pharmacol Exp Ther 1994;271:125–33.
Colrain IM, Trinder J, Swan GE. The impact of smoking cessation on objective and subjective markers of sleep: review, synthesis, and recommendations. Nicotine Tob Res 2004;6:913–25.
Cryan JF, Hoyer D, Markou A. Withdrawal from chronic amphetamine induces depressive-like behavioral effects in rodents. Biol Psychiat 2003;34:49–58.
Davis JM, Zhao Z, Stock HS, Mehl KA, Buggy J, Hand GA. Central nervous system effects of caffeine and adenosine on fatigue. Am J Physiol Regul Integr Comp Physiol 2003;284:R399–404.
Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacol 1995;121:66–72.
Domino EF, Tsukada H. Nicotine sensitization of monkey striatal dopamine release. Eur J Pharmacol 2009;607:91–5.
Eltayb A, Wadenberg ML, Svensson TH. Enhanced cortical dopamine output and antipsychotic-like effect of raclopride with adjunctive low-dose L-dopa. Biol Psychiat 2005;58:337–43.
Faraday MM, Elliott BM, Grunberg NE. Adult vs. adolescent rats differ in biobehavioral responses to chronic nicotine administration. Pharmacol Biochem Behav 2001;70:475–89.
Gäddnäs H, Pietilä K, Ahtee L. Effects of chronic oral nicotine treatment and its withdrawal on locomotor activity and brain monoamines in mice. Behav Brain Res 2000;113:65–72.
Geracioti TD, West AS, Baker DG, Hill KK, Ekhator NN, Wortman MD, et al. Low CSF concentration of dopamine metabolite in tobacco smokers. Am J Psychiat 1999;156:130–2.
Hamilton KR, Berger SS, Perry ME, Grunberg NE. Behavioral effects of nicotine withdrawal in adult male and female rats. Pharmacol Biochem Behav 2009;92:51–9.
Hamilton KR, Perry E, Berger SS, Grunberg NE. Behavioral effects of nicotine withdrawal differ by genetic strain in male and female adolescent rats. Nicotine Tob Res 2010;12(12):1236–45.
Helton DR, Modlin DL, Tizzano JP, Rasmussen K. Nicotine withdrawal: a behavioral assessment using schedule controlled responding, locomotor activity, and sensorimotor reactivity. Psychopharmacol 1993;113:205–10.
Hildebrand BE, Nomikos GG, Hertel P, Schilström B, Svensson TH. Reduced dopamine output in the nucleus accumbens but not in the medial prefrontal cortex in rats displaying a mecamylamine-precipitated nicotine withdrawal syndrome. Brain Res 1998;779:214–25.
Hughes JR. Effects of abstinence from tobacco: etiology, animal models, epidemiology, and significance: a subjective review. Nicotine Tob Res 2007;9(3):329–39.
Hughes JR. Smoking and suicide: a brief overview. Drug Alcohol Depend 2008;98:169–718.
Hurt RD, Ahlskog E, Croghan GA, Offord KP, Wolter TD, Croghan IT, et al. Carbidopa/levodopa for smoking cessation: a pilot study with negative results. Nicotine Tob Res 2000;2:71–8.
Jutkiewicz EM, Torregrossa MM, Sobczyk-Kojiro K, Mosberg HI, Folk JE, Rice KC, et al. Behavioral and neurobiological effects of the enkephalinase inhibitor RB101 relative to its antidepressant effects. Eur J Pharmacol 2006;531(1–3):151–9.
Jutkiewicz EM, Baladi MG, Folk JE, Rice KC, Woods JH. The delta-opioid receptor agonist SNC80 [(+)-4-[alpha(R)-alpha-[(2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl]-(3-methoxybenzyl)-N, N-diethylbenzamide] synergistically enhances the locomotor-activating effects of some psychomotor stimulants, but not direct dopamine agonists, in rats. J Pharmacol Exp Ther 2008;324:714–24.
Kannari K, Shen H, Arai A, Tomiyama M, Baba M. Reuptake of L-DOPA-derived extracellular dopamine in the striatum with dopaminergic denervation via serotonin transporters. Neurosci Lett 2006;402(1–2):62–5.
Kelsey JE, Harris O, Cassin J. The CB(1) antagonist rimonabant is adjunctively therapeutic as well as monotherapeutic in an animal model of Parkinson's disease. Behav Brain Res 2009;203:304–7.
Kompagne H, Bárdos G, Szénási G, Gacsályi I, Hársing LG, Lévay G. Chronic mild stress generates clear depressive but ambiguous anxiety-like behaviour in rats. World Health Organization 2008.
LeSage MG, Keyler DE, Shoeman D, Raphael D, Collins G, Pentel PR. Continuous nicotine infusion reduces nicotine self-administration in rats with 23-h/day access to nicotine. Pharmacol Biochem Behav 2002;72:279–89.
Malin DH, Goyarzu P. Rodent models of nicotine withdrawal syndrome. Handb Exp Pharmacol 2009;192:401–34.
Malin DH, Lake JR, Newlin-Maultsby P, Roberts LK, Lanier JG, Carter VA, et al. Rodent model of nicotine abstinence syndrome. Pharmacol Biochem Behav 1992;43:779–84.
Malin DH, Lake JR, Smith TD, Khambati HN, Meyers-Paal RL, Montellano AL, et al. Bupropion attenuates nicotine abstinence syndrome in the rat. Psychopharmacol 2006;184:494–503.
Mannucci C, Tedesco M, Bellomo M, Caputi AP, Calapai G. Long-term effects of nicotine on the forced swimming test in mice: an experimental model for the study of depression caused by smoke. Neurochem Int 2006;49:481–6.
Marks MJ, Pauly JR, Gross SD, Deneris ES, Hermans-Borgmeyer I, Heinemann SF, et al. Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. J Neurosci 1992;12:2765–84.
Miyata R, Sasaki T, Hayashi M, Araki S, Shimohira M, Kohyama J. Low-dose levodopa is effective for laryngeal dystonia in xeroderma pigmentosum group A. Brain Dev 2010;32(8):685–7.
Nomura Y, Segawa M. Neurology of Tourette's syndrome (TS) TS as a developmental dopamine disorder: a hypothesis. Brain Dev 2003;25(Suppl. 1):S37–42.
Paulson PE, Camp DM, Robinson TE. Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine

concentrations during amphetamine withdrawal in rats. Psychopharmacol 1991;103:480–92.
Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266(5604):730–2.
Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 1978;47:379–91.
Quik M, Bordia T, Okihara M, Fan H, Marks MJ, McIntosh JM, et al. L-DOPA treatment modulates nicotinic receptors in monkey striatum. Mol Pharmacol 2003;64: 619–28.
Rada P, Jensen K, Hoebel BG. Effects of nicotine and mecamylamine-induced withdrawal on extracellular dopamine and acetylcholine in the rat nucleus accumbens. Psychopharmacol 2001;157:105–10.
Rahman S, Zhang J, Engleman EA, Corrigall WA. Neuroadaptive changes in the mesoaccumbens dopamine system after chronic nicotine self-administration: a microdialysis study. Neurosci 2004;129:415–24.
Rylkova D, Boissoneault J, Isaac S, Prado M, Shah HP, Bruijnzeel AW. Effects of NPY and the specific Y1 receptor agonist [D-His(26)]-NPY on the deficit in brain reward function and somatic signs associated with nicotine withdrawal in rats. Neuropeptides 2008;42:215–27.
Schlosberg AJ, Harvey JA. Effects of L-dopa and L-5-hydroxytryptophan on locomotor activity of the rat after selective or combined destruction of central catecholamine and serotonin neurons. J Pharmacol Exp Ther 1979;211:296–304.
Takahashi H, Takada Y, Nagai N, Urano T, Takada A. Effects of nicotine and footshock stress on dopamine release in the striatum and nucleus accumbens. Brain Res Bull 1998;45:157–62.
Takayasu S, Sakurai T, Iwasaki S, Teranishi H, Yamanaka A, Williams SC, et al. A neuropeptide ligand of the G protein-coupled receptor GPR103 regulates feeding, behavioral arousal, and blood pressure in mice. Proc Natl Acad Sci U S A 2006;103:7438–43.
Wachtel SR, Abercrombie ED. L-3,4-dihydroxyphenylalanine-induced dopamine release in the striatum of intact and 6-hydroxydopamine-treated rats: differential effects of monoamine oxidase A and B inhibitors. J Neurochem 1994;63:108–17.
World Heatlh Organization. The ICD-10 classification of mental and behavioural disorders. Diagnostic criteria for research. Geneva, Switzerland: World Health Organization; 1993.