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Stimulus Effects of *d*-Amphetamine II: DA, NE, and 5-HT Mechanisms

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WEST, W. B., B. J. VAN GROLL AND J. B. APPEL. *Stimulus effects of* d-*amphetamine II: DA, NE, and 5-HT mechanisms.* PHARMACOL BIOCHEM BEHAV 51(1) 69-76, 1995.—Activation of dopaminergic (DA) systems is a necessary component of the behavior effects of *d*-amphetamine, but other neurotransmitters such as norepinephrine (NE) and serotonin (5-HT) appear to modulate DA input; thus, they might have an important role in the stimulus (subjective) effects of this drug. Therefore, rats were trained to discriminate *d*-amphetamine (1 mg/kg) from saline and given combination (antagonism, potentiation) or substitution (generalization) tests with drugs that act through DA, noradrenergic, or serotonergic (5-HT) mechanisms. In the first of two experiments, the D_1 antagonist SCH 39166 blocked the effects of *d*-amphetamine (1 mg/kg) at doses of 0.05, 0.1, and 0.2 mg/kg. NE and 5-HT antagonists including prazosin (0.5–2 mg/kg), idazoxan (1.25–5 mg/kg), ketanserin (0.06–0.15 mg/kg), and metergoline (5–20 mg/kg) had no significant effects on the *d*-amphetamine cue. In the second experiment, neither the α_2-NE agonist clonidine (0.0025–0.1 mg/kg), the β-NE agonist salbutamol (0.05–0.25 mg/kg), nor the NE uptake inhibitor nisoxetine (5–15 mg/kg) had *d*-amphetamine-like effects. The α_2-NE antagonist yohimbine (0.5–2 mg/kg) and the β-NE antagonist propranolol (0.5–3 mg/kg) failed to alter the *d*-amphetamine cue. ICS 205-930 (10 mg/kg) neither mimicked nor blocked the effects of 1 mg/kg of *d*-amphetamine. Indeed, this 5-HT_3 antagonist potentiated the actions of lower doses of *d*-amphetamine (0.25–0.4 mg/kg); the potentiation of the 0.25-mg/kg dose was blocked significantly by the α_1-NE antagonist prazosin (1 mg/kg). These results suggest that the discriminative stimulus effects of *d*-amphetamine are mediated primarily by DA neuronal systems and that these effects are similar to those of drugs that act through 5-HT_3 receptors; this similarity could be mediated by α_1-NE mechanisms.

d-Amphetamine CNS stimulants Dopamine Drug discrimination Norepinephrine Serotonin Clonidine Salbutamol Nisoxetine Yohimbine Propranolol ICS 205-930 Prazosin SCH 39166 Idazoxan Ketanserin Metergoline Rats

THE IDENTIFICATION of receptor subtypes has raised interesting questions about the actions underlying the subjective effects of drugs of abuse. For example, CNS stimulants are thought to act through presynaptic dopaminergic mechanisms (by facilitating release and inhibiting reuptake) (13,25,28,31), but the extent to which these mechanisms are modulated by other neuronal events is not clear. In drug discrimination experiments, the effects of cocaine generalize reliably to dopamine (DA) uptake inhibitors such as mazindol, bupropion, and nomifensine (13,39), and are blocked by DA antagonists, primarily those that act at D_1 receptor subtypes (35); this suggests that inhibition of DA reuptake is the primary action underlying the subjective (as well as other) effects of cocaine. However, the role of DA in the actions of amphetamines has been more difficult to characterize (31,38).

Although DA antagonists block the stimulus properties of *d*-amphetamine (13), DA agonists such as apomorphine, lisuride, SKF 38393, and quinpirole do not have *d*-amphetamine-like effects (13,31,38). In addition, the reinforcing properties of *d*-amphetamine (unlike those of cocaine) are not correlated with ability to bind to DA transporters (31). Finally, rats depleted of DA by 6-hydroxydopamine (6-OHDA) can discriminate *d*-amphetamine from saline, although the dose–response curve in depleted rats is less potent than that of nondepleted controls (40). All of these results suggest that nondopaminergic neuronal systems may contribute to the discriminable as well as other effects of this abused substance. For example, it is known that DA transmission is modified by a number of substances including γ-aminobutyric acid (GABA) (14,36), acetylcholine (14,36), norepinephrine (NE), and 5-hydroxy-

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tryptamine (5-HT) (3,12,15,41). In addition, *d*-amphetamine can facilitate interactions between DA and other neurotransmitters or neuromodulators (29,38).

The contribution of NE to the effects of amphetamine-like compounds has been called minimal, primarily because *d*-amphetamine and its less active *l*-isomer inhibit locus coeruleus activity to the same extent (30,33); nevertheless, both stereoisomers stimulate α_1-NE receptors and sensitize D_2 receptors (18). In addition, *d*-amphetamine binds to α_2-NE receptors and may act as an antagonist at this site (31). The increased locomotor activity that may follow treatment with relatively low doses of *d*-amphetamine (and is blocked by the α-NE antagonist prazosin) is probably the result of drug-induced increases in NE release (15). Psychosis and more pronounced stereotypies that may accompany higher doses of *d*-amphetamine (37) have been attributed to DA release, possibly as a consequence of passive diffusion and subsequent displacement of DA from cytosolic sites (44). Although NE receptors generally have not been thought to play a major role in the stimulus effects of psychomotor stimulants (36), low doses (0.5–2 mg/kg) of *d*-amphetamine have been reported to generalize to nisoxetine, an NE uptake inhibitor, in mice and rhesus monkeys (34). Because higher doses of *d*-amphetamine (2 mg/kg) do not have this effect, different mechanisms may be involved in the in vivo effects of high and low doses of this substance. It has also been found that the nonselective β-NE antagonist propranolol blocks the *d*-amphetamine cue (36).

Serotonin systems also can modulate DA activity such as that which follows treatment with *d*-amphetamine. In particular, 5-HT_3 receptor agonists have been found to release DA in the striatum (3,7,41,43) and nucleus accumbens (24). Moreover, 5-HT_3 receptor antagonists such as ICS 205-930, odansetron (Zophran), and MDL 72222 reverse the hyperactivity caused by direct injections of *d*-amphetamine into the nucleus accumbens, although these compounds do not alter the effects of systemic injections of *d*-amphetamine; it is interesting that ICS 205-930 and odansetron attenuate drug-induced locomotor activity in mice and rats when they are given in combination with cocaine or caffeine (3). When injected directly into the medial prefrontal cortex, 5-HT_3 agonists increase presynaptic dopamine release in vivo (23,34), perhaps by activating 5-HT_3 receptors located on or near DA axon terminals (15). This 5-HT_3-mediated release is accompanied by a corresponding decrease in the extracellular DA metabolites DOPAC and HVA, an effect that is also characteristic of amphetamine-like compounds and may be the result of the release of DA from newly synthesized pools rather than reuptake blockade (15).

There is also evidence that DA may not be the only neurotransmitter mediating reward. In rats, neither rate nor frequency of self stimulation of the medial forebrain bundle are directly correlated with DA levels in the accumbens; that is, the pulse intensity and duration that are consistently rewarding (e.g., for at least 1 h), decrease DA levels (11). In the dorsal raphe nucleus (DRN), cocaine, *d*-amphetamine, and fluoxetine (a 5-HT reuptake inhibitor), but not NE or DA reuptake inhibitors, inhibit the tonic activity of 5-HT_{1A} receptors (17). It has been suggested that this effect is mediated by an increase in 5-HT that interacts with 5-HT_{1A} autoreceptors in the DRN (17). 8-OHDPAT, a 5-HT_{1A} agonist, appears to inhibit DA turnover in the striatum and limbic forebrain (32); however, this effect is not dose dependent, and no change in striatal DA release after 8-OHDPAT has been reported (4). The 5-HT_2 agonist DOI may also increase DA turnover (to 121% of control levels) in the limbic forebrain (32), but the reinforcing properties of amphetamine-like compounds are, if anything, inversely related to their ability to bind to 5-HT_2 receptors (28).

Thus, although the primary actions of *d*-amphetamine are DA release and reuptake inhibition, it is likely that NE and 5-HT systems can modulate these actions and their consequent behavioral (and clinical) manifestations. For these reasons, the effects of DA, NE, and 5-HT agonists and antagonists on the *d*-amphetamine (1 mg/kg) cue were assessed or, more accurately, reassessed in two related experiments.

METHODS

Subjects

Male albino rats of Sprague–Dawley strain (Charles River Breeding Laboratories, Wilmington, MA), 90 days old at the beginning of training, were used. The animals were housed individually in a colony maintained on a 12 L : 12 D schedule; lights were on from 0700 to 1900 h. Temperature and humidity were held constant at 20–22°C and 39–50%, respectively. After a 2-week habituation period in which food and water were freely available, water was restricted to that available during training sessions on weekdays, a 10-min period after test sessions, and at least 24 h on weekends.

Apparatus

Eight commercially available experimental chambers (BRS/LVE 143-23) housed in light- and sound-attenuating shells (BRS/LVE 132-04) were used for both training and testing. Each chamber contained a dipper mounted equidistant between two levers that delivered 0.1 ml of water. A 28-V house light in the chambers was illuminated to signal the onset of training or test sessions.

Training

Following deprivation of water for 23 h, the animals were injected intraperitoneally (IP) with either 1 mg/kg of *d*-amphetamine sulfate or 0.9% saline solution, 15 min before being placed into the chambers. Half the rats in each group were trained to press the right lever to obtain water following *d*-amphetamine, and the left lever following a comparable volume of saline (control); the lever associated with the two training conditions was reversed in the remaining animals. Responding on the incorrect lever was recorded but had no other consequences. *d*-Amphetamine or saline was given randomly, with the restriction that neither condition continued for more than 3 consecutive days. Initially, water reinforcers were provided after each correct response (FR 1). As rates of lever pressing increased, the ratio of correct responses required for reinforcement was raised gradually until all animals were responding under an FR 20 schedule. During this phase of the experiment, sessions were 30 min in duration and occurred 5 days/week. Session length was then decreased to 20 min/day and training continued until performance under the FR 20 schedule stabilized. When all animals reached a criterion of 10 consecutive sessions in which the number of correct responses before the first reinforcer divided by the total number of responses ($\times 100$) equaled or exceeded 80%, test sessions were conducted as described below for each experiment.

Testing

Animals were placed in the chambers as during training, but were given either a novel compound (or compounds) along with *d*-amphetamine (combination or potentiation tests), dif-

ferent doses of *d*-amphetamine (doses-response tests), or a novel compound (agonist or uptake inhibitor) in place of the training drug (substitution tests). All tests ended without reinforcement as soon as 20 responses on one or the other lever occurred or 20 min elapsed, whichever came first. Periodically, the accuracy of discrimination was assessed by exposing the animal to the training drugs (*d*-amphetamine or 0.9% saline) under test conditions. Combination, dose-response, or substitution tests were conducted once or twice per week on different days of the week in a random order provided the animals maintained an accuracy of 80% correct for 3 consecutive training days.

Pharmacologic Procedures

The drugs used in both experiments, routes of administration, and suppliers were: *d*-amphetamine sulfate (IP; Sigma Chemical Co., St. Louis, MO); clonidine (IP; Boehringer Ingelheim, Ridgefield, CT); ICS 205-930 (IP; Sandoz Research Institute, Hanover, NJ); idazoxan HCl (IP; Research Biochemicals, Inc., Natick, MA); ketanserin tartrate (IP; Janssen Pharmaceutica, New Brunswick, NJ); metergoline (IP; Farmitalia Carlo Erba, Milan, Italy); nisoxetine HCl (IP; Eli Lilly, Indianapolis, IN); prazosin HCl (IP; Research Biochemicals, Inc.); propranolol HCl (IP; Ayerst Research Laboratories, Montreal, Canada); salbutamol (IP; Glaxo Research, Ware, Hertfordshire, UK); SCH 39166 HCl [subcutaneously (SC), Schering Co., Bloomfield, NJ]; and yohimbine (IP; Ayerst).

All drugs were dissolved in 0.9% saline and given in a volume of 1 ml/kg, except for metergoline and prazosin. Metergoline was dissolved in 1 : 10 ascorbic acid : deionized water and given in volume of 1 ml/kg; prazosin was either dissolved in 0.9% saline, heated, and given in a volume of 2 ml/kg, or dissolved in 5.0% dextrose, heated, and given in a volume of 1 ml/kg.

Data Analysis

The effects of each drug except those of ICS 205-930 and the combination of ICS 205-930, *d*-amphetamine, and prazosin were analyzed by one way repeated-measures analyses of variance (ANOVAS). When significant F values were obtained ($p < 0.05$), the effects of each dose were assessed further with Student-Newman-Keuls tests. Paired t-tests were conducted to evaluate the effects of the single dose (10 mg/kg) of ICS 205-930; two-way ANOVAS were used to assess the effects of ICS 205-930 in combination with different doses of *d*-amphetamine. When animals did not complete at least 20 responses during a test, percent or rate data were estimated according to a general linear model (26); tests in which fewer than half of the animals did not emit at least 20 responses were discarded.

EXPERIMENT 1

Procedure

Thirty-two rats were given combination tests with *d*-amphetamine (1 mg/kg, 15 min before testing) and the D_1 antagonist SCH 39166 (0.0125, 0.025, 0.05, 0.1, and 0.2 mg/kg, 30 min before testing); prazosin (0, 0.5, 1, and 2 mg/kg, 30 min before testing); idazoxan (0, 1.25, 2.5, and 5 mg/kg, 30 min before testing); ketanserin (0, 0.0625, 0.125, and 0.25 mg/kg, 60 min before testing); and metergoline (0, 5, 10, and 20 mg/kg, 90 min before testing).

Results

Figure 1 shows that, of all the compounds tested, only the D_1 antagonist SCH 39166 reduced the percentage of responding occurring on the *d*-amphetamine lever. The ANOVA indicated that the effect was significant [$F(5, 60) = 14.536$, $p < 0.001$] and occurred at doses of 0.05, 0.1, and 0.2 mg/kg (Student-Newman-Keuls tests). SCH 39166 did not significantly alter response rate [$F(5, 60) = 1.563$, $p = 0.186$].

Prazosin had no effects on percent of *d*-amphetamine lever responding [$F(3, 66) = 0.288$, $p = 0.834$] or response rate [$F(3, 66) = 0.443$; $p = 0.723$]. Idazoxan also failed to alter the tendency of animals to choose the *d*-amphetamine lever [$F(3, 60) = 0.803$, $p = 0.498$] or their rates of responding [$F(3, 60) = 0.215$, $p = 0.886$].

The 5-HT_2 antagonist ketanserin did not change the percentage of *d*-amphetamine lever responding [$F(3, 66) = 0.529$, $p = 0.664$] but did alter the response rate [$F(3, 66) = 3.456$, $p < 0.05$]. However, this effect was neither dose related nor systematic; none of the posthoc comparisons between control and ketanserin treatments were significant. Metergoline had no effect on the percentage of *d*-amphetamine lever responding [$F(3, 60) = 1.563$, $p = 0.208$] but did increase the rate [$F(3, 60) = 2.836$, $p < 0.05$] at the highest dose tested (20 mg/kg).

EXPERIMENT 2

Procedure

Nine animals were given dose-response tests with *d*-amphetamine (0, 0.25, 0.4, 0.5, 1.0, 1.5, and 2.0 mg/kg) and substitution tests with the α_2-NE agonist clonidine (0, 0.0025, 0.025, 0.05, and 0.10 mg/kg), the β_2-NE agonist salbutamol (0, 0.05, 0.1, and 0.2 mg/kg), and the NE uptake inhibitor nisoxetine (0, 5, 10, and 15 mg/kg); in all of these tests, drugs were injected 15 min before behavioral testing. In combination tests, rats were given 1 mg/kg of *d*-amphetamine (15 min before testing) after administration of one of the following drugs: a) the α_2-NE antagonist yohimbine (0, 0.5, 1, 1.5, and 2 mg/kg, 30 min before testing), and b) the β-NE antagonist propranolol (0, 0.5, 2, and 3 mg/kg, 15 min before testing). They then received both the 5-HT_3 antagonist ICS 205-930 (10 mg/kg, 30 min before testing) and *d*-amphetamine (0.25, 0.4, and 1 mg/kg, 15 min before testing). Finally, rats treated with both *d*-amphetamine (0.25 mg/kg) and ICS 205-930 (10 mg/kg) as before were given prazosin (1 mg/kg, 30 min before testing).

Results

The results of dose-response tests with *d*-amphetamine and substitution tests with three compounds that act through various NE mechanisms (clonidine, salbutamol, and nisoxetine) are shown in Fig. 2. Among these agents, only *d*-amphetamine engendered significant amounts of responding on the drug-appropriate lever [$F(6, 55) = 38.701$, $p < 0.001$]; doses of 0.4–2 mg/kg substituted for the training drug (1 mg/kg). d-Amphetamine also enhanced the response rate significantly [$F(6, 55) = 3.842$; $p < 0.005$], but this effect was not dose related and occurred only after 0.25 mg/kg.

Clonidine had no significant effect on the percentage of *d*-amphetamine lever responding [$F(4, 36) = 0.909$, $p = 0.470$]; however, this α-NE agonist did increase the response rate [$F(4, 36) = 6.517$, $p < 0.001$], but only at a dose of 0.0025 mg/kg (Fig. 2).

Salbutamol decreased both the amount of responding on

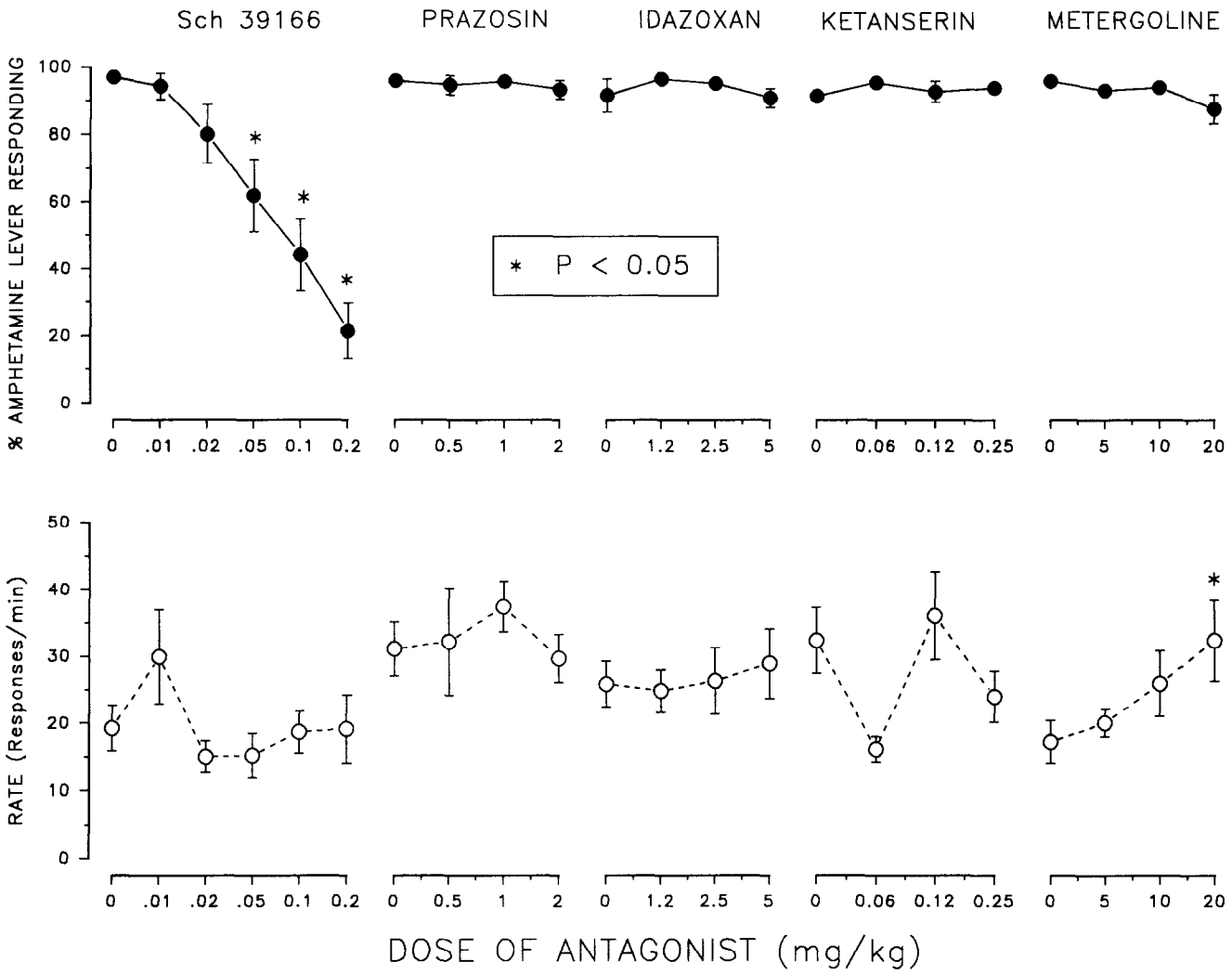

FIG. 1. Results of combination tests with D_1 (SCH 39166), NE (prazosin, idazoxan), and 5-HT (ketanserin, metergoline) antagonists and *d*-amphetamine in rats trained to discriminate *d*-amphetamine (1 mg/kg) from saline. The effects of each combination on response choice (mean percentage of responding on the lever associated with *d*-amphetamine) during test sessions (± SEM) are shown in the top panels (●, —); effects on mean response rates (± SEM) are shown in the bottom panels (○, ---). *Significant difference between the specific combination of a test drug and *d*-amphetamine (1 mg/kg) and the test drug vehicle and *d*-amphetamine (Student-Newman-Keuls test).

the *d*-amphetamine lever [$F(3, 24) = 3.128$, $p < 0.05$] and the response rate [$F(3, 24) = 5.871$, $p < 0.001$]; however, none of the posthoc tests (on the rate variable) were significant. Nisoxetine had no effect on the percentage of *d*-amphetamine lever responding [$F(3, 20) = 1.969$, $p = 0.157$] but did increase the response rate [$F(3, 20) = 6.669$, $p = 0.001$] at a dose of 5 mg/kg.

The results of combination tests with two NE antagonists are shown in Fig. 3. At the doses tested, yohimbine had no significant effect on either percent responding on the *d*-amphetamine lever [$F(4, 29) = 0.604$, $p = 0.664$] or the response rate [$F(4, 27) = 0.949$, $p = 0.454$]. Similarly, propranolol had no effects on either the percentage [$F(3, 24) = 0.804$, $p = 0.506$] or the rate [$F(3, 23) = 1.113$, $p = 0.367$].

The effects of ICS 205-930 given alone, in combination with *d*-amphetamine (0.25, 0.40, and 1 mg/kg), and in combination with both *d*-amphetamine (0.25 mg/kg) and prazosin (10 mg/kg) are shown in Fig. 4. When given alone (Fig. 4, left side of dotted line), this 5-HT_3 antagonist engendered no more *d*-amphetamine lever responding than vehicle control [$t(7) = 0.566$, $p = 0.589$]; it also had no effect on the response rate [$t(7) = 0.098$, $p = 0.925$]. However, when given in combination with *d*-amphetamine, ICS 205-930 increased the percentage of choice responding that occurred on the *d*-amphetamine lever significantly [$F(2, 12) = 15.168$, $p < 0.001$]; posthoc tests indicated that this potentiating effect occurred after doses of both 0.25 and 0.4 mg/kg of *d*-amphetamine. The effect of ICS 205-930 on the percentage of responding induced by 0.25 mg/kg of *d*-amphetamine was blocked completely by prazosin [$F(2, 15) = 55.550$, $p < 0.001$], a drug which, when given in combination with *d*-amphetamine (without ICS 205-930), has no significant effect on *d*-amphetamine lever responding (see earlier discussion). Neither the combination of ICS 205-930 and *d*-amphetamine [$F(2, 11) = 3.741$, $p =$

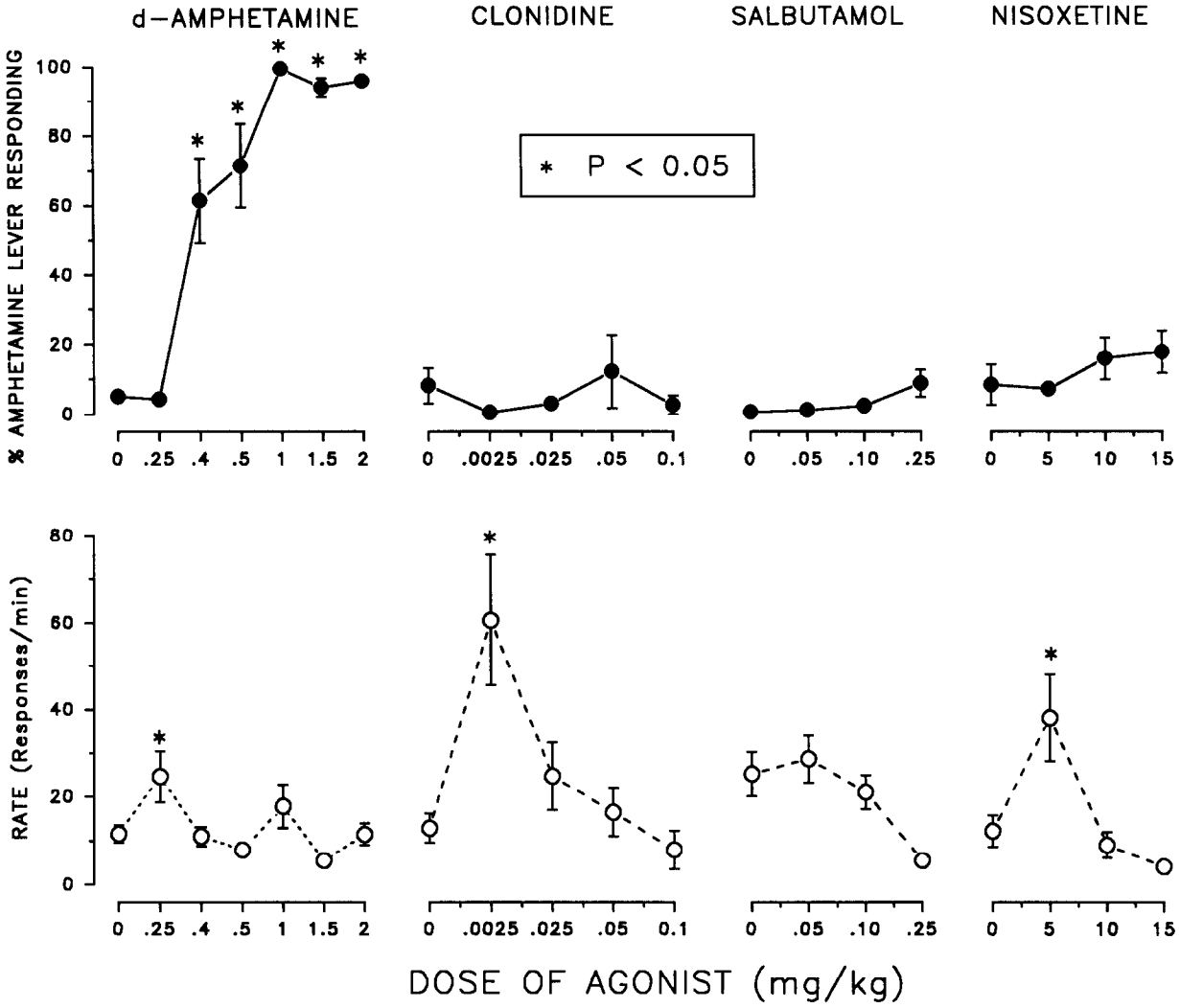

FIG. 2. Results of dose-response tests with *d*-amphetamine and substitution tests with three compounds that act primarily through adrenergic mechanisms in rats trained to discriminate *d*-amphetamine (1 mg/kg) from saline. The effects of each compound on response choice during test sessions (±SEM) are shown in the top panels (●, —); effects on mean response rates (±SEM) are shown in the bottom panels (○, ---). *Significant difference between a particular dose and vehicle control (Student-Newman-Keuls test).

0.058] nor ICS 205-930, *d*-amphetamine, and prazosin [$F(2, 12) = 2.152$, $p = 0.159$] had significant effects on the response rate.

DISCUSSION

The results of these experiments can be summarized as follows: The D_1 antagonist SCH 39166 (0.05–0.2 mg/kg) blocked the discriminative stimulus properties of *d*-amphetamine (1 mg/kg), whereas neither NE (prazosin, 0.5–2 mg/kg; idazoxan, 1.25–50 mg/kg) nor 5-HT (ketanserin, 0.06–0.15 mg/kg; metergoline 5–20 mg/kg) antagonists had similar effects. In a second experiment, neither the α_2-NE agonist clonidine (0.0025–0.1 mg/kg), β-NE agonist salbutamol (0.05–0.25 mg/kg), nor NE uptake inhibitor nisoxetine (5–15 mg/kg) had amphetamine-like effects. The α_2-NE antagonist yohimbine (0.5–2 mg/kg) and β-NE antagonist propranolol (0.5–3 mg/kg) did not alter the stimulus properties of the training drug. ICS 205-930 (10 mg/kg) neither mimicked nor blocked the effects of *d*-amphetamine (1 mg/kg); however, this 5-HT_3 antagonist appeared to potentiate the effects of relatively low doses of *d*-amphetamine (0.25–0.4 mg/kg); the potentiation of the 0.25 mg/kg dose was blocked by prazosin (1 mg/kg).

Although the role of DA in the behavioral effects of *d*-amphetamine is by no means clear, it is better understood than that of most other biogenic amine neurotransmitter systems. Therefore, the most interesting aspects of these data are probably: a) similarities in the effects of *d*-amphetamine and compounds that act at 5-HT_3 receptor sites; and b) how these effects appear to be modulated by other (e.g., NE) neuronal systems. As mentioned previously, 5-HT antagonists such as ketanserin, metergoline, and ICS 205-930 normally block amphetamine-like behavioral effects, but in the present experiments, ICS 205-930 potentiated low doses (0.25–0.4 mg/kg) of the stimulus effects of this compound. Because 5-HT antagonists usually reduce DA concentration in both the caudate

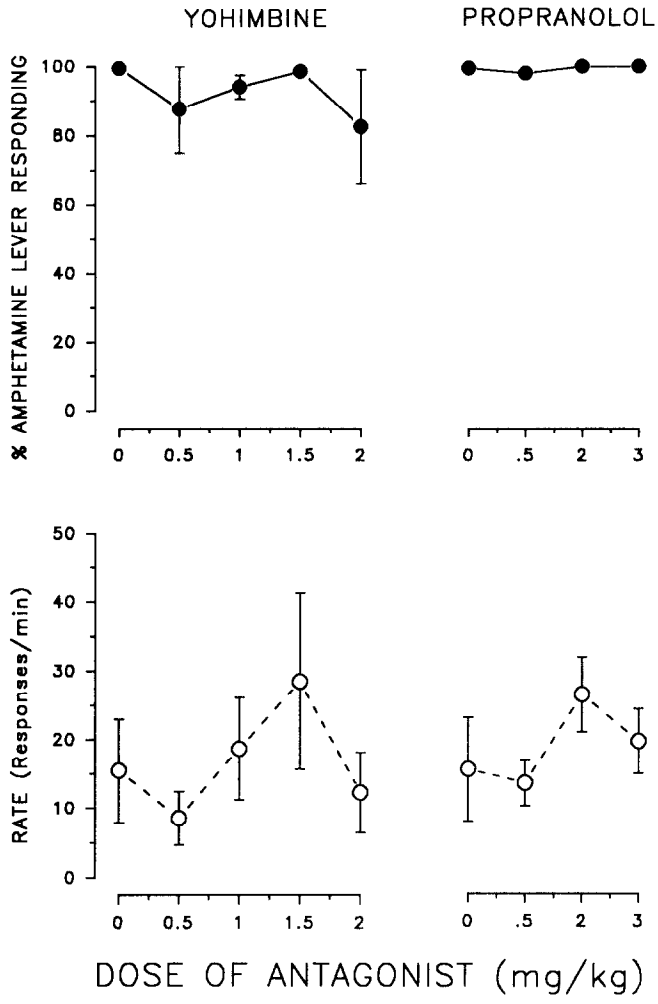

FIG. 3. Results of combinations of *d*-amphetamine (1 mg/kg) with two additional NE antagonists in rats trained to discriminate *d*-amphetamine from saline.

and nucleus accumbens (1-3), this potentiation was unexpected. However, the ability of 5-HT antagonists to block elevated DA levels in these areas of the brain is commensurate with their ability to block behavioral correlates of drugs that increase DA levels in the CNS (8). Moreover, both ICS 205-930 and MDL 72222 block place preferences and place aversions conditioned with methylenedioxymethamphetamine (MDMA), nicotine, and morphine but, interestingly, not *d*-amphetamine (6). It has been suggested that this effect may be the result of the failure of *d*-amphetamine to increase the firing rate of DA neurons, and thus does not depend on presynaptic 5-HT_3 receptors (2,3).

The potentiation of low doses of *d*-amphetamine by ICS 205-930 was reversed (at least, partially) by prazosin. Because the rate of NE synthesis is regulated by the same mechanism(s) as DA synthesis, *d*-amphetamine may have similar effects on postsynaptic DA and NE neurons (10,11,21). In addition, *d*-amphetamine binds to α_2 receptors and is thought to act as an antagonist at this site (21). α_2-NE antagonists such as yohimbine and idazoxan increase NE release subsequent to blocking α_2-NE receptors (16). Reserpine-induced depletion of α_2-NE increases tyrosine hydroxylase activity and abolishes the synthesis-enhancing effects of these antagonists. The enhanced release of NE may result in increased stimulation of α_1 receptors that are thought to act in concert with D_1 receptors to increase the sensitivity of postsynaptic D_2 neurons (18).

Although the potentiation of *d*-amphetamine by ICS 205-930 was reversed by prazosin, it was not reversed completely. This suggests, among other things, that α_1-NE agonists reduce the ability of biogenic amine transmitters to bind to postsynaptic receptors (24). Because NE interacts with both DA and 5-HT systems (21), the extent to which either or both of these neurotransmitters may be involved in the reversal of the effects of ICS 205-930 on the *d*-amphetamine cue is not clear. In addition, a decrease in the sensitivity of postsynaptic D_2 receptors has the same effect (at least in the substantia nigra) as a decrease in activity of 5-HT_3 receptors (23,29). The failure of prazosin to block an amphetamine-induced conditioned response in this experiment might indicate that prazosin primarily affects 5-HT systems; however, this α_1-NE antagonist has a substantial inhibitory effect on amphetamine-induced unconditioned behaviors by DA systems (unpublished observations).

Although α-NE receptors may play a role in the stimulus effects of *d*-amphetamine, it is important to note that neither clonidine, salbutamol, nor nisoxetine had significant amphetamine-like effects; in addition, neither propranolol, yohimbine, prazosin, nor idazoxan blocked the *d*-amphetamine (1

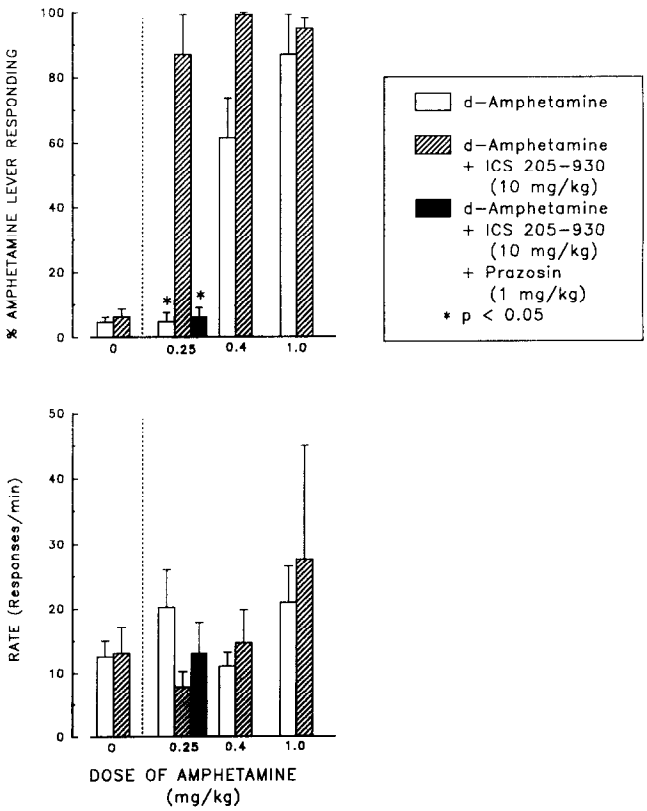

FIG. 4. Results of dose-response tests with *d*-amphetamine (open bars); combination (potentiation) tests with *d*-amphetamine and the 5-HT_3 antagonist ICS 205-930 (10 mg/kg, striped bars); and the combination of *d*-amphetamine (0.25 mg/kg), ICS 205-930 (10 mg/kg), and the α_1-NE antagonist prazosin (1 mg/kg; solid bars).

mg/kg) cue. Thus, neither activation nor blockade of NE is sufficient to alter the discriminative stimulus properties of *d*-amphetamine. Similarly, even though ICS 205-930 may potentiate the effects of low doses of *d*-amphetamine (see earlier discussion), the failure of ketanserin and metergoline to block the *d*-amphetamine cue indicates that 5-HT receptor activity is not sufficient to explain the occurrence of amphetamine-like stimulus effects.

Thus, the present results suggest that the primary mechanisms underlying the discriminable and other behavioral effects of *d*-amphetamine involve DA, primarily D_1 receptors, but activity at these sites can be modulated by α-NE and 5-HT mechanisms. This does not rule out the possibility that interactions between D_1 and D_2 receptors could also affect the *d*-amphetamine cue. Because the only drug that blocked *d*-amphetamine completely, SCH 39166, is a full-efficacy D_1 antagonist (37), the results are consistent with the limited antagonism of the behavioral effects of CNS stimulants reported previously with SCH 23990 (5,10,33). SCH 23990 has less affinity for DA receptors than SCH 39166 (35) and may be a partial 5-HT antagonist at higher doses (10).

Although these data suggest that D_1 receptors are involved in the stimulus effects of *d*-amphetamine, the D_2 antagonist sulpiride has also been reported to block the *d*-amphetamine cue (27,28). Thus, it is possible that various subtypes of DA receptors interact, and that antagonism of D_2 mechanisms decrease D_1-mediated neurotransmission. Such interactions have been reported both in vitro (9) and in vivo (31,32), but their functional significance is not clearly understood and may depend on the type of behavior being studied. Indeed, both synergistic (31) and oppositional (20) interactions at different DA receptor subtypes have been identified.

Perhaps more relevant to the present results is a lack of evidence that D_1-D_2 interactions occur in drug discrimination situations. The D_2 agonist quinpirole has been reported to potentiate the effects of SKF 38393, but SKF 38393 does not potentiate the effects of quinpirole (42). Such data suggest that D_2 receptors may modulate neuronal systems mediating specific behaviors; they also suggest that D_1 receptor activation may be necessary, but not sufficient to activate D_2 receptors. Increases in D_1 activity may stimulate D_2 receptors, but not necessarily to their maximal levels of activity. Maximal activation of D_2 receptors by a full-efficacy agonist such as quinpirole precludes further activation as a result of increasing D_1 activity. Endogenous DA released by *d*-amphetamine may stimulate D_1 receptors at relatively low doses (23) and both D_1 and D_2 receptors at higher doses (22). Moreover, the latency of onset of D_2 receptor activity is probably a function of dose of *d*-amphetamine. Thus, differences in dose and interinjection interval may account for differences in extent of antagonism of, and substitution for, *d*-amphetamine (8,19).

In conclusion, the results of these experiments indicate that activation of D_1 receptors is necessary for *d*-amphetamine to function as a discriminative stimulus; they also suggest that interactions between D_1 and D_2 (and perhaps, other subtypes of DA) receptors are likely to play a role in the *d*-amphetamine cue. Serotonin receptor antagonists such as ketanserin, metergoline, and ICS 205-930 do not alter the *d*-amphetamine cue, which suggests that 5-HT receptor activity is not sufficient to explain the occurrence of amphetamine-like effects. Although ICS 205-930 potentiates the stimulus properties of low doses of *d*-amphetamine, it is likely that the mechanism subserving this potentiation (unlike that of *d*-amphetamine) involves D_2 (rather than 5-HT_3) receptors. Because ICS 205-930-induced potentiation of *d*-amphetamine can be blocked (at least partially) by the α_1-NE antagonist prazosin (which, when given alone does not block the *d*-amphetamine cue), the effects of prazosin on the interaction between *d*-amphetamine and ICS 205-930 may result from actions at 5-HT rather than DA receptor systems.

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