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Nicotine-Induced Hyperlocomotion Is not Modified by the Estrous Cycle, Ovariectomy and Estradiol Replacement at Physiological Level

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Abstract

The present study was designed to investigate whether nicotine's effect on locomotion might be modulated by the ovarian hormone at physiological level. Rats at normal cycling of estrus and diestrus were selected for the comparison of nicotine-induced hyperlocomotion based on the document that the release of striatal dopamine was greatest at the estrous phase. Ovariectomized rats primed with or without estrogen at physiological level were also selected for comparison. Increase in spontaneous locomotion by nicotine was statistically significant at the doses of 0.15 and 0.3 mg/kg ($p < 0.001$). The stimulating effect of nicotine led the locomotor response to almost the same magnitude in all hormonal groups studied. Nicotine-induced hyperlocomotion appeared to be mediated by central nicotinic receptor because it was blocked by mecamylamine (0.5 and 1.0 mg/kg, i.p.). Also it was blocked by haloperidol (0.04 and 0.08 mg/kg, i.p.) indicating the involvement of dopaminergic neurotransmission. These effects were similar in all groups regardless of the estrous cycle or ovariectomy. The observed data provided behavioral evidence to suggest that the effect of nicotine on locomotion-related dopaminergic neurons might not be modified by the physiological action of estrogen.

Key Words: nicotine, dopaminergic system, locomotor behavior, estrous cycle, ovariectomy

Introduction

Effects of nicotine on locomotor activity are complex; nicotine may produce a stimulation or depression of locomotion depending on the dosage or the duration of treatment (35), as well as previous experience of animal in the testing environment (26, 33). Stimulatory effects of nicotine on locomotor activity are suggested to act through the nicotinic receptors located in neurons of the nigrostriatal or mesolimbic dopaminergic system (6, 8, 9). For example, nicotine can increase the release of dopamine (DA) (7, 23, 32, 35) in dopaminergic neurons; microinjection of nicotine into the nucleus accumbens or ventral tegmental area caused hyperlocomotion that can be reversed by dopamine antagonists (21, 24, 27). Previously we reported (25) that nicotine (0.15 or 0.3 mg/kg, s.c.) increased spontaneous locomotion in female rats at the cycling stage of diestrus. In this experiment, we further examined whether the locomotor response was the same when the nicotine was administered to cycling rats at different stages or to ovariectomized (OVX) rats primed with estrodiol or not.

Given that fluctuation of hormones during the estrous cycle can modulate the striatal DA system

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(14), the ovarian hormone may modulate the action of drugs affecting on dopaminergic transmission (18, 31). Estrogen-related motor behavior are introduced to mediate through the action on dopaminergic system (4). Estrogenic effects on dopaminergic neuron are controversial. Estrogen decreased both general activity and apomorphine-induced stereotyped behavior (27). Some studies have demonstrated that estrogen has an antidopaminergic effect (3, 11,27) using behavioral (17) and neurochemical detection (11, 31). However, it was also documented that estrogen increased the striatal DA turnover rate (13), potentiated amphetamine-induced DA release (2), and increased the number of DA receptors within the striata (10, 22). There are estrous cycle-dependent variations in some nonsexual behaviors, such as general motor activity, running wheel activity (1) and sensorimotor performance (4). All these behaviors increase after estrogen treatment.

These discripancies may be due to several parameters. In the present study, we examine the effect of estradiol on nicotine-induced locomotion using the estradiol replacement at physiological level. The obtained data suggested that estrodiol failed to modulate the nicotine-induced hyperlocomotion in cycling or OVX rats.

Materials and Methods

Animals

Female Wistar rats, weighing 180-280 g, were housed in a room under a 12L:12D (lights were on from 06:00-18:00 h) at 24±2°C and 60±10% relative humidity, with free access to food and tape water. Vaginal smears were taken daily from intact female rats to determine different stages of the estrous cycle. Only the rats exhibiting two or more consistent 4-day estrous cycles were used. Since the release of endogenous DA from striatal tissue was greatest at estrous phase (14), we took diestrous and estrous phase for the treatment of drugs.

Another batch of rats was bilaterally ovariectomized under light ether anesthesia. Fourteen days after surgical removal of the ovaries, rats were randomly divided into two groups that received either estradiol benzoate (EB, 4 μg/rat) or the sesame oil vehicle (0.2 ml/rat), injected intraperitoneally 48 h before the administration of drugs.

Drug Treatment

(−)-Nicotine (free base), β-estradiol 3-benzoate (1,3,5[10]-Estratriene-3,17 β-diol 3-benzoate), mecamylamine, haloperidal and sesame oil were purchased from Sigma Chemical Co. (St. Louis, MD USA). In Experiment 1, rats were treated with nicotine (0.15 or 0.3 mg/kg, s.c.) 5 min before the behavioral testing in a volume of 1 ml/kg. During the initial 5 min, hypoactivity or prostration were observed at some rats. Since previous report (19) revealed that haloperidol induced locomotor hypoactivity at a dose higher than 0.1 mg/kg (i.p.), rats were pretreated with haloperidol (0.04 or 0.08 mg/kg, i.p.) 60 min before nicotine testing in Experiment 2. In Experiment 3, rats were pretreated with mecamylamine (0.5 or 1.0 mg/kg, i.p.) 30 min before nicotine (0.3 mg/kg) testing. All of the three experiments were applied to four hormonal groups studied.

Motor Activity Recording

The activity monitor Video Path Analyzer (VPA) (Model E61-21, Coulbourn Instruments) measured motor activity. The VPA followed the animal's path with the TV camera and analyzed a variety of behaviors including motor activity with the analyzer. The analyzer was fully automatic, it took the camera picture, established an X-Y coordinate from 50×50 cm edge floor which was divided into 16×16 sets of coordinates, generated the cursor block to superimpose over the animal's image on the video monitor, and logged the coordinate data.

The experiment was carried out between 8:00 and 12:00 a.m. Each animal was allowed to adapt the open field 60-min every day for at least one week before drug administration. Motor activity was monitored for 30 min immediately after the animal had been placed into the open field.

Statistical Analysis

All data were analyzed by two-way analysis of variance (ANOVA) followed by Dunnette's multiple comparisons against the saline control group. Results were expressed as Mean±SEM of each group with a sample size of 8. A probability of 0.05 or less was accepted as significant statistically.

Results

Effect of Nicotine on Spontaneous Locomotion

Figure 1 depicts the effects of two doses of nicotine on spontaneous locomotion in a 30-min period. In control animals, the total distance of locomotion was significantly different between estrous and OVX groups using one-way ANOVA analyses, $F(3, 28) = 4.65$, $p < 0.05$. A two-way ANOVA revealed a significant dose-related effect of nicotine on this behavior, $F(2, 84) = 17.17$, $p < 0.0001$. The

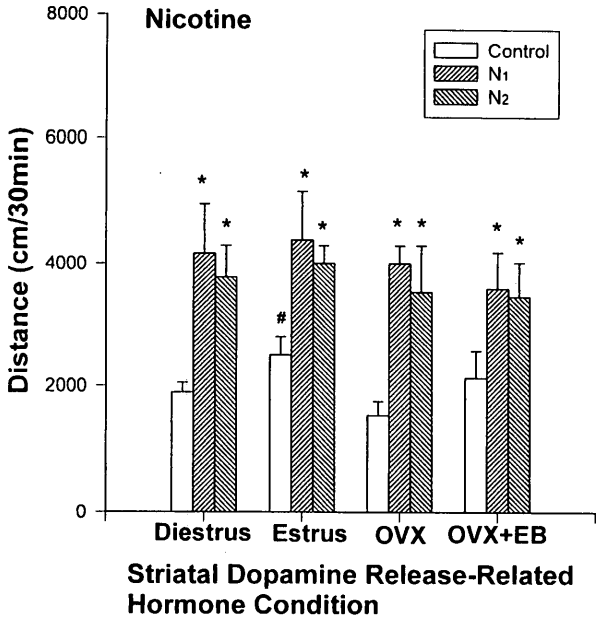

Fig. 1. Influence of ovarian hormone at physiological level on nicotine-induced hyperlocomotion. Each column represents the mean of each group (n = 8) with ±SEM bar indicating the distance (cm) in locomotion during 30 min of observation. Comparisons were made using two-way ANOVA followed by Dunnette's test: * $p < 0.05$ when compared with its control group. #$p < 0.05$ when compared control groups between Estrus and OVX. OVX = ovariectomized; OVX+EB = ovariectomized with estradiol benzoate. $N_1 = 0.15$ mg/kg dose of nicotine; $N_2 = 0.3$ mg/kg dose of nicotine.

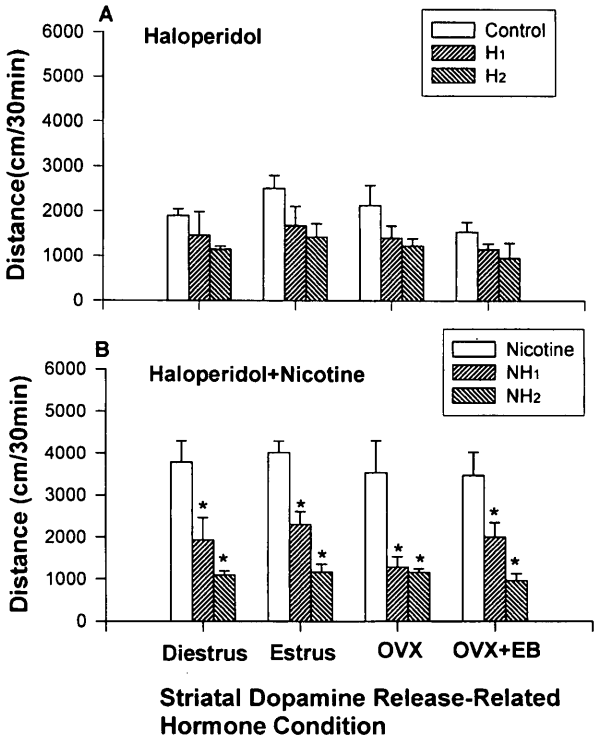

Fig. 2. Influence of hormonal status and haloperidol treatment on spontaneous locomotion (upper panel) and nicotine-induced hyperlocomotion (lower panel). Each column represents the mean of each group (n = 8) with ±SEM bar indicating the distance (cm) in locomotion during 30 min of observation. Comparisons were made using two-way ANOVA followed by Dunnette's test: *$p < 0.05$ when compared with its nicotine control group. OVX = ovariectomized; OVX+EB = ovariectomized with estradiol benzoate. H_1 = haloperidol (0.04 mg/kg, i.p.); H_2 = haloperidol (0.08 mg/kg, i.p.). NH_1 = nicotine (0.3 mg/kg, s.c.) with haloperidol (0.04 mg/kg, i.p.) pretreatment; NH_2 = nicotine (0.3 mg/kg, s.c.) with haloperidol (0.08 mg/kg, i.p.) pretreatment.

hormonal status and the interaction between dose and hormonal status were insignificant. Further comparison by Dunnette's tests revealed that two doses (0.15 and 0.3 mg/kg) of nicotine significantly increased the locomotion in all hormonal groups when compared to their controls ($p < 0.05$).

Effect of Haloperidol on Locomotion

The data illustrated in Fig. 2 revealed that two doses of haloperidol attenuated the spontaneous locomotion (upper panel) and nicotine-induced hyperlocomotion (lower panel). A two-way ANOVA revealed a significant dose effect of haloperidol on nicotine-induced hyperlocomotion, $F(2, 84) = 46.66$, $p < 0.0001$. The hormone effect and the interaction effect between the dose and the hormone were not significant. Further comparison by Dunnette's tests revealed that two doses (0.04 and 0.08 mg/kg) of haloperidol decreased the nicotine-induced hyperlocomotion in all hormonal groups when compared to the control ($p < 0.05$).

Effect of Mecamylamine on Locomotion

Figure 3 showed the effect of mecamylamine on spontaneous locomotion (upper panel) and nicotine-induced hyperlocomotion (lower panel). A two-way ANOVA revealed a significant dose effect of mecamylamine on nicotine-induced hyperlocomotion, $F(2, 84) = 20.37$, $p < 0.0001$. However, the hormone effect and the interaction effect were not significant. Further comparison by Dunnette's test revealed that 1.0 mg/kg dose decreased nicotine-induced hyperlocomotion in all groups, and that 0.5 mg/kg dose had decreasing effect only in estradiol-treated OVX group, when compared to the control ($p < 0.05$).

Discussion

The main findings of the present study were that the ovarian hormone at physiological level failed to modify the nicotine-induced hyperlocomotion, and that the potentiating effect of nicotine led the locomotor response to almost the same magnitude in all hormonal groups studied. From the results, we

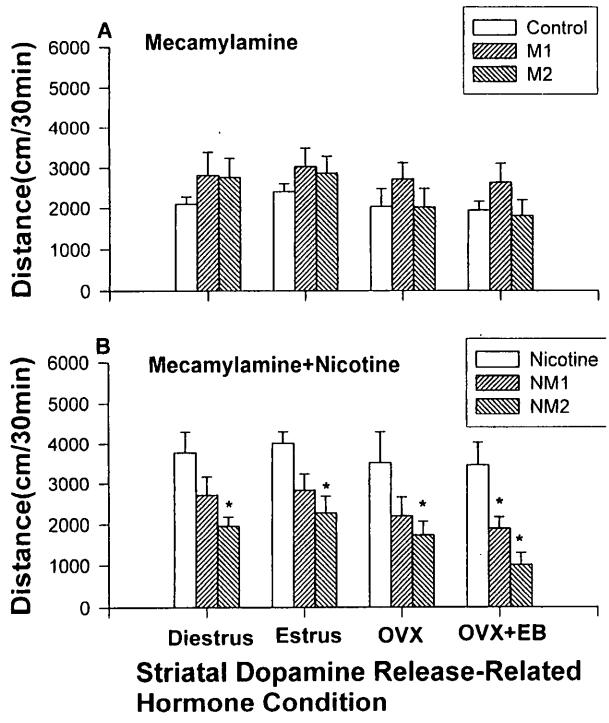

Fig. 3. Influence of hormonal status and mecamylamine treatment on spontaneous locomotion (upper panel) and nicotine-induced hyperlocomotion (lower panel). Each column represents the mean of each group (n = 8) with ±SEM bar indicating the distance (cm) in locomotion during 30 min of observation. Comparisons were made using two-way ANOVA followed by Dunnette's test: *$p < 0.05$ when compared with its nicotine control group. OVX = ovariectomized; OVX+EB = ovariectomized with estradiol benzoate. M_1 = mecamylamine (0.5 mg/kg, i.p.); M_2 = mecamylamine (1.0 mg/kg, i.p.). NM_1 = nicotine (0.3 mg/kg, s.c.) with mecamylamine (0.5 mg/kg, i.p.) pretreatment; NM_2 = nicotine (0.3 mg/kg, s.c.) with mecamylamine (1.0 mg/kg, i.p.) pretreatment.

suggest that the pharmacological action of nicotine on locomotion-related dopaminergic neurons might not be modified by the physiological action of estrogen.

Although the physiological change of sex hormones in cycling rat can modify the activity of striatal DA system, our results reveal that such modification can not influence nicotine-induced hyperlocomotion. In this experiment, the dosage of nicotine (0.15 mg/kg and 0.3 mg/kg) was higher than the dosage pattern of habitual cigarette smokers. Daytime plasma nicotine level in habitual cigarette smokers average around 35 ng/kg, and in rats, similar concentration is obtained following subcutaneous administration of approximately 0.1 mg/kg (30). In rodents, brain concentration is typically four times higher than plasma concentration after subcutaneous injection of nicotine (36). Peak brain concentration occurs within 15 min of injection, and the half-life in brain is 60-90 min after systemic injection in rat (34). If this is so, high concentration of nicotine in the brain might mask the physiological action of estrodiol on dopaminergic system in cycling rats at any cycling phase. That is to say, the physiological action of estrogen on central dopaminergic neurons could not modulate the pharmacological action of nicotine. In our experiment, physiological dose of estrogen did not affect the potentiating level of nicotine-treated groups, leading to the suggestion that the locomotor action of nicotine might not be influenced by the physiological action of estrogen on the dopaminergic system.

In this study, we chose cycling rats at estrus and diestrus for drug treatment. According to previous report (14), release of DA from the striatum was greatest at estrous phase. Cycling rats at estrus are associated with both lower serum estradiol and higher striatal dopamine release, while those at diestrus are associated with lower serum estradiol and dopamine release. Such difference might affect the dopamine-related motor behaviors, such as locomotor activity, of cycling rats at different stage. Cycling rats at proestrus are associated with higher serum estradiol and lower striatal dopamine release, so we did not choose them for experiment. In OVX rats, the hormonal condition is the same as that of cycling rats at diestrus, while in estradiol-treated OVX rats, the hormonal condition is the same as that of cycling rats at estrus (17). Physiological dose (4 μg/rat) of estrogen used in this study was sufficient to reverse the typical changes induced by ovariectomy in endocrine target organs (12). Also, it has been established that this treatment produces serum levels approximately equal to those observed at proestrus (5). Previous reports have demonstrated that the administration of estradiol to OVX rats can influence active avoidance behavior that has been associated with the activity of central dopaminergic system. A profound influence of this behavior, similar to that observed in the intact rat at estrus, was evident 48 hr after estradiol (approximately 2 μg/rat) administration (15, 16).

The mechanisms for the behavioral effect of nicotine on dopaminergic function are well known. Nicotine-induced hyperactivity appears to be mediated by nicotinic receptors located on dopaminergic neurons and requires the release of brain DA, which in turn stimulate DA receptors to increase locomotion (6, 20, 26). In agreement with this viewpoint, our results revealed an involvement of both nicotinic receptors and dopamine receptors in the regulation of nicotine's effect on dopaminergic neurons. Mecamylamine, a nicotinic antagonist acting centrally in the brain (25, 26, 28), and haloperidol, a dopaminergic antagonist (25), were effective to decrease nicotine-induced hyperlocomotion in all groups studied.

In control animals, the baseline level of

locomotion were similar at various estrous stage in haloperidol- and mecamylamine-treated studies (Fig. 2 and 3), but those were different in nicotine-treated study (Fig. 1). Such differences were also observed in previous reports (16,17). The dopaminergic system is apparently modulated by the estrogenic system. This modulation is not strong or overpowering, as is the case for most modulatory systems, so it probably would not significantly alter a strong interaction of the dopaminergic system with nicotinic system.

In conclusion, from the obtained data, we suggest that nicotine's effect on hyperlocomotion might not be influenced by the physiological action of estrogen on locomotor-related dopaminergic neurons.

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