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Short communication

Differential discriminative-stimulus effects of cigarette smoke condensate and nicotine in nicotine-discriminating rats

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HIGHLIGHTS

- Cigarette smoke condensate (CSC) fully substitutes for nicotine and produces nicotine-like effects in rats.
- The α4β2, but not the α7, nAChR is involved in the discriminative stimulus effects of CSC and nicotine.
- DHβE pretreatment was less effective in attenuating the discriminative-stimulus effects of CSC compared with nicotine.
- Compared with nicotine alone, CSC had a relatively longer half-life in terms of its discriminative stimulus effects.

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ABSTRACT

Although it is widely accepted that nicotine plays a key role in tobacco dependence, nicotine alone cannot account for all of the pharmacological effects associated with cigarette smoke found in preclinical models. Thus, the present study aimed to determine the differential effects of the interoceptive cues of nicotine alone versus those of cigarette smoke condensate (CSC) in nicotine-trained rats. First, the rats were trained to discriminate nicotine (0.4 mg/kg, subcutaneous [s.c.]) from saline in a two-lever drug discrimination paradigm. Then, to clarify the different neuropharmacological mechanisms underlying the discriminative-stimulus effects in the nicotine and CSC in nicotine-trained rats, either the α4β2 nicotinic acetylcholine receptor (nAChR) antagonist dihydro-β-erythroidine (DHβE; 0.3–1.0 mg/kg, s.c.) or the α7 nAChR antagonist methyllycaconitine citrate (MLA; 5–10 mg/kg, intraperitoneal [i.p.]) was administered prior to the injection of either nicotine or CSC. Separate set of experiments was performed to compare the duration of action of the discriminative-stimulus effects of CSC and nicotine. CSC exhibited a dose-dependent nicotine generalization, and interestingly, 1.0 mg/kg of DHβE antagonized the discriminative effects of nicotine (0.4 mg/kg) but not CSC (0.4 mg/kg nicotine content). However, pretreatment with MLA had no effect. In the time-course study, CSC had a relatively longer half-life in terms of the discriminative-stimulus effects compared with nicotine alone. Taken together, the present findings indicate that CSC has a distinct influence on interoceptive effects relative to nicotine alone and that these differential effects might be mediated, at least in part, by the α4β2, but not the α7, nAChR.

The primary role of nicotine in the maintenance of tobacco use and the development of tobacco dependence is widely accepted, but there are many chemicals other than nicotine present in tobacco and tobacco smoke [1]. Thus, recent studies have focused on the neurobehavioral effects of the non-nicotinic constituents of tobacco and tobacco smoke that might make a critical contribution to tobacco dependence. Furthermore, preclinical studies have suggested that these constituents may play a key role in the abuse potential of cigarettes independent of nicotine [2].

Recent studies have suggested that the minor alkaloids, such as nornicotine, and acetaldehyde that are present in cigarette smoke have reinforcing and/or rewarding effects in and of themselves. For instance, rats self-administered intravenously nornicotine and acetaldehyde [3–5], which indicates that both of these compounds function as positive reinforcers. Additionally, several drug discrimination studies have reported that minor alkaloids produce

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nicotine-like effects and that nornicotine fully substitutes for the interoceptive effects of nicotine in mice [6]. Similarly, high doses of anabasine result in full substitution in rats trained to discriminate nicotine from saline [7]. Based on these findings, it appears that both nornicotine and anabasine share interoceptive effects with nicotine. On the other hand, other behavioral studies have suggested that non-nicotine compounds can alter the reinforcing or interoceptive effects of nicotine. For example, anatabine reduces the rate of nicotine self-administration in non-human primates [8] and nornicotine, anatabine, and anabasine can either enhance or reduce nicotine self-administration and/or discriminative-stimulus effects in rodents [6].

Recently, Harris et al. [9] found that an extract of a smokeless nicotine produced discriminative-stimulus effects similar to those produced by nicotine in nicotine-trained rats. Additionally, Costello et al. [10] found that rats responded to an aqueous cigarette smoke extract derived from a saline extract of tobacco smoke and that this cigarette smoke extract-maintained responding was attenuated by the nicotinic acetylcholine receptors (nAChRs) antagonist mecamylamine and the partial agonist of α4β2 nAChRs and agonist of α7 nAChRs varenicline. These results indicate that nAChRs mediate the reinforcing effects of cigarette smoke extract, at least in part, which is supported by data from a ligand binding study showing that cigarette smoke extract and nicotine have an equal affinity for all tested nAChR subtypes (e.g., α4β2, α3β4, α3β2, α7) [10].

To date, the interoceptive effects of liposoluble cigarette smoke condensate (CSC) remain unclear. Therefore, the primary objective of the present study was to compare discriminative-stimulus effects of CSC with those of nicotine alone. Additionally, the present study examined the neuropharmacological mechanisms underlying the interoceptive effects in the nicotine and CSC in nicotine-trained rats via the α4β2 and α7 nAChRs. For the present study, CSC was prepared from a dimethyl sulfoxide (DMSO) extract of cigarette smoke because it was easier to prepare CSC and administer it to animals compared with cigarette smoke. To the best of our knowledge, this is the first study to investigate the discriminative-stimulus effects of CSC using a drug discrimination paradigm.

The present study utilized male Sprague-Dawley rats (Orient Bio Inc., Seoul, Korea) that weighed between 235 and 275 g. The rats were individually housed in an animal room with controlled temperature (23 ± 3 °C) and humidity (30–70%) and were food-restricted to 13–15 g/day of rat chow (Rodent Diet 5001; PMI feeds Inc., St. Louis, MO, USA) throughout the experiment. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the Korea Institute of Toxicology and met the National Institutes of Health guidelines for the care and use of laboratory animals.

(−)-Nicotine tartrate (Fisher Scientific, Pittsburgh, PA, USA), dihydro-β-erythroidine (DHβE; Tocris, Bristol, UK), and methyllycaconitine citrate (MLA; Tocris) were dissolved in saline and adjusted to a pH of 7.2–7.4 with NaOH. The CSC was prepared by the Research Center for Inhalation Toxicology of the Korea Institute of Toxicology (Jeongeup, Korea) using a procedure that was similar to that of a previously published study [11]. The nicotine, nornicotine, anabasine, and anatabine contents of the CSC samples were analyzed and the contents of each minor alkaloid are shown in Table 1.

Table 1
Contents of nicotine and minor alkaloids in cigarette smoke.

Analysis of nicotine and minor tobacco alkaloids (μg/mL)			
Nicotine	(R,S)-Nornicotine	(R,S)-Anabasine	(R,S)-Anatabine
2734.35	12.68	66.04	5.08

The drug discrimination experiment was conducted in operant conditioning chambers (Coulbourn Instruments, Whitehall, PA, USA). To facilitate lever-pressing behavior, all rats were initially trained to press a lever for 45 mg food pellets (Bio-serv, Frenchtown, NJ, USA) on a fixed-ratio (FR) 1 schedule of reinforcement during daily sessions lasting 1 h. The schedule of reinforcement was gradually increased to FR10 and the lever press training continued under this schedule until the criterion level of performance (60 food pellets for 3 consecutive days) was achieved. Following the lever press training, the rats were trained to discriminate nicotine (0.4 mg/kg, subcutaneous [s.c.]) from saline (1 ml/kg, s.c.).

Immediately after the administration of either nicotine or saline, the rats were placed into the operant chamber with all lights terminated and the levers retracted for 10 min. After the 10-min delay, the house light was illuminated and both levers were extended into the operant chamber to signal the beginning of the 15-min session. The completion of 10 responses on the injection-appropriate (correct) lever resulted in the delivery of a food pellet followed by a 15-s time-out (TO) period during which the house light was turned off and the lever responses had no programmed consequences. Responses on the inappropriate (incorrect) lever reset the FR response requirement for food delivery but otherwise had no programmed consequences.

Each training session was terminated after 15 min or after the rat received 20 food pellets, whichever occurred first. During this phase of training, the nicotine and saline training days varied according to a semi-random alternation schedule and training continued until the following criteria were satisfied for 8 of 10 consecutive sessions: (1) the percentage of correct responses during the entire session was >80%, and (2) the number of responses on the inappropriate lever prior to the first trial was less than 5 lever responses. Test sessions were conducted once or twice per week with intervening training sessions. These test sessions occurred only if the rats met all of the criteria listed above for at least 2 prior training sessions. The test sessions were identical to the training sessions except that the completion of 10 responses on either lever resulted in the delivery of a food pellet.

During the generalization (substitution) tests, either nicotine (0.0178, 0.056, 0.178, or 0.56 mg/kg, s.c.) or CSC (0.0056, 0.0178, 0.056, or 0.178 mg/kg nicotine content, s.c.) were administered 10 min prior to each test session. In a different set of experiments, either DHβE (0.3, 1.0, and 3.0 mg/kg, s.c.) or MLA (5 and 10 mg/kg, intraperitoneal [i.p.]) was administered either 5 or 15 min prior to the administration of nicotine (0.4 mg/kg, s.c.) or CSC (0.4 mg/kg nicotine content, s.c.), respectively. The dose ranges for DHβE and MLA were chosen based on previous studies [12,13]. Finally, in the time-course study, either nicotine (0.56 mg/kg, s.c.) or CSC (0.178 mg/kg nicotine content, s.c.) was administered at 10, 40, or 70 min prior to the onset of a test session.

All statistical analyses were performed using Graphpad Prism software 5.0 (GraphPad software Inc., La Jolla, CA, USA). The drug discrimination results were expressed as the percentage of total lever presses made on the nicotine-appropriate lever and the response rate was represented as the number of presses on both levers per second during the test session. To compare the potency of CSC versus that of nicotine, the ED_{50} values were analyzed using a nonlinear regression analysis and the findings were considered to be significantly different when the 95% confidence intervals (CI) of the ED_{50} values did not overlap. All data except the ED_{50} values were analyzed using a two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests.

In the present study, nicotine (0.0178–0.56 mg/kg) produced a dose-dependent increase in nicotine-appropriate responding, and the calculated ED_{50} value (0.12 mg/kg, 95% CI: 0.098–0.147) was similar to that of previous studies using the same training dose (0.4 mg/kg) of nicotine [9]. Although the rats underwent several