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Acute Effects of Nicotine Injection Into the Nucleus Accumbens on Locomotor Activity in Nicotine-Naive and Nicotine-Tolerant Rats

H. WELZL,[1] K. BÄTTIG AND S. BERZ

Federal Technical Institute, Laboratory of Behavioral Biology
CH-8092 Zürich, Switzerland

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WELZL, H., K. BÄTTIG AND S. BERZ. *Acute effects of nicotine injection into the nucleus accumbens on locomotor activity in nicotine-naive and nicotine-tolerant rats.* PHARMACOL BIOCHEM BEHAV **37**(4) 743–746, 1990.—To assess the role of nicotine receptors in the nucleus accumbens on locomotor activity we bilaterally implanted guide cannulae for later injection of (−)-nicotine into the nucleus accumbens of Wistar rats. Motor activity was tested in a complex tunnel maze equipped with photocells for automatic recording. This system of dark tunnels elicits spontaneous exploration even after repeated exposure. Half of the rats were made nicotine-tolerant by daily systemic injections of (−)-nicotine for 15 days (nicotine pretreatment); the other half remained nicotine-naive (saline pretreatment). Whereas (−)-nicotine (40 nmol/0.3 μl) bilaterally injected into the nucleus accumbens of nicotine-naive animals suppressed locomotor activity, the same amount injected into the nucleus accumbens of nicotine-tolerant rats had no effect on locomotor activity. Systemic injections of nicotine (0.4 mg/kg) induced a depression and stimulation of locomotor activity in saline-pretreated and nicotine-pretreated rats, respectively. Our results support a dual role for nicotine in locomotor activity with the initial depressant effect in nicotine-naive animals due to stimulation of the nucleus accumbens and perhaps other structures.

Locomotor activity Exploratory activity Nicotine Nicotine tolerance Nucleus accumbens Intracranial injection

NICOTINE, the alkaloid of the tobacco plant, acts on a subset of cholinergic receptors, the nicotinic receptors, which can be found in the peripheral and central nervous system (1). In the brain, a high concentration of nicotinic receptors is found in the interpeduncular nucleus, most of the thalamic nuclei, the medial habenula, the superior colliculus, the ventral tegmental area (VTA) and the substantia nigra; the striatum and the nucleus accumbens (NAc) show a moderate density of nicotinic receptors [e.g., (6,7)]. Systemically administered nicotine is quickly distributed throughout the body. Nicotine passes the blood-brain barrier and reaches a high concentration in the brain where it stimulates nicotinic receptors (1). Stimulation of nicotine receptors induces a number of behavioral changes; the most prominent effects in rodents are changes in locomotor activity. Whereas an acute injection of nicotine usually reduces locomotor activity, repeated daily injections of nicotine increase locomotor activity (4, 12, 18). These effects are very likely mediated via central nervous system nicotine receptors (4,12).

The demonstration of nicotine's strong effects on locomotor activity led to the question of where in the brain nicotine acts to produce its effect on locomotor activity. And further, if there are different sites for nicotine's locomotor depressant and stimulant effect. The VTA and the NAc have been implicated in the control of locomotor activity in a number of studies [e.g., (17,28)]. Both sites contain nicotine receptors (5,6), and the neurons of the VTA respond to systemic application of nicotine [e.g., (9,15)]. Indeed, recent studies have demonstrated a crucial role for the NAc and the VTA in nicotine's effect on locomotor activity. Selective destruction of the dopaminergic terminals in the NAc abolished the stimulant effect of nicotine on locomotor activity (3), and direct injection of nicotine into the VTA stimulated locomotor activity (22).

The present study tried to answer two questions. Firstly, how does direct bilateral injection of nicotine into the NAc of rats affect locomotor activity? And secondly, is there a difference in the effect dependent on whether or not the injected animals are nicotine-tolerant? We therefore implanted cannulae for intra-NAc injection of nicotine in nicotine-naive and nicotine-tolerant rats. After intra-NAc injection of nicotine, locomotor activity was measured in a complex environment (hexagonal tunnel maze).

[1]Requests for reprints should be addressed to H. Welzl, ETH Zürich, Lab. Behavioral Biology, Turnerstrasse 1, CH-8092 Zürich, Switzerland.

The same animals were later systemically injected with nicotine and locomotor activity was again measured in the tunnel maze.

METHOD

Subjects

Thirty-six male Wistar rats weighing 280–300 g at the time of operation were used. They were housed in groups of two rats per cage with free access to food and water. A 12-h light/12-h dark cycle (lights on 07:00–19:00) was used.

Surgery

Surgery was performed under Equithesin anesthesia (3 ml/kg). Guide cannulae (inner dia. 0.41 mm, outer dia. 0.71 mm) were bilaterally implanted with their tip 1 mm above the injection site in the NAc. The coordinates for the injection site (tip of the injection cannula) were +1.7 mm anterior to bregma, 1.5 mm lateral to the midline, and −6.8 mm below skull surface (21). After implantation, the guide cannulae were fixed to the skull with anchoring screws and dental cement. Stainless steel plugs prevented the guide cannulae from clogging.

Drugs and Drug Injections

(−)-Nicotine-(+)-tartrate (BDH Chemicals Ltd., UK) was dissolved in physiological saline and the pH of the solution was adjusted to 6.5–7.0 by adding small amounts of 1 N NaOH. The 0.3 μl solution injected into each NAc contained 40 nmol of (−)-nicotine. A dose of 40 nmol (−)-nicotine was selected on the basis of a short pilot study. Doses lower than 40 nmol (−)-nicotine (10 nmol and 20 nmol) did not induce a measurable change in locomotor activity. Drug solutions or vehicle were injected into the NAc through a stainless steel cannula (outer dia. 0.37 mm). The cannula was connected via polyethylene tubing to a 10 μl Hamilton microsyringe driven by a pump. A volume of 0.3 μl solution was injected into the hand-restrained animal at a rate of 1 μl/60 s. After termination of the injection, the cannula was left in place for another 60 s.

For systemic injections, 1 ml contained 0.4 mg (−)-nicotine and 0.4 mg/kg body weight were injected SC (subcutaneously) in the dorsal surface of the neck (for details see below). Control injections consisted of corresponding amounts of vehicle solution.

Apparatus

To measure locomotor activity, a hexagonal tunnel maze (150 cm dia.) was used [Fig. 1; for details see (26,30)]. The tunnels of this maze (8 cm wide, 15 cm high) form an outer and an inner hexagonal ring which are connected by six short tunnels. The inner hexagonal ring is further connected by six tunnels with a central field. The walls and roof are opaque so that animals are tested in complete darkness. These dark tunnels provide a sufficient stimulus to elicit locomotor activity, i.e., exploratory activity, in rats. Locomotor activity is automatically recorded by 42 photocells which are evenly distributed throughout the maze. The total number of photobeam interruptions provides a measure of locomotor activity. Repeated activations of the same photocell (e.g., AA) or pair of photocells (e.g., ABAB) were not counted to avoid counting stereotyped behaviors in front of a photocell as activity.

Procedure

Three to five days after surgery the 36 rats were divided into four groups (N = 9/group). During Days 1–15, two groups were daily SC injected (between 17:00–18:00) with saline (1 ml/kg; saline-pretreated groups) and two groups with (−)-nicotine (0.4 mg/kg; nicotine-pretreated groups). To familiarize the rats with the apparatus and to obtain stable baseline values for locomotor activity, all animals were allowed to freely explore the hexagonal tunnel maze for four minutes on Days 11–15 (between 09:00–11:00). Locomotor activity stabilized in all groups over trials (changes in activity between days smaller than 5%) and the mean locomotor activity for Days 14–15 was taken as the baseline locomotor activity for each animal. On Day 16, (−)-nicotine was bilaterally injected into the NAc of one saline-pretreated group (SN) and of one nicotine-pretreated group (NN). The second saline-pretreated group (SS) and the second nicotine-pretreated group (NS) received corresponding bilateral intra-NAc injections of saline. Four minutes after the termination of the injection, each animal was placed into the center of the tunnel maze. It was then allowed to freely explore the maze for four minutes while locomotor activity was measured automatically. On Day 17, nicotine was SC injected (0.4 mg/kg) in animals of all four groups. Locomotor activity was measured as described for Day 16.

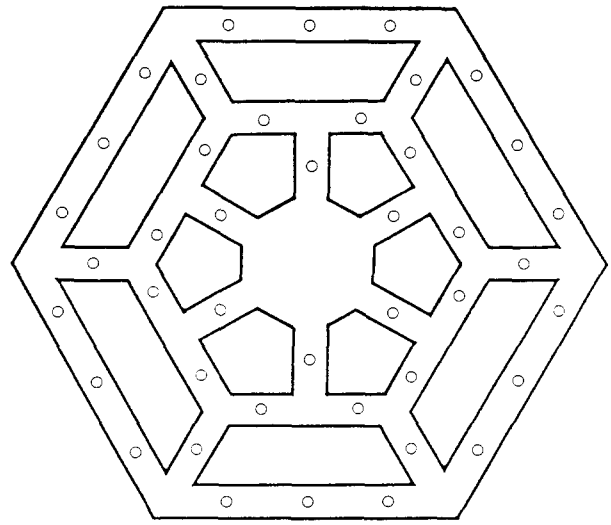

FIG. 1. Ground plan of the hexagonal tunnel maze (150 cm dia.). The positions of the 42 photocells which register the movements of a rat are shown as circles in the hexagonal maze (for details see text).

Histology

Immediately after the last test, 0.3 μl of a saturated Thionin solution were bilaterally injected to mark the injection site. The animals were then perfused with formaline and their brains were cut into 40 μm thick slices with a cryostat. The sections were stained with cresyl violet to allow verification of the injection site.

Statistical Analysis

Differences between baseline and treatment data were statistically evaluated with the Wilcoxon matched-pairs signed-ranks test for related samples (27).

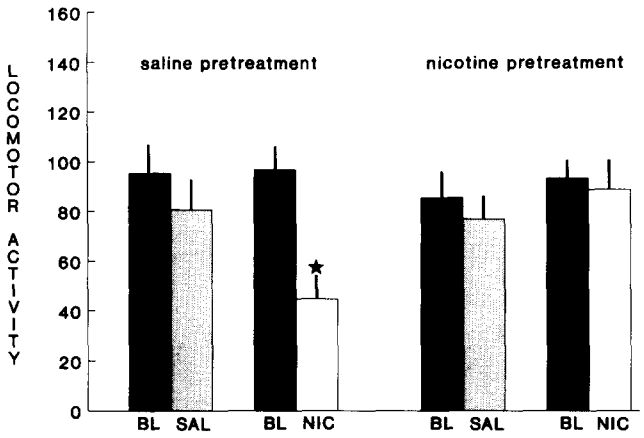

FIG. 2. Locomotor activity (expressed as the total number of photocell interruptions per trial) of saline-pretreated (nicotine-naive) and nicotine-pretreated (nicotine-tolerant) rats during baseline activity (BL; Days 14 and 15) and after intracranial injection of saline (SAL) or nicotine (NIC) on Day 16; mean ± SEM; *$p<0.05$.

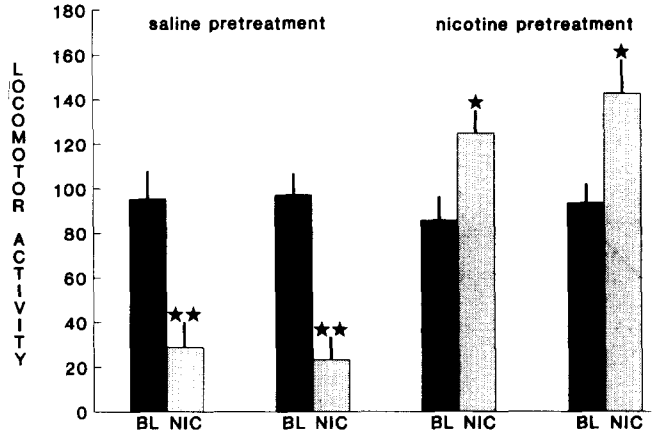

FIG. 3. Locomotor activity (expressed as the total number of photocell interruptions per trial) of the two groups of saline-pretreated (SS,SN) and the two groups of nicotine-pretreated (NS,NN) rats during baseline activity (BL; Days 14 and 15) and after systemic injection (SC) of nicotine (NIC) on Day 17; mean ± SEM; *$p<0.05$, **$p<0.01$.

RESULTS

Histological analysis confirmed that all injection sites of animals included in this study were within the boundaries of the NAc. No significant differences in baseline locomotor activity (BL) could be found between the four groups (SS, SN, NS, and NN). Bilateral intra-NAc injection of saline did not significantly reduce locomotor activity below baseline activity in the nicotine-tolerant (nicotine-pretreated) or the nicotine-naive (saline-pretreated) groups. However, bilateral intra-NAc injection of (−)-nicotine reduced locomotor activity in the nicotine-naive group ($p<0.01$), but not in the nicotine-tolerant group (n.s., Fig. 2).

Intracranial injections of vehicle as well as (−)-nicotine were often followed by a loss of muscle tone, teeth chattering and/or compulsive gnawing. Both phenomena, however, disappeared within three minutes of the termination of injection, i.e., before testing in the tunnel maze began.

Systemic injections of (−)-nicotine on Day 17 influenced locomotor activity differently in saline-pretreated rats (independent of whether or not nicotine was intracranially injected on Day 16) and nicotine-pretreated rats (Fig. 3). An SC injection of (−)-nicotine in saline-pretreated animals (groups SS and SN) drastically reduced their locomotor activity compared to baseline locomotor activity as measured during Days 14 and 15 ($p<0.01$). In contrast, nicotine-pretreated rats (groups NS and NN) increased their locomotor activity in response to a SC injection of (−)-nicotine ($p<0.05$).

DISCUSSION

Our results confirm earlier findings that nicotine has both a depressant and stimulant effect on locomotor activity. Intra-NAc injection of nicotine depressed locomotor activity, suggesting that this site contributes to the depression of activity seen after nicotine injection. With the development of nicotine tolerance this effect disappears, due to as yet unknown mechanisms.

Systemic injection of nicotine also depressed locomotor activity in nicotine-naive animals, but in contrast to intra-NAc injection of nicotine, it stimulated activity in nicotine-tolerant rats. This suggests that systemically injected nicotine acts on nicotine receptors lying outside the NAc to stimulate locomotor activity.

Nicotine has a regulatory function on the release of a number of neurotransmitters (25) including stimulation of dopamine release in the NAc in in vivo preparations (10, 11, 13, 16). Since an increase in dopamine release in the NAc is associated with an increase in locomotor activity (20), one would expect, in contrast to our results, that intra-NAc nicotine would stimulate locomotor activity. However, approximately 60% of all nicotinic receptors in the NAc are located on nondopaminergic axons (5). These nicotine receptors seem to mediate the initial depressant effect that blocks or masks the stimulant effect caused by the simultaneous release of dopamine. Nicotine could, for example, increase the release of GABA in the NAc. An increase in the release of GABA in the ventral striatum due to cholinergic stimulation has been demonstrated in anesthetized rats (8). Stimulation of GABA receptors in the NAc by GABA or GABA analogues induces hypoactivity (29). Further, hyperactivity induced by intra-NAc injection of dopamine or by systemic injection of d-amphetamine can be blocked by intra-NAc injection of GABA or GABA analogues (23).

After tolerance to nicotine's depressant effects on locomotor activity, the locomotor stimulant effects of nicotine become visible. The increase in locomotor activity observed after repeated systemic nicotine injections [(4, 12, 18), data presented above] might be due to nicotine's ability to stimulate the dopaminergic projection from the VTA to the NAc (10, 11, 13, 16). Experimental data support such a hypothesis. Direct injection of nicotine into the VTA stimulates locomotor activity already in nicotine-tolerant rats (19, 22, 24), and systemic nicotine no longer stimulates locomotor activity in nicotine-tolerant rats when the dopaminergic terminals are selectively lesioned in the NAc (3).

Our results confirm those of a previous study reporting depressant effects of cytisine, a nicotine agonist, on locomotor activity when injected into the NAc (22). These results and our results, however, are in contrast with the data obtained by Austin and Kalivas (2). They reported a locomotor stimulant effect of intra-accumbens injection of the cholinergic agonist carbachol and this effect could be blocked by a systemic injection of the nicotine antagonist mecamylamine. The use of different techniques to stimulate nicotinic receptors in the NAc and different techniques to measure locomotor activity might explain the different results

obtained in these studies. Further, the possibility cannot be excluded that the drugs diffuse away from the injection site and stimulate structures lying outside the NAc [see, e.g., (14)].

Recently, Reavill and Stolerman (24) reported that an infusion of nicotine or cytisine into the nucleus accumbens neither increased nor decreased locomotor activity (cage crosses). Since they presented their data as the mean of 1-h sessions in photocell activity cages, an initial depressant action of the drugs might have been covered up.

In summary, the present results and those from other laboratories suggest that nicotine's depressant effect on locomotor activity is mediated via stimulation of nicotine receptors in the NAc. With the development of nicotine tolerance, the depressant effect of nicotine on locomotor activity, produced by systemic or intra-NAc injections, disappears. No tolerance, however, develops for nicotine's stimulant effect on locomotor activity. Stimulation of nicotine receptors lying outside the NAc seems to be responsible for the stimulant effect on locomotor activity.

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