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Effects of Nicotine on the Threshold for Rewarding Brain Stimulation in Rats

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HUSTON-LYONS, D. AND C. KORNETSKY. *Effects of nicotine on the threshold for rewarding brain stimulation in rats.* PHARMACOL BIOCHEM BEHAV **41**(4) 755–759, 1992. – The rewarding effects of nicotine alone and nicotine challenged with mecamylamine, a nicotine receptor blocker, or naloxone were determined using a rate-independent discrete-trial threshold measure of brain-stimulation reward in rats. If nicotine acts as other drugs of abuse, it would be expected to lower the reward threshold, that is, increase an animal's sensitivity to rewarding brain stimulation, and naloxone would be expected to block this effect, as it does other stimulants in this paradigm. Nicotine was found to significantly lower the reward threshold and mecamylamine blocked this effect. However, although naloxone increased the variability of nicotine's effect on the reward threshold, it failed to dose dependently block nicotine's threshold-lowering effect.

Nicotine Naloxone Brain-stimulation reward Self-stimulation Mecamylamine

ALTHOUGH nicotine is generally considered an abused substance, it has been more difficult to demonstrate the reinforcing properties of nicotine than other drugs of abuse using animal models such as brain-stimulation reward (BSR). Early investigation of nicotine's effects on rewarding brain stimulation examined changes in rate of lever pressing for stimulation (25,26,28). In general, increases in rate of responding were found at low to moderate doses or after high doses if sufficient time had elapsed. Because nicotine, however, also increases locomotor activity (5,19), these increases in rates of responding are difficult to interpret.

More recent studies that examined nicotine's effects on rewarding brain stimulation yielded mixed results. Clarke and Kumar (7), using a Y-maze in which animals shuttled between the arms of the maze to obtain stimulation, concluded that the increased movement between arms after administration of nicotine was due to increased motor activity and not an increase in the reward value of the stimulation. These investigators (8), however, in a later study based on a technique less dependent on motor activity concluded that increased locomotion after nicotine was not responsible for the observed facilitation of BSR. Schaefer and Michael (31) found that nicotine caused an increase in rates of lever pressing for brain stimulation to the medial forebrain bundle under an FR:15 schedule; however, no change in threshold for rewarding brain stimulation was found using the autotitration threshold method. Similarly, Druhan et al. (10) found that although nicotine facilitated rates of responding it failed to appreciably shift the rate-intensity function to the left, which would have indicated a change in the reward threshold.

Due to the variability in results, it remains unclear whether nicotine increased the sensitivity of animals to rewarding brain stimulation or simply caused nonspecific increases in rate of response. In addition, previous studies, with one exception (7), used measures in which rate of operant response was an integral part of the procedure. To avoid the possible role of nonspecific stimulation effects caused by nicotine, the present study determined the effects of nicotine on BSR using a rate-independent discrete-trial threshold method. If nicotine acts as other drugs of abuse and increases the sensitivity of neurons in the medial forebrain bundle, the threshold for electrical brain stimulation would be expected to be lowered. Furthermore, if nicotine acts on precisely the same neural substrate as, for example, cocaine and amphetamine, naloxone would be expected to block the threshold lowering of nicotine as it does these other stimulants in this paradigm (2,12).

METHOD

Subjects and Surgical Procedure

Four male F-344 rats (Charles River Laboratories, Inc., Wilmington, MA) weighing approximately 300 g were anesthetized with either xylazine and ketamine or pentobarbital. Bipolar stainless steel electrodes, 0.13 mm in diameter and insulated except at the tips (Plastic Products, Roanoke, VA), were stereotaxically implanted into the lateral hypothalamic

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region of the medial forebrain bundle (MFB-LH coordinates: 3.8 mm posterior to bregma, 1.4 mm lateral from the midline suture, and 8.5 mm ventral to the skull surface). The electrodes were placed through small burr holes in the skull and attached permanently to the surface with an acrylic platform. After surgery, animals received 60,000 units of penicillin (IM) and were given at least 1 week for postoperative recovery before behavioral testing was begun. Animals were maintained on a 12 L:12 D cycle (lights on at 0700 h), housed individually in stainless steel cages, and had access to food and water ad lib.

Training and Testing Procedure

Animals were trained and tested in an acrylic chamber (20 × 20 × 34 cm). A cylindrical manipulandum (15 cm long and 7.5 cm wide) was located within one wall of the test chamber. Four equally spaced cams on one endplate of the manipulandum operated a microswitch that resulted in immediate delivery of a stimulation, depending on the schedule, when the cylinder was rotated. A constant-current stimulator (Sunrise Systems, Pembroke, MA) was used to deliver the biphasic symmetrical square-wave pulses. Each stimulus consisted of a 500-ms train at a frequency of 160 Hz with a pulse width of 0.2 ms and a delay of 0.2 ms between the positive and negative pulses. Thresholds were determined by a rate-independent procedure for rewarding brain stimulation that has been previously described (11).

Animals required approximately six 1-h training sessions to learn the task and approximately four additional sessions for establishment of a stable threshold level whereupon experimental vehicle-control sessions were begun. No more than one experimental session was run per day, and at least five control sessions preceded drug treatment sessions. During an experimental session, the reward threshold was determined twice: once pre- and once postinjections. Postinjection sessions began 5 min after the administration of nicotine or vehicle; when mecamylamine or naloxone were given, they were administered 5 min before administration of nicotine or vehicle. All injections were subcutaneous. Each animal was tested after the administration of a drug two to three times a week. On alternate days, animals were tested after saline administration. In most cases, a specific dose was given to each animal only once. In the few cases where a dose was repeated, the average for the two treatments was used as the datum. All drugs were dissolved in isotonic saline; nicotine doses refer to the base. All injections were made in volumes of 1 ml/kg body weight, and the sequence of doses was roughly counterbalanced.

Statistical Analysis

For each animal, threshold values were calculated for both the pre- and postinjection sessions with the difference (post minus pre) between the two scores taken as the dependent measure. To take into account the variance seen after saline (control) treatment, the difference scores for drug test days were transformed to standard scores (z-scores) based on the mean and standard deviation of the difference scores for all vehicle control days. A z-score of ± 2.0 (approximately the 95% confidence level) was preselected as the level of significance for individual animals. Paired t-tests were also performed using z-scores to compare the effects of nicotine and saline and the effects of naloxone alone and nicotine plus naloxone.

Experiment 1

The effects of nicotine (0.06–1.0 mg/kg, SC) on the threshold for rewarding brain stimulation were determined in four rats. Subsequently, mecamylamine (4 mg/kg, SC) was used to challenge a threshold-lowering dose of nicotine in each animal.

Experiment 2

The effects of various doses of naloxone alone (0.5–16 mg/kg) and naloxone coadministered with a maximally effective dose of nicotine (0.5 or 0.75 mg/kg) were determined in the same animals used in Experiment 1.

Histology

At the completion of the experiment, animals were killed with an overdose of pentobarbital and perfused intracardially with saline followed by a 10% formaldehyde solution. Brains were subsequently removed from the skull, fixed, embedded, and sliced at 40 μm. Mounted sections were stained with cresyl violet or thionin and examined under a light microscope to determine the placement of the electrode tips.

RESULTS

The mean threshold for vehicle preinjection sessions was 48.2 μA. The mean vehicle postinjection threshold was 53.0 μA.

Experiment 1

As shown in Fig. 1, nicotine treatment resulted in a significant lowering of the threshold (paired t-test, $p < 0.025$) at doses between 0.125 and 0.75 mg/kg. Figure 2 depicts mean z-score changes in the reward threshold for maximally effective threshold-lowering doses of nicotine alone (0.5 or 0.75 mg/kg), mecamylamine alone (4 mg/kg), and the interaction

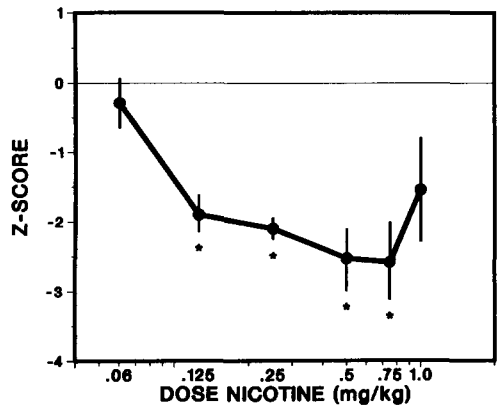

FIG. 1. Mean z-score (standard score) ± SEM changes in reward threshold from pre- to postdrug after administration of various doses of nicotine. Saline post- minus predrug threshold is indicated by a z-score of 0. Asterisks identify the doses of nicotine that caused a statistically significant lowering of the threshold ($p < 0.025$) determined by two-tailed paired t-tests.

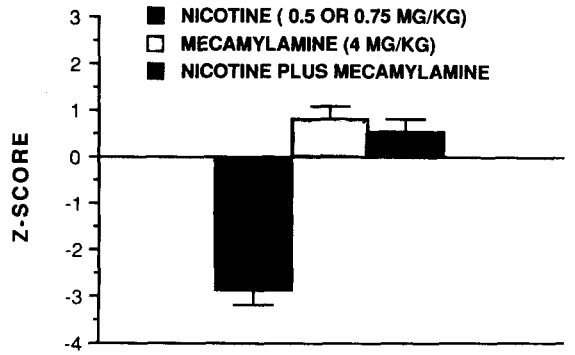

FIG. 2. Mean z-score changes in reward threshold from pre- to post-drug after administration of doses of nicotine that maximally lower the reward threshold, administration of mecamylamine alone, and coadministration of both drugs. Saline post- minus predrug threshold is indicated by a z-score of 0.

of these drugs when coadministered. Although mecamylamine by itself had no effect on the reward threshold, it effectively blocked the threshold-lowering effect of nicotine.

Experiment 2

Figure 3 shows the effect of naloxone alone and the naloxone challenge of a maximally effective dose of nicotine. As in previous studies using this discrete-trial threshold paradigm, naloxone had no effect on the threshold for rewarding brain stimulation (2,12,20,27). In the present experiment, no dose of naloxone significantly blocked the effect of nicotine ($p > 0.1$); it did, however, increase variability of the threshold lowering induced by nicotine.

Histology

Histological examination revealed the electrode tips of three of the four animals were located in the medial forebrain bundle at the level of the lateral hypothalamus. One animal died before histological analysis could be performed.

DISCUSSION

The present study demonstrated that the administration of nicotine effectively lowered the threshold for rewarding brain stimulation. This effect was blocked by mecamylamine, a ganglionic (C6) blocker that has been shown to bind to central nicotine receptors (4). To the extent that this paradigm is an animal model of drug-induced euphoria, nicotine's ability to increase an animal's sensitivity to the rewarding impact of brain stimulation to the medial forebrain bundle suggests the neural substrate of nicotine reward is similar to other abused substances. However, naloxone failed to block the threshold-lowering effects of nicotine. Although naloxone has no effect on threshold when administered alone (27), it blocks the threshold-lowering effects of other drugs of abuse, including morphine (21,24), amphetamine (12), cocaine (2), amfonelic acid (20), and methylenedioxymethamphetamine (MDMA) (3). That nicotine's threshold-lowering effect was not blocked by naloxone indicates that nicotine's rewarding effects are mediated, at least in part, by mechanisms distinct from other drugs of abuse. To our knowledge, there have been two previous studies of the effect of nicotine on the threshold for BSR. Using the autotitration method, Schaefer and Michael (31) found no change in threshold, as did Druhan et al. (10), who used a shift in the rate-intensity function as an indicator of a shift in the reward threshold. Both groups did find, however, that rate of responding was facilitated. The reason these previous studies did not find threshold changes is unclear. However, the most obvious methodological difference is the use of a rate-independent measure in the present study that, in addition to limiting artifacts resulting from changes in locomotor activity, provides considerably fewer total pulse trains per experimental session.

Pharmacologic studies of the rewarding properties of drugs of abuse indicate that dopaminergic systems mediate, in part, these reinforcing effects (13,33). Several lines of evidence also indicate that nicotine activates the dopaminergic mesocorticolimbic and nigrostriatal systems (1). Nicotine receptors exist on dopamine axons and cell bodies in the ventral tegmental area, hypothalamus, nucleus accumbens, and olfactory tubercle, as well as in the substantia nigra and striatum (9,32). Electrophysiological studies of dopaminergic midbrain neurons have demonstrated increased firing rates after systemic administration of nicotine (6,16) or iontophoretic administration into the substantia nigra (22). Increased dopamine turnover after nicotine administration has been well documented in mesolimbic, for example, nucleus accumbens and olfactory tubercle, and nigrostriatal terminal fields (1,14,15,18,29). Using the microdialysis technique to assess extracellular brain neurotransmitter levels, systemic (18) or intraaccumbens (23) injections of nicotine increased dopamine levels in the nucleus accumbens, an effect that was blocked by mecamylamine. In addition, Mifsud and colleagues argue that in their microdialysis study (23) intraaccumbens nicotine, unlike cocaine, which decreases dihydroxyphenylacetic acid (DOPAC) levels (17), altered neither DOPAC nor homovanillic acid (HVA) levels, which, in turn, suggests that nicotine does not act as a dopamine reuptake blocker. Thus, previous experiments sug-

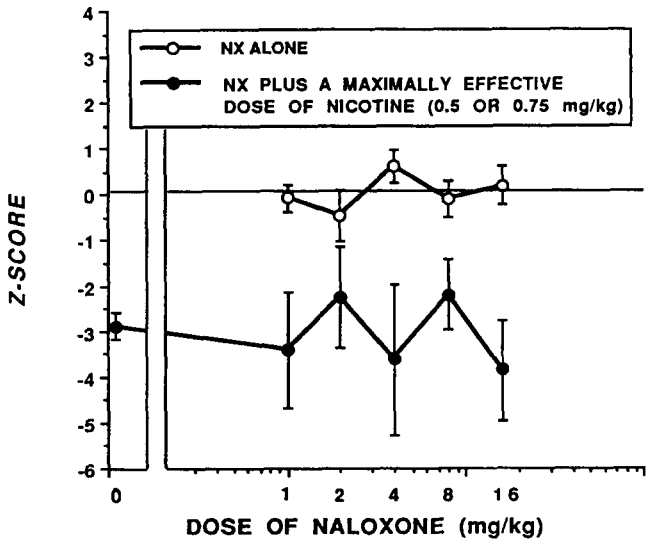

FIG. 3. Mean z-score changes in reward threshold from pre- to post-drug after administration of naloxone (NX) alone and in combination with a maximally effective dose of nicotine. Saline threshold change is indicated by a z-score of 0.

gest that nicotine acts at nicotinic receptors in the forebrain and midbrain causing the release of dopamine. The present study further demonstrates that nicotine produces increased behavioral sensitivitiy to the rewarding impact of electrical stimulation in the vicinity of the mesocorticolimbic and nigrostriatal pathways.

Naloxone's effects on behavior motivated by rewarding brain stimulation have been review by Schaefer (30), who concluded that when naloxone was administered alone it consistently reduced responding for intermittent schedules of responding, for example, fixed-ratio or fixed-interval schedules, but had no effect on thresholds for stimulation that were rate independent, such as the paradigm employed in the present study. Schaefer (30) also concluded naloxone modified the effects on rewarding brain stimulation of dopaminergic drugs such as cocaine and amphetamine (2,12). However, in the present study naloxone failed to block nicotine's threshold-lowering effect over the dose range that blocked these other drugs. These data, therefore, set nicotine apart from other drugs of abuse. However, the mechanism by which naloxone blocks the threshold-lowering effect of cocaine or amphetamine has not been elucidated. The reason that naloxone failed to attenuate nicotine's effect in the present study remains equally unclear.

In conclusion, the present study established that nicotine increases the rewarding impact of electrical stimulation to the medial forebrain bundle in a discrete-trial rate-independent paradigm, and this effect is due to receptor occupation of nicotine receptors. Furthermore, nicotine reward as measured by effects on rewarding brain stimulation is less susceptible to opiate antagonist modulation than other psychomotor stimulants. Future research of the neuronal substrate of nicotine reward is required to establish whether a causal link between dopaminergic activity and nicotine rewards exists.

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