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Mixed D_2/5-HT_{2A} Antagonism of Amphetamine-Induced Facilitation of Brain Stimulation Reward

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FRANK, R. A., V. TSIBULSKY, S. GROCKI, B. DASHEVSKY, J. H. KEHNE, C. J. SCHMIDT, S. M. SORENSEN. *Mixed D_2/5-HT_{2A} antagonism of amphetamine-induced increases in brain stimulation reward.* PHARMACOL BIOCHEM BEHAV **52**(4) 799–804, 1995. – Recent experiments have demonstrated that 5-HT_{2A} antagonists can modify electrophysiological, neurochemical, and behavioral responses to psychostimulants. These findings led to an interest in using 5-HT_{2A} antagonists to block the effects of psychostimulants on brain reward mechanisms. The present experiments assessed the ability of mixed D_2/5-HT_{2A} antagonists to reverse amphetamine-induced facilitation of self-stimulation. The D_2/5-HT_{2A} antagonists MDL 28,133A and risperidone attenuated the effects of cocaine and amphetamine, but only at antagonist doses that elevated baseline self-stimulation thresholds. A comparison of the effects of the mixed antagonists to those of haloperidol and eticlopride revealed that all four antagonists produced similar anti-stimulant effects when the influence of the drugs on baseline responding was considered. The D_2 activity of the antagonists appears to account for their ability to reduce the effects of psychostimulants on self-stimulation. 5-HT_{2A} antagonism makes a negligible contribution to the anti-amphetamine effects.

Dopamine Serotonin D_2 Receptor 5-HT_{2A} Receptor Self-stimulation Amphetamine Cocaine Drug abuse Haloperidol MDL 28,133A Risperidone Eticlopride

CONSIDERABLE evidence can be mustered supporting the view that amphetamine and other psychostimulants influence a wide variety of behaviors by affecting dopaminergic neurotransmission (2,3,8,11,17,22). In particular, the incentive properties of psychostimulants and their ability to support self-administration appear to involve dopaminergic neurons that project from the ventral tegmental area to the nucleus accumbens (5,9,18), and it has been argued that the addiction liability of psychostimulants is mediated by this neural substrate (7,11). We have recently assessed the possibility that 5-HT_{2A} receptor anatagonists could be used to regulate dopaminergic responses to psychostimulants as part of our efforts to develop pharmacotherapies for stimulant abuse. Serotonergic regulation of dopaminergic function has been well documented [e.g., (1,16)], and recent work has shown that 5-HT_{2A} antagonists reduce the effects of some psychostimulant known to induce dopaminergic hyperactivity. For example, the 5-HT_{2A} antagonist MDL 100,907 attenuates amphetamine-induced hyperlocomotion, and the slowing of A_{10} dopaminergic neuron firing produced by amphetamine (14). MDL 100,907 also attenuates dopamine release induced by the amphetamine analog MDMA, as well as MDMA neurotoxicity (20). These findings raise the possibility that drug or disease-induced patterns of dopaminergic hyperactivity may be rectified by blocking 5-HT_{2A} receptors.

In several recent experiments, the ability of specific 5-HT_{2A} antagonists to block the effects of psychostimulants on brain reward mechanisms was evaluated by assessing their ability to reverse amphetamine and cocaine-induced facilitation of brain stimulation reward. Two highly specific 5-HT_{2A} antagonists, MDL 100,907 and MDL 26,508 (14), had no effect on cocaine, nicotine, or amphetamine-induced increases in brain stimula-

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tion reward (10,12,24). Thus, 5-HT_{2A} antagonism alone was not sufficient to block the psychostimulants' ability to enhance reward. However, the possibility remains that a combination of serotonergic and dopaminergic activity might be more effective than selective dopaminergic or serotonergic antagonism alone. This argument has recently been made for the antipsychotic effects of some atypical neuroleptics such as clozapine, a mixed D_2/5-HT_{2A} antagonist (13). It has been shown that dopaminergic antagonists can reverse amphetamine-induced increases in brain stimulation reward, but this only occurs at antagonist doses that attenuate brain stimulation reward when they are administered alone (4). An appropriate mix of D_2 and 5-HT_{2A} antagonism might reverse the effects of amphetamine without influencing baseline self-stimulation behavior.

The effects of two mixed D_2/5-HT_{2A} antagonists, risperidone and MDL 28,133A, on amphetamine-induced facilitation of brain stimulation reward were assessed in the present experiment, and the effects of the mixed antagonists were compared to two D_2 compounds, eticlopride and haloperidol. A comparison of the mixed antagonists and dopaminergic compounds allowed us to evaluate the contribution of 5-HT_{2A} antagonism to the antistimulant actions of the mixed antagonists.

METHOD

Subjects and Surgery

Male Sprague–Dawley rats (Zivic-Miller Labs, Pittsburgh, PA), weighing between 330–600 g at the time of surgery, were housed individually in stainless steel wire hanging cages. They were maintained on 12 L : 12 D (600–1800, lights on), at 21°C. Food and water were available at all times, except during testing. After 2 weeks of acclimatization, each rat was implanted with bipolar stainless steel electrode (diameter = 0.25 mm, Plastics One Inc., Roanoke, VA) under sodium pentobarbital anesthesia (65 mg/kg, IP). Electrodes were aimed at the ventral tegmental area (AP = 4.4 from bregma, L = 1.4 from the midline, V = 8.5 mm from skull surface, with the skull flat between bregma and lambda).

Apparatus

Rats were trained and tested in 12 metal and acrylic chambers (28 × 21 × 21 cm) with an aluminum rod floor (diameter = 0.5 cm and 1 cm apart). One wall of the chamber had a round hole (the center was 4.5 cm above the floor and had a diameter of 5.0 cm) opening into a dark chamber (9 × 8 × 5 cm) containing an infrared, motion detector. A 1.0 cm excursion of any part of the rat's body (such as its nose) into the small chamber resulted in a signal to a computer. Test chambers were equipped with mercury swivel commutators and 5 W light bulbs, and each test chamber was housed in an individual light and sound attenuation chamber. Two 80286 computers controlled the train duration of bipolar square pulses delivered to the rats' brains by Grass SD9 stimulators. Stimulation frequency and pulse width were set at 100 Hz and 0.5 ms, respectively. Constant current was maintained using a high-impedance stimulation circuit. Stimulating currents were selected such that train-duration response functions were centered around 40–80 ms (see below).

Drugs

D-Amphetamine sulfate was provided courtesy of National Institute on Drug Abuse and Research Triangle Institute, NC. Risperidone was provided by Janssen Pharmaceutica, Beerse, Belgium, and MDL 28,133A [1-(4-fluorophenyl)-2-[4-[(4-methanesulfon-amido-phenyl)carbonyl]-1-piperidinyl]-ethanone hydrochloride] was provided by Marion Merrell Dow, Inc., Cincinnati, OH. Haloperidol lactate was obtained from McNeil Pharmaceutical, Spring House, PA. The doses of each drug are shown in Table 1. The amphetamine doses were selected to include the lowest doses that consistently produces facilitation of brain stimulation reward, and the highest dose that did not induce stereotypies that interfere with operant responses. Antagonist doses were selected so that they produced a minimal effect on self-stimulation response rates and thresholds when administered alone.

Procedure

Subjects were screened for self-stimulation behavior following at least a 10-day, postoperative recovery period. Those that exhibited reliable self-stimulation were trained to respond for stimulation during 45 s trials separated by 15 s time-out periods. Small, 5.0 W house lights signalled the availability of current and were turned off during time-out periods. Training sessions consisted of 45 trials, with each trial composed of a 15 s warm-up and a 30-s test period. The train duration of the stimulation was set at 250 ms during the first phase (7–9 days) of training. Once the subjects were responding reliably during the test, but not time-out periods, the train durations were varied from 100 to 240 ms in random order with steps divisible by 10 ms. The second phase of training lasted 5–7 days. Finally, the computers were programmed to randomly vary the stimulation train duration from 0 to 140 ms, resulting in 15 different test durations. Three blocks of these 15 test durations were used in all subsequent testing. Current levels were adjusted so that the train duration that supported 50% of the maximal self-stimulation response rate fell between 40 and 80 ms. The third training phase lasted 7–9 days until appropriate stimulation currents had been selected, and responding did not vary substantially from day to day.

Each experiment started with all animals receiving the three amphetamine doses across 3 consecutive days of testing, with order of doses counterbalanced across animals. Next, randomized combinations of the stimulants and antagonists were evaluated during 3-day blocks of testing separated by 2-day blocks of saline, baseline testing. The amphetamine-only injections were repeated once all combinations of amphetamine and the antagonist had been administered.

Two experiments using combinations of amphetamine and MDL 28,133A were completed, one with 23 subjects (Experiment A) and the other using 11 (Experiment B). The second experiment was performed to increase the range of doses that

TABLE 1
DRUGS AND DOSAGE LEVELS

Compound	Doses (mg/kg)
Amphetamine	0.33, 0.66, 1.0
Haloperidol	0.05, 0.075
MDL 28,133A	
Experiment A	1.5, 3.0, 6.0
Experiment B	5.0, 7.5
Risperidone	0.1, 0.2, 0.3

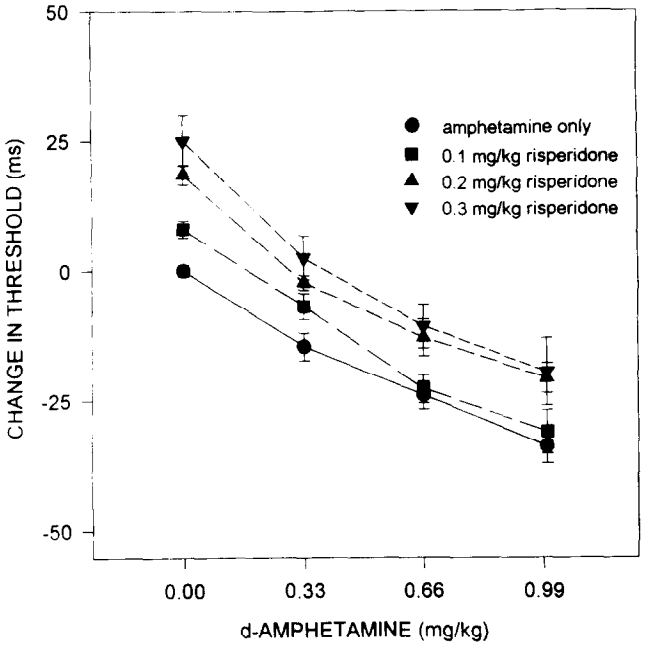


FIG. 1. The effect of risperidone on amphetamine-induced decreases in self-stimulation thresholds. The bars above and below each data point show plus and minus one standard error of the mean.

were tested. Ten rats were used in the amphetamine-haloperidol experiment, while 12 served in the amphetamine-risperidone tests.

Histology

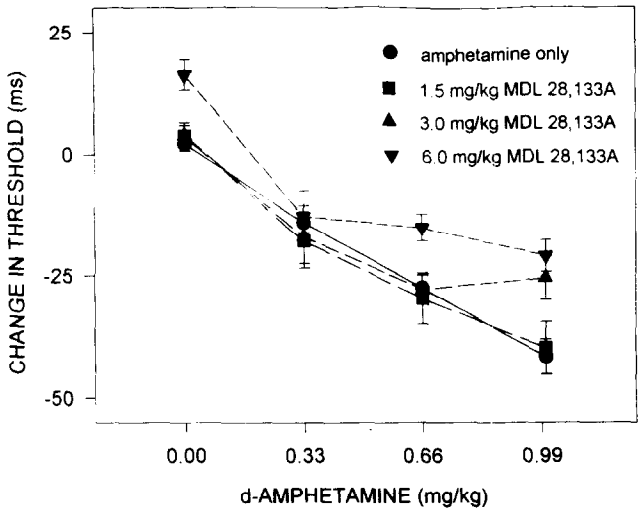
The rats were sacrificed with injection of 130 mg/kg pentobarbital and intracardially perfused with 10% formalin solution. Brains were removed, further fixed, and then frozen and sectioned. Slices (80 μm) were treated as photographic negatives. The location of the electrode tip placements were determined using a stereotaxic atlas (Konig and Klippel, 1963). All placements were found to be along the course of the medial forebrain bundle from the level of the ventral tegmental area to the posterior hypothalamus.

RESULTS

Self-stimulation rates at each train duration were calculated as a mean rate of sampled trials, i.e., trials when rats began self-stimulation during the warm-up period. The average was calculated across the three blocks of trials completed for each testing session. Response rates collected from test trials on which no warm-up sampling occurred were excluded from the analyses. Thresholds were calculated for each test session, and were defined as the train duration that supported 50% of maximal self-stimulation rate observed for the session.

Linear regression was used to fit least-squares equations to the no-drug threshold data of each subject across the course of each experiment. These equations were used to a) determine whether each subject's self-stimulation threshold was stable across the course of the experiment, and b) to calculate baseline self-stimulation thresholds for each stimulant-antagonist pair, adjusted for any threshold drift over the course of the experiment. Thresholds were considered stable if the standard deviation for the no drug, baseline thresholds was less than 10 ms across the course of the experiment. It should also be mentioned that amphetamine's effects were compared before and after coadministration of the antagonists, and no evidence for either sensitization or tolerance to amphetamine's effects was noted in any of the experiments. This is consistent with previous findings from our laboratory (24).

The effects of risperidone, haloperidol, and MDL 28,133A on amphetamine- induced facilitation of brain stimulation reward are shown in Figs. 1-3. Analysis of variance (ANOVA) was used to evaluate the drug interactions in each of the experiments. The main effect F-ratios for both amphetamine and antagonist treatment were significant in all cases (all $p < 0.01$), but no significant interactions between amphetamine and the antagonists were observed (all $p > 0.05$).



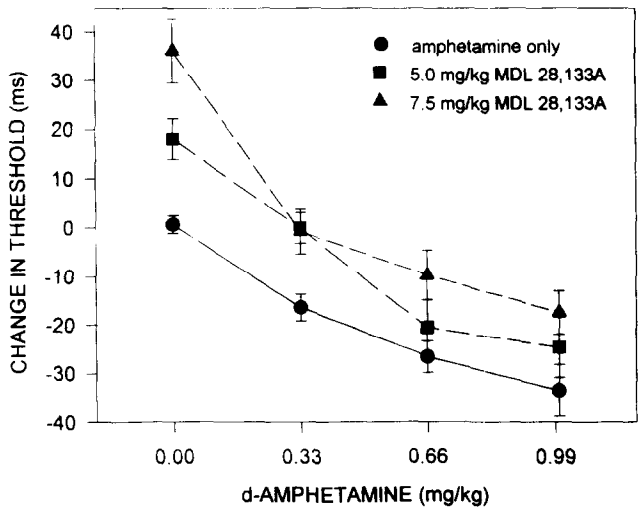


FIG. 2. The effect of MDL 28,133A on amphetamine-induced decreases in self-stimulation thresholds. The results of two experiment that used different dosage ranges are shown. The bars above and below each data point show plus and minus one standard error of the mean.

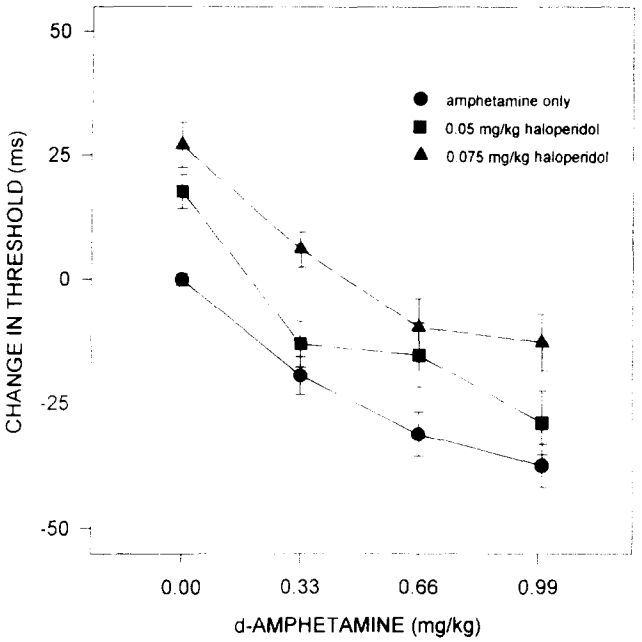


FIG. 3. The effect of haloperidol on amphetamine-induced decreases in self-stimulation thresholds. The bars above and below each data point show plus and minus one standard error of the mean.

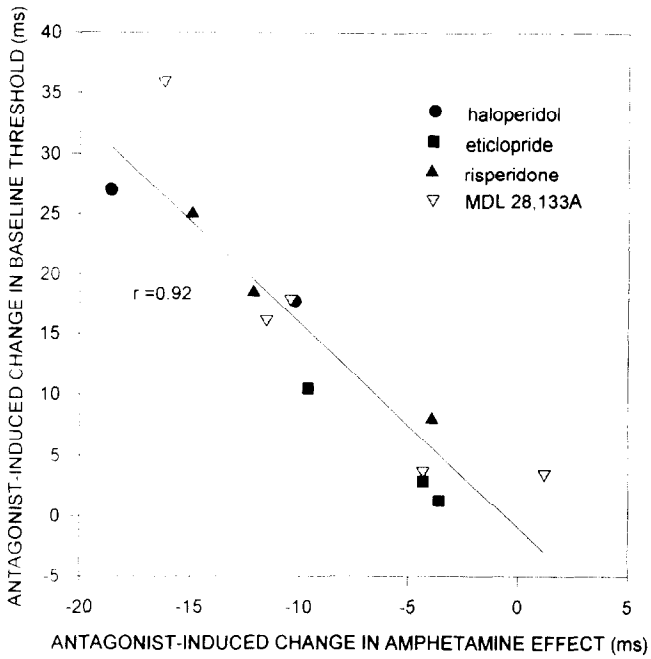


FIG. 4. The relationship between antagonist-induced changes in amphetamine's effects on self-stimulation threshold and threshold changes induced by administration of the antagonists alone. The data points are for each dose of antagonist collapsed across three doses of amphetamine.

Visual inspection of Figs. 1-3 suggests that the ability of the antagonists to attenuate the effects of the stimulants is related to increases in baseline self-stimulation threshold produced when the antagonist is administered alone. This idea was further evaluated by plotting the reduction in amphetamine's effect produced by each dose of antagonist (collapsed across doses of amphetamine) against the threshold increase produced by administering the antagonist alone (see Fig. 4). Data from a recent experiment (24) that examined the same doses of amphetamine combined with the specific D_2 antagonist eticlopride have also been included in the figure. As shown in the figure, the ability of a particular dose of antagonists to attenuate amphetamine's effects is highly correlated with the threshold-increasing effect of that dose, for both the D_2 and mixed D_2/5-HT_{2A} antagonists. A single, linear function provides an excellent fit to the data for both the mixed and specific antagonists.

DISCUSSION

A recent study (24) demonstrated that the 5-HT_{2A} antagonist MDL 100,907 neither elevated self-stimulation thresholds when administered alone, nor enhanced the ability of the specific D_2 antagonist eticlopride to attenuate amphetamine or cocaine-induced facilitation of brain stimulation reward. Likewise, no evidence was found for an advantage of mixed antagonists over D_2 compounds in the present set of experiments. The effects of amphetamine on self-stimulation thresholds were attenuated equally well by D_2 and mixed antagonists. In fact, the ability of the antagonists to attenuate or reverse amphetamine's effects appears to be related to their affinity for the D_2 receptor. Table 2 shows the antagonist doses required to reduce the effects of 0.33 mg/kg amphetamine by 50% (calculated by interpolating between doses). The potency of these drugs as antiamphetamine agents closely resembles their affinity for the D_2 receptor (14,21). This finding is consistent with a large body of evidence supporting a link between the rewarding effects of psychostimulants and dopamine (7,11,15).

5-HT_{2A} antagonism modulates some of the effects of psychostimulants, but not others. For example, the specific 5-HT_{2A} antagonist MDL 100,907 reverses amphetamine's effects on the firing rates of A_{10} neurons (14), locomotion, and latent inhibition of conditioned emotional responses (12). However, it does not affect the cue properties of amphetamine or amphetamine's effect on self-stimulation (12). Several distinct, neurochemical mechanisms appear to mediate the effects of amphetamine on dopaminergic systems, and only some of these mechanisms involve serotonergic regulation. A similar dissociation of neurochemical control was demonstrated in a recent study by Robledo, Maldonado, and Koob (19). These researchers reported that neurotensin injected into the nucleus accumbens blocked the locomotor-activating properties of co-

TABLE 2

ANTAGONIST DOSES PREDICTED TO REDUCE THE SELF-STIMULATION THRESHOLD-LOWERING EFFECTS OF 0.33 mg/kg AMPHETAMINE BY 50%

Antagonist	Dose
Eticlopride	0.033 mg/kg
Haloperidol	0.063 mg/kg
Risperidone	0.10 mg/kg
MDL 28,133A	2.5 mg/kg

caine, but had no effect on cocaine self-administration. This result is reminiscent of MDL 100,907's ability to block amphetamine hyperlocomotion, but not amphetamine-induced facilitation of brain stimulation reward. Perhaps amphetamine's ability to increase locomotion involves neurochemical mechanisms that are distinct from those that mediate these drug's effects on brain reward systems.

As has been reported by others [e.g., (3,4)], there is a mutual antagonism between the effects of psychostimulants and D_2 antagonists on self-stimulation thresholds. Dopamine antagonists appear to attenuate activity in the neural substrate that mediates brain stimulation reward while psychostimulants such as amphetamine amplify activity in this system. The anatomical locus of the drug interaction has not been established, but the available evidence suggests that dopaminergic synapses in the nucleus accumbens are a prime candidate for consideration. The accumbens receives dopaminergic input from the ventral tegmental area, and this pathway has been implicated in the reinforcing effects of psychostimulants and brain stimulation (7,15). According to one view, psychostimulant-induced increases in synaptic dopamine increase postsynaptic binding of dopamine receptors in the accumbens, and thereby augment the reward signal produced by the brain stimulation. D_2 antagonists attenuate the reward signal produced by either brain stimulation or psychostimulants by blocking accumbens dopamine receptors. If postsynaptic receptors in the nucleus accumbens mediate the effects of amphetamine and D_2 antagonists on ventral tegmental area self-stimulation, lesions of the accumbens should block or attenuate the effect of BOTH psychostimulants and neuroleptics on brain stimulation reward. Interestingly, Johnson and Stellar (6) have shown that excitotoxic lesions of the accumbens do not alter thresholds for medial forebrain bundle self-stimulation. It would be interesting to determine whether psychostimulant and neuroleptic-induced changes in brain stimulation reward could be demonstrated in animals with this type of lesion. These studies would further elucidate the neuroanatomical and neuropharmacological mechanisms by which dopaminergic agents influence brain stimulation reward, and would further clarify the relationship between drug and stimulation-induced reward.

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