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Dopamine $D_{1/5}$ and $D_{2/3}$ agonists differentially attenuate somatic signs of nicotine withdrawal in rats

Yu Ohmura [1], Emily M. Jutkiewicz, Andrew Zhang, Edward F. Domino *

Department of Pharmacology, University of Michigan, Ann Arbor, MI, 48109–5632, USA

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ABSTRACT

Abrupt tobacco/nicotine cessation after chronic use causes various withdrawal symptoms/signs. There is evidence that dysfunction of brain dopaminergic system might be responsible for some nicotine withdrawal symptoms. The hypothesis for the present study was that different dopaminergic agonists would relieve different nicotine withdrawal signs. Adult male Sprague–Dawley rats were used. (−)-Nicotine bitartrate (9 mg/kg/day, salt content) or equimolar sodium tartrate was infused into each rat via a subcutaneous (s.c.) osmotic minipump for 7 days. To assess nicotine withdrawal signs, several somatic abstinence signs including teeth-chattering/chews, stretches/gasps, ptosis, shakes, and yawns were counted one day after removal of pumps. These signs were attenuated by the s.c. injection of 0.4 mg/kg nicotine bitartrate. Both a dopamine $D_{1/5}$ agonist (SKF81297) and a $D_{2/3}$ agonist (pramipexole) relieved abstinence signs dose-dependently but differentially. SKF81297 (0.32 mg/kg, s.c.) reduced teeth-chattering/chews but not shakes. Pramipexole (1 mg/kg, s.c.) decreased both teeth-chattering/chews and shakes. A low dose of pramipexole (0.1 mg/kg, s.c.) significantly increased yawns, consistent with previous studies that the stimulation of D_3 receptors induces yawning. These results indicate that a D_2-selective agonist should be considered a candidate to relieve nicotine withdrawal symptoms.

1. Background

A role of dopamine (DA) in the reinforcing effects of nicotine/tobacco smoking is well established. On the other hand, the precise role of DA during nicotine/tobacco abstinence is less well known. Fung et al. (1996) found a significant reduction of DA content in nucleus accumbens in rats after 24 h withdrawal from chronic nicotine administration. Duchemin et al. (2009) showed that basal DA release in mice striatal slices was decreased 12 and 24 h after chronic nicotine discontinuation. Some chronic nicotine rat studies using *in vivo* microdialysis have demonstrated that abrupt nicotine cessation or mecamylamine decreases basal levels of DA release in the nucleus accumbens (Hildebrand et al., 1998; Takahashi et al., 1998; Rada et al., 2001; Rahman et al., 2004). A monkey study showed that basal levels of DA release in the dorsal striatum also decreased after overnight abstinence from daily nicotine (Domino and Tsukada, 2009). A human study demonstrated that smokers abstinent from tobacco for 11 to 17 h have only 54% of the cerebrospinal fluid concentration of the DA metabolic homovanillic acid (HVA) of nonsmokers (Geracioti et al., 1999). Dagher et al. (2001) reported reduced D_1 receptor binding in the ventral striatum of cigarette smokers.

Recently, we found that L-DOPA reduces signs of nicotine withdrawal in rats (Ohmura et al., 2011). The next question to answer was whether D_1 and D_2 receptor families affect different signs of nicotine abstinence. Therefore, we examined whether a dopamine $D_{1/5}$ agonist (SKF81297), and a DA $D_{2/3}$ agonist (pramipexole) are effective in relieving nicotine withdrawal signs. If decreased DA levels after nicotine abstinence were a cause of nicotine withdrawal signs, selective dopaminergic agonists would be differentially effective. This manuscript describes the results obtained.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Harlan, Indianapolis, IN), weighing 260–320 g at 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.

* Corresponding author. Tel.: +1 734 764 9115; fax: +1 734 763 4450.
E-mail address: efdabcde@umich.edu (E.F. Domino).
[1] Present address: Department of Pharmacology, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060–8638, Japan.

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2.2. Drugs

(−)-Nicotine bitartrate salt, sodium tartrate and SKF81297 were purchased from Sigma-Aldrich, St. Louis, MO, USA. Pramipexole was obtained from Boehringer Ingelheim Co. SKF81297 (R-(+)-6-Chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide) was dissolved in distilled water and other drugs were dissolved in saline. The pH of the nicotine 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 doses are expressed as salt. They 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 were surgically 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). Moreover, the blood concentrations resulting from this dose in rats are almost the 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. Spontaneous somatic signs of nicotine withdrawal

Behavioral observations were performed from 9 to 12 a.m. in a clear plastic observation chamber (48×23×20 cm). Rat behaviors were observed 20–24 h after pump removal, based upon previous studies (Malin et al., 1992; Rylkova et al., 2008; Paterson et al., 2008). Rat behaviors were counted for 30 min by observers blind to the experimental conditions. Teeth-chattering/chews with empty mouth, stretches/gasps, shakes, ptosis, and miscellaneous other less frequent signs (*e.g.* diarrhea and yawns) were counted. Although the counting method is based on previous studies (Malin et al., 1992, 2006), "stretches" instead of "writhes" were noted because writhes were not observed. Tremors were not recorded because none was observed. To accurately assess teeth-chattering/chews with an empty mouth, bedding was not used in the observation chamber. Ptosis was not counted when a rat took a resting position to avoid confounding ptosis with sleeping. Ptosis was counted only once per min.

2.4. Experiment 1: effects of acute nicotine administration on nicotine withdrawal signs

Nicotine or equimolar sodium tartrate was infused into each rat via an s.c. osmotic minipump for 7 days. One day after removal of the pump, behavioral signs were counted. Rats received an s.c. injection of 0.4 mg/kg nicotine or saline 3 min prior to behavioral observation. Malin et al. (1992) showed that this dose of nicotine alleviated nicotine withdrawal signs.

2.5. Experiment 2: effects of acute $D_{1/5}$ agonist administration on nicotine withdrawal signs

Nicotine was infused into each rat via an s.c. osmotic minipump for 7 days. One day after removal of the pump, behavioral signs were counted. Rats received an s.c. injection of SKF81297 (0.032 mg/kg or 0.32 mg/kg) or sterilized water 10 min prior to behavioral observation.

2.6. Experiment 3: effects of acute $D_{2/3}$ agonist administration on nicotine withdrawal signs

Nicotine was infused into each rat via an s.c. osmotic minipump for 7 days. Rats received s.c. injection of pramipexole (0.1 mg/kg or 1 mg/kg) or saline 10 min prior to behavioral observation.

2.7. Data analysis

The number of nicotine withdrawal signs was analyzed by using one-way analysis of variance (ANOVA). Multiple comparisons with Bonferroni's correction were also conducted following each ANOVA if necessary. There was no significant difference among groups that received 7 day control tartrate infusion and acute saline or sterile water injection in each experiment despite the different time points of the injection (see above). Hence, that data were combined and used as the control groups for statistical analysis to decrease the number of sacrificed animals. 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 acute nicotine administration on nicotine withdrawal signs

One-way ANOVA showed a significant main effect of treatment conditions on overall withdrawal signs ($F(2, 30) = 24.95$, $P<0.01$, see

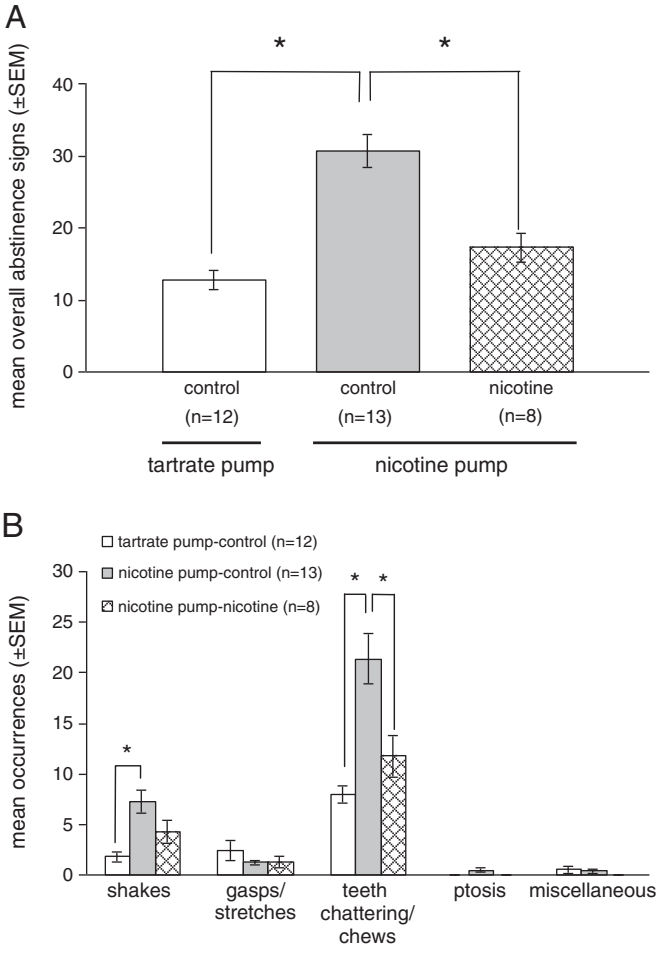

Fig. 1. Effects of nicotine on (A) overall somatic abstinence signs and (B) each category of somatic signs. Behavioral observation was conducted 20–24 h after removal of pump. Nicotine (0.4 mg/kg, s.c.) was injected 3 min before the observation. * $P<0.05$.