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Research report

Estradiol promotes the rewarding effects of nicotine in female rats

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HIGHLIGHTS

- Greater rewarding effects of nicotine in female versus male rats.
- Greater rewarding effects of nicotine in intact female versus OVX female rats.
- Greater nicotine reward in E2-supplemented OVX female rats versus controls.

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ABSTRACT

It is presently unclear whether ovarian hormones, such as estradiol (E2), promote the rewarding effects of nicotine in females. Thus, we compared extended access to nicotine intravenous self-administration (IVSA) in intact male, intact female, and OVX female rats (Study 1) as well as OVX females that received vehicle or E2 supplementation (Study 2). The E2 supplementation procedure involved a 4-day injection regimen involving 2 days of vehicle and 2 days of E2 administration. Two doses of E2 (25 or 250 μg) were assessed in separate groups of OVX females in order to examine the dose-dependent effects of this hormone on the rewarding effects of nicotine. The rats were given 23-hour access to nicotine IVSA using an escalating dose regimen (0.015, 0.03, and 0.06 mg/kg/0.1 mL). Each dose was self-administered for 4 days with 3 intervening days of nicotine abstinence. The results revealed that intact females displayed higher levels of nicotine intake as compared to males. Also, intact females displayed higher levels of nicotine intake versus OVX females. Lastly, our results revealed that OVX rats that received E2 supplementation displayed a dose-dependent increase in nicotine intake as compared to OVX rats that received vehicle. Together, our results suggest that the rewarding effects of nicotine are enhanced in female rats via the presence of the ovarian hormone, E2.

1. Introduction

Epidemiological reports suggest that women are more susceptible to tobacco use than men. This is based on previous studies showing that women consume more tobacco products and they have a harder time quitting smoking than men [1,2]. Nicotine has been identified as the main compound that motivates tobacco use, and clinical reports indicate that women rate nicotine as more pleasurable than men [3]. In addition, women that use tobacco regularly report higher positive subjective effects following presentation of smoking-related stimuli as compared to men [1,4]. Clinical reports have also shown that nicotine replacement therapy is a less effective smoking cessation therapy in women as compared to men [5]. Despite the evidence suggesting that nicotine is a strong reinforcing agent in women, very little is known about the underlying mechanisms that promote tobacco use in females.

Reviews of pre-clinical studies have provided converging lines of evidence suggesting that the rewarding effects of nicotine are greater in adult female versus male rodents [1,6–8]. One of the first studies in this area demonstrated that female adult rats display faster acquisition rates of low doses of nicotine IVSA than males [9]. The latter study also revealed that adult females reach a higher break point for nicotine infusions on a PR schedule of reinforcement than males. Subsequent studies from the same laboratory also showed that female rats display 2-fold higher levels of nicotine IVSA as compared to males in the presence of a visual stimulus that signals a nicotine infusion [10]. Female rats also display higher levels of nicotine intake in procedures involving oral (11) and IVSA procedures under both short [12] and long [13] access conditions. Female rodents also display conditioned place preference (CPP) produced by nicotine that is more robust across a wider range of doses as compared to male rats [14] and mice [15]. However, we acknowl-

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edge another report showing that nicotine-induced CPP is larger in male versus female rats [16].

Clinical reports have suggested that ovarian hormones, such as estrogen, promote tobacco use in women. Indeed, high levels of estrogen are positively correlated with a greater sensitivity to the rewarding effects of nicotine in women [17]. Consistent with the latter finding, women display higher levels of nicotine craving and relapse rates during the follicular phase of the menstrual cycle when estrogen levels are highest [18]. Although these studies suggest that estrogen promotes tobacco use in women, the role of specific ovarian hormones in promoting nicotine reward has not been examined in pre-clinical animal studies.

The goal of the present study was to examine the role of the ovarian hormone, E2 in promoting the rewarding effects of nicotine in female rats. Nicotine IVSA was compared in intact male, intact female, and OVX female rats (Study 1) as well as OVX female rats that received vehicle or E2 supplementation (Study 2). Two doses of E2 were included to examine the dose-dependent effects of this hormone on nicotine IVSA. We used an extended-access model of IVSA whereby rats were given 23-hour access to increasing doses of nicotine separated by 3-day periods of drug abstinence. We hypothesized that E2 plays a primary role in modulating nicotine reward. This is based on previous studies showing that OVX females display a reduction in cocaine IVSA that is normalized to intact female levels following E2 supplementation [19]. Also, another report revealed that OVX rats that received E2 supplementation display greater motivation to obtain cocaine relative to OVX rats that received vehicle [20].

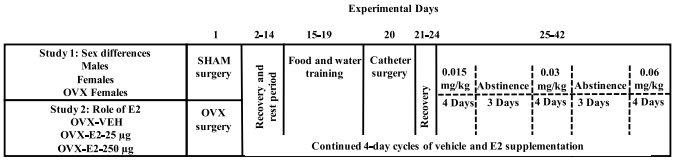
2. Materials and methods

2.1. Subjects

Male and female Wistar rats were obtained from an out-bred stock of animals (Harlan, Inc., Indianapolis, IN). On post-natal day (PND) 21, the rat pups were weaned and paired with a same-sex littermate until PND 60, at which point they were individually housed for the remainder of the study. The rats were housed in a humidity- and temperature-controlled (22 °C) vivarium on a 12-hour light/dark cycle (lights off at 6:00 am and on at 6:00 pm). Prior to beginning the experiment, the rats were handled for 5 days and they had ad libitum access to food and water. The UTEP Institutional Animal Care and Use Committee approved our procedures prior to experimentation.

2.2. Overall experimental design

This project consisted of 2 studies with different experimental goals (see inset below). Study 1 compared nicotine intake in intact male (n = 10), intact female (n = 14), and OVX female (n = 9) rats. Both male and female rats received a sham surgery at PND 60 as a control procedure for the OVX surgeries. Study 2 examined the role of E2 in modulating the rewarding effects of nicotine in OVX females that received vehicle (peanut oil; OVX-VEH; n = 8) or an E2 supplementation procedure involving 2 different doses in separate groups of animals (E2-25 μg; n = 8 and E2-250 μg; n = 10).



2.3. Operant procedures

The present study utilized extended access procedures that are established in our laboratory [21,22]. IVSA was assessed in standard operant chambers (MED associates, St. Albans, VT) that were kept on the same light cycle as the holding room. Operant sessions were conducted using 2 retractable levers (active and inactive) that extended 2.5 cm into the chamber. A 28 V white cue light was located above the active lever and a dummy light was above the inactive lever. A pellet dispenser mounted between the inactive and active lever allowed the rats to nose-poke for food. A separate hole located in the back of the chamber allowed the rats to nose-poke for water that was released into an adjacent metal dipper cup. The exit port of the catheter fitting was connected to a polyethylene tubing within a metal spring that was connected to a liquid swivel above the operant chamber.

During the first 4 days of operant procedures, the rats received food and water training. The rats were allowed to nose-poke for the delivery of food pellets (45 mg; Bio-Serv; Frenchtown, NJ) or water (0.1 mL) on a fixed-ratio 1 (FR-1) schedule of reinforcement. Throughout the operant procedures, the rats were removed from the chambers between 11:00 am and 12:00 pm in order to clean the cages and replenish the water and food levels. Immediately after being removed from the chambers, the rats were weighed and placed individually into their home cage.

On the first day of IVSA, the rats were presented with a novel active and inactive lever at 12:00 pm. The rats were given access to various doses of nicotine IVSA on an FR-1 schedule of reinforcement using an escalating dose regimen of nicotine (0.015, 0.03, and 0.06 mg/kg/0.1 mL infusion; base). When the active lever was pressed, the nicotine solution was delivered at a rate of 0.1 mL per s. At the onset of the 1 s infusion, a cue light was illuminated above the lever for 20 s. This was followed by a 20 s time out period. Responses on the inactive lever had no scheduled consequences. The nicotine solutions were prepared daily based on the animals' weight from the previous day. A nicotine stock solution was prepared for each IVSA dose using (−) nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, MO) dissolved in 0.9% sterile saline (pH of 7.4). Each dose of nicotine was administered in a 4-day cycle with 3 intervening days of drug abstinence. During the 3-day abstinence period, the rats were individually housed in their home cage with ad libitum access to food and water.

2.4. Surgical procedures

At PND 65, the rats were anesthetized with an isoflurane/oxygen vapor mixture (1–3%) and were prepared with jugular catheters, as described previously [21,22]. Following surgery, the rats were allowed to recover for 4 days and the catheters were flushed daily with a 0.2 mL infusion of an antibiotic solution containing Timentin (100 mg/mL) and heparinized saline (30 USP units/mL). Prior to nicotine IVSA, the catheter patency was verified using a 0.1 mL IV infusion of the short-acting barbiturate Brevital® sodium (10 mg/mL). Patency tests were also conducted when aberrant shifts in behavior were detected, and non-patent animals were excluded from the study.

Some female rats received surgical removal of ovarian tissue at PND 45–46, as described previously [14]. In order to assess the role of E2 in modulating the rewarding effects of nicotine, we removed ovarian tissue and immediately began an E2 supplementation procedure. The OVX procedure was done at PND 45–46 based on previous work in our laboratory showing that adult female rats that received OVX procedures at PND 45 display a reduction in the rewarding effects of nicotine [14] and a suppression of anxiety-like behavior and stress-associated gene expression during nicotine withdrawal [23,24]. These studies suggest that after PND 45 ovar-