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Nicotine and Brain-Stimulation Reward: Interactions With Morphine, Amphetamine and Pimozide

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HUSTON-LYONS, D., M. SARKAR AND C. KORNETSKY. *Nicotine and brain-stimulation reward: Interactions with morphine, amphetamine, and pimozide.* PHARMACOL BIOCHEM BEHAV 46(2) 453–457, 1993.—Using a rate-independent discrete trial method of determining thresholds for rewarding electrical intracranial stimulation in rats, we evaluated the pharmacological interaction of nicotine plus morphine, *d*-amphetamine, or the D_2 receptor antagonist, pimozide. Both morphine and amphetamine shifted the dose-response curve for nicotine down and to the left, indicating increased efficacy and potency, respectively. Pimozide at doses that have no effect on performance and only minimal effect on brain-stimulation reward blocked the effect of nicotine. These data suggest that the same dopaminergic substrate that supports the positive reinforcing effects of other drugs of abuse also supports nicotine reward.

Self-stimulation Opiate Psychomotor stimulant Dopamine receptor antagonist

IT has been well documented that a variety of abused substances increase an experimental animal's sensitivity to intracranial electrical stimulation (17,27). Despite obvious differences in receptor specificity, abused opioids and psychomotor stimulants similarly facilitate brain-stimulation reward (BSR). Studies such as these have led to a final common pathway theory for drugs of abuse in which the action of pharmacologically distinct compounds with positive reinforcing properties converge on the same neural substrate that supports drug-induced pleasure (13,18,19,27). We recently reported that nicotine also dose dependently increases sensitivity for BSR to the medial forebrain bundle (MFB) (14), suggesting the same substrate that supports opiate and stimulant abuse may support tobacco smoking behavior, as well.

The present study endeavored to examine the common pathway hypothesis further by testing the ability of morphine or amphetamine to shift the nicotine reward-threshold dose-response. A positive interaction may be viewed as additional evidence of the similar impact these drugs manifest in the MFB-lateral hypothalamus. Further, in light of the extensive literature detailing the importance of dopamine in BSR (17,26) and drug reward processes (6,20), we also tested the ability of the D_2 receptor antagonist, pimozide, to attenuate the rewarding effect of nicotine on the BSR threshold. Pimozide itself decreases sensitivity to stimulation at doses that do not affect attention or perception (2), demonstrating that the rewarding effect of stimulation is influenced by dopaminergic activity in this model. It was further hypothesized that if nicotine reward is at least partially dependent upon dopaminergic transmission then pimozide would also reverse nicotine threshold lowering at these moderate doses that do not diminish performance.

METHOD

Two experiments were conducted. Experiment 1 involved the effects of nicotine alone and in combination with morphine or *d*-amphetamine. Experiment 2 determined the effect of pimozide on the threshold-lowering effect of nicotine.

Subjects and Surgical Procedure

Of the six male F-344 rats (Charles River Laboratories, Inc., Wilmington, MA) used in the two experiments, five were employed in Experiment 1; one of these and two new animals were used in Experiment 2. Rats, weighing approximately 300 g, were anesthetized with either xylazine and ketamine or pentobarbital and 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

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hypothalamic region of the medial forebrain bundle (coordinates: 3.8 mm posterior to bregma, 1.4 mm lateral to the midline suture, and 8.5 mm ventral to the skull outer 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 U penicillin IM and were given at least 1 week for postoperative recovery before behavioral testing was started. Animals were maintained on a 12 L : 12 D cycle (light on 0700 h), housed individually in stainless steel cages, and had access to food and water ad lib. Some of the same animal data contained in the nicotine dose-response has appeared in a previous article (14).

Training and Testing Procedures

Animals were trained and tested in acrylic chambers (20 × 20 × 34 cm) with a cylindrical manipulandum (15 cm long and 7.5 cm wide) 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 when the cylinder was rotated, depending upon the schedule. 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 discrete trial, rate-independent procedure for rewarding brain stimulation that has been previously described (2,8,12,16).

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. At least five control sessions preceded the start of drug treatment sessions, and no more than one experimental session was run per day throughout the study. During an experimental session, the reward threshold was determined twice: once pre- and once postinjection. Postinjection sessions began 5 min after administration of nicotine (Sigma Chemical Co., St. Louis, MO) or nicotine vehicle. Morphine sulfate (Penick, New York) or *d*-amphetamine (Sigma) were injected 5 min and pimozide (Sigma) was injected 4 h before nicotine (or vehicle) injections. Nicotine and morphine injections were SC; amphetamine and pimozide were injected IP. Each animal was tested after administration of a drug two to three times per week, and on alternate days animals were tested after vehicle control 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, except pimozide, were dissolved in isotonic saline; nicotine refers to the base. Pimozide was dissolved in 10% emulfar, 5% ethanol, and saline (85%) by volume. All injections were made in volumes of 1 ml/kg body weight, and the sequence of doses was roughly counterbalanced within each experiment.

Statistical Analysis

For each animal, threshold values were calculated for both the pre- and postinjection sessions, and the difference (post-

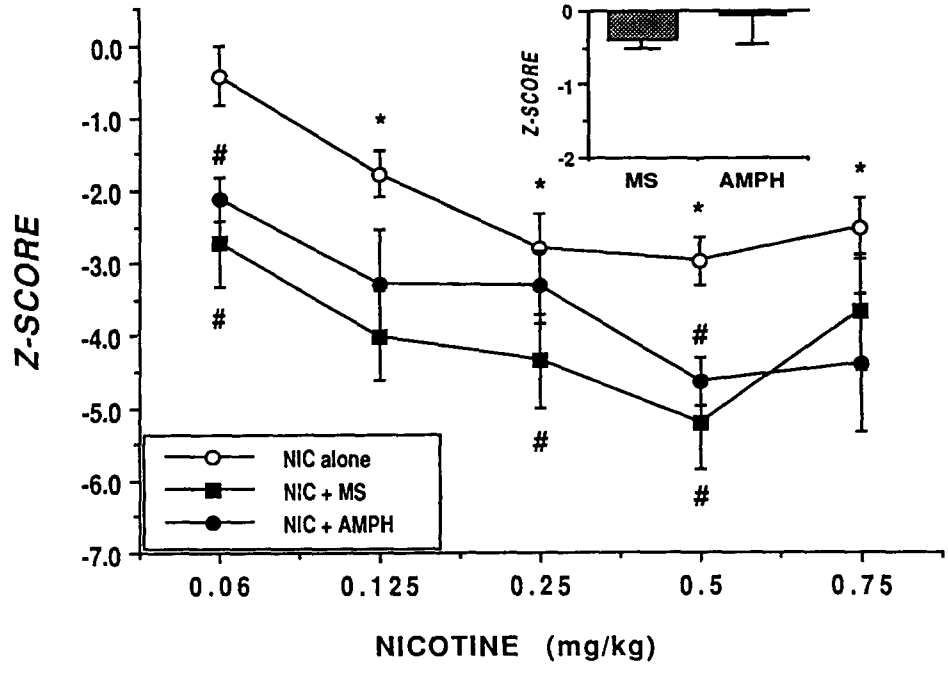

FIG. 1. Mean *z*-score changes in the reward threshold after administration of nicotine (NIC) alone or NIC coadministered with morphine (MS) or amphetamine (AMPH). The inset illustrates the lack of significant effect of the low doses of MS (0.125 or 0.25 mg/kg) alone or AMPH (0.06 mg/kg) alone. Vehicle control threshold change is indicated by a *z*-score of zero (0.0). *Significant change in the threshold after a single injection of NIC compared to vehicle control (paired *t*-test, $p < 0.01$, df = 3 or 4, not all animals were given every dose). #Significant difference between the threshold after a single NIC injection and the combination of NIC plus either MS or AMPH (paired *t*-test, $p < 0.02$, df = 3 or 4).

minus preinjection) between the two values was taken as the dependent measure. To control for individual differences in the variance of threshold, pre- to postinjection differences for drug test days were transformed to standard scores (z-scores) based upon the mean and SD of the respective pre- to postinjection differences for all vehicle control days. Paired *t*-tests were performed using z-scores to compare treatment groups.

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 thionine and examined under a light microscope to determine the placement of the electrode tips.

RESULTS

The mean of the averages of each rat's post- and presaline treatment threshold was 44.1 and 41.4 μA, respectively. The mean of the individual animal's SD of the postsaline minus the presaline treatment threshold was +3.87. This translates to an approximate threshold of 44.1, 40.2, 36.4, and 32.5 μA for z-scores of 0, −1, −2, and −3, respectively. Because the effects of saline treatment were determined two to three times a week throughout the course of the experiment and all these vehicle treatments contributed to the mean saline threshold and SD used to compute the z-score for each animal, a drift in the control threshold values would be reflected in a large SD. If there were such a drift in the control threshold changes for whatever reason, including repeated measurement effects, it would result in a spuriously large SD that would preclude the achievement of any significant treatment effect. In this study, there was no drift in control values throughout the experiment.

Experiment 1

As depicted in Fig. 1, nicotine lowered the threshold for rewarding electrical stimulation to the medial forebrain bundle (0.125–0.75 mg/kg, SC; paired *t*-test, $p < 0.01$). The inset of Fig. 1 illustrates the failure of low doses of morphine (0.125 or 0.25 mg/kg, SC) or amphetamine (0.06 mg/kg, IP) to alter the reward threshold. These doses of morphine or amphetamine when combined with nicotine shifted the nicotine dose-response curve to the left and down. A leftward shift was evidenced by the fact that an ineffective dose of nicotine (0.06 mg/kg) plus a low dose of morphine or amphetamine yielded a significant lowering of the reward threshold (paired *t*-test, $p < 0.02$). Downward shifts were seen at the maximally effective dose of nicotine (0.5 mg/kg) as coadministration with morphine or amphetamine produced thresholds significantly lower than this dose of nicotine alone (paired *t*-test, $p < 0.02$).

Experiment 2

The D_2 receptor antagonist, pimozide (0.05–0.2 mg/kg, IP, 4 h after pimozide injection), significantly raised the threshold for rewarding brain stimulation at 0.15 and 0.20 mg/kg (Fig. 2). This figure also illustrates that 0.20 mg/kg pimozide reversed the threshold-lowering effect of a maximally effective dose of nicotine (0.5 or 0.75 mg/kg, SC, 5–90 min after nicotine injection).

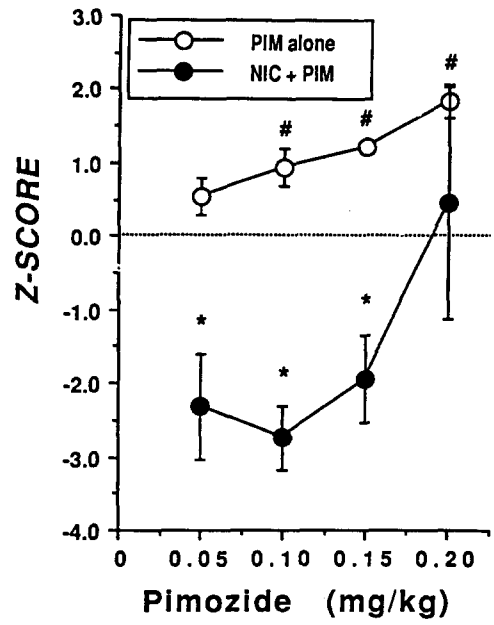

FIG. 2. Mean z-score changes in the reward threshold after administration of various doses of pimozide (PIM) alone or PIM plus a maximally effective dose of nicotine (NIC, 0.5 mg/kg). *Significant threshold lowering for the combination of PIM plus NIC (paired *t*-test, $p < 0.05$, df = 2). #Significant difference (paired *t*-test, $p < 0.05$) between vehicle control treatment and pimozide treatments. Vehicle control threshold change is indicated by a z-score of zero (0.0).

Histology

Electrode placements were verified histologically. The tips of the electrodes were located in the medial forebrain bundle at the level of the lateral hypothalamus in all but one animal that died and for which histological analysis could not be carried out.

DISCUSSION

Nicotine lowered the threshold for stimulation to the medial forebrain bundle using a discrete trial rate-independent method, and although three of the five animals used in the nicotine curve (alone) appeared earlier (14) nicotine lowered the stimulation threshold in the two additional drug-naive animals, adding evidence to the reliability of this effect. Interestingly, the magnitude of nicotine's effects were remarkably similar to opiate and stimulant effects previously observed, an approximate maximum z-score lowering of −3.0 to −4.0 (12,16). We also confirmed the D_2 receptor antagonist, pimozide, decreases an animal's sensitivity to rewarding stimulation using the same experimental paradigm (2). Other previous nicotine work has shown that nicotine clearly enhances rates of responding in self-stimulation (continuous reinforcement) studies (22–24); however, due to nicotine's locomotor stimulant properties it cannot be concluded that increased rates correspond to changes in the rewarding impact of stimulation. For this reason, changes in reward threshold may be preferable. To our knowledge, two other groups using paradigms different from the present procedure tested the effect of nicotine on reward thresholds and failed to show that nicotine increased the sensitivity of animals to the rewarding stimulation (7,25). Methodology differences may explain the disparity

in results. In the present paradigm, the threshold is not dependent upon rate of response and requires a much smaller motor output and the animal receives considerably fewer total pulse trains, approximately six a minute, than that used in other BSR threshold methods.

Moderate doses of morphine (21) or amphetamine (9) reliably lower the threshold for BSR, and when these drugs are given together they potentiate each other's effects (12). Experiment 1 demonstrated that low doses of morphine or amphetamine, with nominal effects on the reward threshold, potentiated the threshold-lowering effect of nicotine by shifting the nicotine dose–response curve both to the left (increased potency) and down (increased efficacy) (see Fig. 1). These data appear to indicate that the same neural substrate, mediating self-stimulation to the MFB, is not only similarly altered by nicotine, morphine, and amphetamine but also synergistically affected by mixtures of them. Moreover, in as far as drug effects on BSR adequately model the hedonic action of drugs in humans these data suggest nicotine consumption augments the pleasurable effects of opiates and psychomotor stimulants. Although there is consistent evidence of dopamine's role in the rewarding effects of the abused opiates and psychomotor stimulants (6,17,18,20,26,28) and extensive evidence that nicotine stimulates the dopamine system (1,3,11,15), a direct link between nicotine's stimulation of dopaminergic activity and nicotine reward was only recently demonstrated by Corrigall et al., in which dopamine receptor antagonists (4) or 6-hydroxydopamine lesions of the nucleus accumbens (5) decreased responding for nicotine self-administration in the rat. The present study adds further support to a dopamine involvement in the reinforcing effects of nicotine by demonstrating that the BSR threshold-lowering effect of nicotine is reversed by the D_2 receptor antagonist pimozide. Even though pimozide, by itself, raises the threshold for rewarding stimulation, there is clear evidence that this is a specific decrease in sensitivity to the rewarding stimulation, not a result of an effect on attention or perception (2) or an effect on motor performance (2,10). Thus, these data demonstrate that nicotine's threshold-lowering effects are not independent of D_2 receptor activity.

In summary, the experiments reported here, along with the reports from other laboratories mentioned above, strongly support the hypothesis that nicotine's rewarding effects are similarly dependent, as are the opiates and the psychomotor stimulants, upon dopaminergic transmission.

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