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Effects of Cinanserin and p-Chlorophenylalanine and Their Interaction with d-Amphetamine on DRL Performance in Rats[1]

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MELE, P. C. AND M. A. CAPLAN. *Effects of cinanserin and p-chlorophenylalanine and their interaction with d-amphetamine on DRL performance in rats.* PHARMAC. BIOCHEM. BEHAV. 12(6) 883–891, 1980.—The effects of the serotonin antagonist cinanserin and the serotonin depletor p-chlorophenylalanine (PCPA) were compared with the effects of d-amphetamine on responding maintained by differential-reinforcement-of-low-rate schedule (DRL). d-Amphetamine (0.25–2.0 mg/kg) increased response rates and shortened interresponse times (IRTs). Cinanserin at low doses (8, 16 and 32 mg/kg) did not alter DRL responding; high doses (48 and 64 mg/kg) decreased response rates and shortened IRTs. PCPA (200 and 300 mg/kg) decreased DRL response rates and disrupted the IRT distributions for up to 72 hours post-injection, but had few effects over the subsequent 7–8 day period. d-Amphetamine given in combination with cinanserin or administered 3, 8 and 12 days post-PCPA administration resulted in decreased response rates relative to those induced by d-amphetamine alone; the d-amphetamine-induced shortening of IRTs persisted. These results suggest that cinanserin and PCPA do not exert general response-stimulant effects and that serotonergic systems are not of major functional significance in the maintenance of low rate DRL responding. These results do suggest that serotonergic systems are involved in the manifestation of the behavioral response to amphetamine, possibly as a result of a serotonergic-catecholaminergic interaction.

Cinanserin p-Chlorophenylalanine Amphetamine Drug interactions DRL Response rate Serotonin

SCHEDULE-controlled operant behavior has been shown to be affected by drugs which interfere with the normal functioning of central serotonin systems. A number of studies have reported that these drugs increased rates of responding which had been suppressed by punishment (electric shock). Such punishment attenuating effects were demonstrated in rats with the serotonin depleter p-chlorophenylalanine (PCPA) [10, 13, 40, 58], and in rats or pigeons with the structurally diverse serotonin antagonists cinanserin [9,14], cyproheptadine [16] and the lysergic acid derivatives methysergide and bromolysergic acid [9, 16, 18, 49, 57]. Although some reports have failed to demonstrate increases in punishment-suppressed responding with either PCPA [3] or cinanserin [57], most authors have interpreted the punishment-attenuating effects of the serotonin antagonists and depletors as indicating that serotonin acts as a mediating factor in punishment-induced response suppression [9, 40, 49, 58].

When examining the effects of drugs on schedule-controlled behavior, the ongoing control rate of responding has been shown to be an important determinant of the behavioral effects of various drugs, regardless of the specific reinforcement contingencies that maintained the responding [27]. In studies where drugs have been shown to increase both punished and unpunished rates of responding, the punishment-attenuating effects of these drugs have often been attributed, either in whole or in part, to their nonspecific rate-enhancing effects. This has been true for several of the benzodiazepines as well as for pentobarbital, a barbiturate, and ethanol [15, 32, 59]. Of the antiserotonergic agents, methysergide, bromolysergic acid and cyproheptadine have been reported to increase unpunished as well as punished responding in rats and pigeons performing on a variety of reinforcement schedules [9, 17, 18, 46, 49, 57], suggesting that a similar relationship may hold for these drugs as well.

Reports of cinanserin and PCPA-induced increases in unpunished responding have been limited and inconsistent [41,58]. In most instances these drugs have either not altered or have decreased unpunished rates of responding [9, 14, 57]. Only Tanaka, Yoh and Takaori [51], using a Sidman avoidance procedure, have reported substantial increases in the response rates of rats following PCPA administration. They also reported that there was a direct relationship be-

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tween the magnitude of these rate increases and the degree to which central serotonin levels were depleted over a 10 day post-injection period. It is possible that these results reflect the functional equivalent of animals performing under increased shock intensities and are not representative of nonspecific rate-enhancing effects. This is suggested by studies showing that PCPA decreased the aversive thresholds of rats to foot shock [11,52] and that avoidance response rates were directly related to shock intensities [39].

It appears that the degree to which cinanserin and PCPA increase rates of responding in general is presently unclear. The purpose of the present study was to examine cinanserin and PCPA on responding maintained by a differential-reinforcement-of-low rate (DRL) schedule. The DRL schedule, by specifying that responses must be spaced by a minimum time interval in order for reinforcement to occur, typically engenders a stable low rate of responding. Responding maintained by a DRL schedule has been shown to be sensitive to the rate-increasing effects of various drugs, such as amphetamine [26,45], methyl-phenidate [50], pentobarbital [45,50], phenobarbital [26], chlordiazepoxide [42], and diazepam [5]. Responding maintained by a DRL schedule has not been systematically examined with any of the antiserotonergic agents. A second objective of the present study was to determine whether serotonin has a functional role in the maintenance of this particular schedule-suppressed behavior, as has been previously suggested for punishment-suppressed responding [9, 40, 49, 58].

It has been shown that alterations in DRL response rate may be due to alterations either in the schedule-induced temporal spacing of responses (e.g. temporal discrimination) or in the length of the post-reinforcement pause [33,34]. These response measures are inherently confounded in the typical DRL situation where the time between responses is recorded on a single manipulandum. A two-lever DRL task has been used previously in attempts to distinguish between alterations in these different measures of DRL responding [33,34]. Both single-lever and two-lever DRL procedures were compared in the present study to be able to differentially examine the effects of cinanserin and PCPA on the temporal discrimination and on the length of post-reinforcement pauses.

Amphetamine was used as a comparison drug to assess the potential response rate increasing effects of cinanserin and PCPA on DRL performance. Combinations of cinanserin or PCPA with amphetamine were also studied since previous reports have shown that the various antiserotonergic agents are capable of modifying the behavioral effects of amphetamine. These agents have been reported to potentiate [4, 17, 23, 31, 46, 53], to antagonize [48,53], or to have no effect on [1, 37, 48] amphetamine-induced behavioral effects, depending upon the particular antiserotonergic agent and procedure used. It was therefore decided to directly compare cinanserin and PCPA in conjunction with amphetamine on DRL performance to determine whether similar effects could be obtained with a serotonin antagonist and a depletor within this particular behavioral paradigm.

METHOD

Animals

Six male Long-Evans hooded rats were individually housed and maintained at 80% of their free feeding weights with water freely available in the home cage. All animals were approximately 90–120 days old at the start of the experiment and had no previous history of drug treatment. For each animal, testing was carried out at the same time each day during the light portion of a 12 hour light/dark cycle.

Apparatus

A conventional operant conditioning rat chamber was used which measured 23 cm long × 17.5 cm wide × 19 cm high. The chamber was constructed of aluminum walls, a Plexiglas top, and a steel grid floor and was enclosed in a sound and light attenuating wooden compartment. Two levers mounted on microswitches protruded into the chamber from the left (lever-A) and right (lever-B) sides of the front wall. A stimulus light was located above each lever and a feeder cup was located between the two levers into which a dipper presented 0.02 ml of liquid (1:1 mixture of sweetened condensed milk and tap water). All programming and data collection were done with solid state logic modules, digital counters, and a cumulative recorder located in an adjoining room.

Behavioral Procedure

All rats were initially shaped to press the right lever (lever-B) for milk reinforcement. Two of the rats (R7 and R8) were maintained on a DRL schedule, which required that the responses on lever-B be spaced by a minimum time interval in order for reinforcement to be obtained; the minimum time interval was gradually increased from 2 to 18 sec in 2 sec steps. The DRL criterion value was increased only when the IRTs showed shifts toward longer values and resulted in an increased frequency of reinforcement, indicating that the animals' performances were being progressively shaped by this schedule (designated as the single-lever DRL schedule). For these two rats responses on lever-A had no programmed consequence. The other four rats (R1, R5, R6, R9) were placed on a schedule under which a single response on each of the two different levers was required for reinforcement [7]; that is, a response on lever-A followed by a response on lever-B [34]. A lever-A response turned on the signal light above this lever, while a lever-B response extinguished this light and operated the dipper. A DRL contingency was then introduced which required that the rats first press lever-A, then press lever-B after a minimum time interval had elapsed. Responses in the sequence A-A or B-B had no programmed consequences. The minimum time interval required between an A response and a B response was increased in a manner similar to that for the single-lever DRL schedule; this schedule was referred to as the A-B DRL schedule. For the animals performing on the A-B DRL schedule, a single A-B response sequence was considered as one response. Once a criterion value of 18 sec had been reached for both the single-lever and the A-B DRL schedules, training was continued for an additional 50–80 sessions at which time responding was considered stable.

Drug Procedure

d-Amphetamine sulfate (Smith, Kline and French Laboratories, Philadelphia, PA) and cinanserin hydrochloride (Squibb Institute for Medical Research, Princeton, NJ) were dissolved in saline at concentrations of 1 mg/ml and 25 mg/ml respectively. p-Chlorophenylalanine methyl ester hydrochloride (ICN Biochemicals, Plainview, NY) was mixed in saline at a concentration of 100 mg/ml with several drops of Tween 80 at pH 7.0. All drug doses are expressed in terms of the salt.

All animals were adapted to the injection procedure by

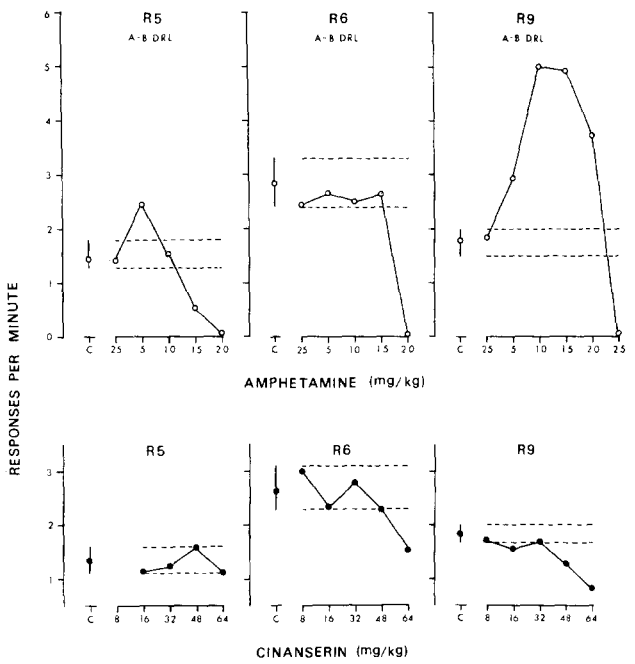

FIG. 1. Effects of amphetamine and cinanserin on A-B DRL 18 sec response rates. A single A-B response sequence was considered as one response. The points labelled C represent the mean control rates of responding of 10 noninjection or saline control days. Vertical lines denote the range of control response rates.

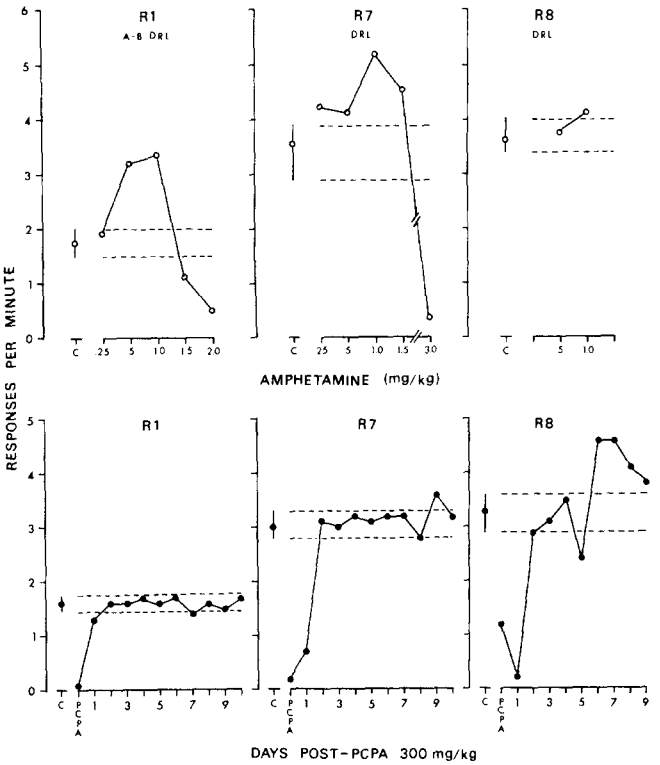

FIG. 2. Effects of amphetamine and PCPA (300 mg/kg) on A-B and single-lever DRL 18 sec response rates. For rat 1 performing on the A-B DRL schedule, a single A-B response sequence was considered as one response. The points labelled C represent the mean control rates of responding of 10 noninjection or saline control days for amphetamine and 6 days for PCPA. Vertical lines denote the range of control response rates.

administering physiological saline in a range of volumes from 0.3 to 1.0 ml. Dose-response functions for amphetamine were then determined for all six rats. Doses of amphetamine were administered in a mixed order, with all doses administered once during the first series of injections which was then repeated. During the second series of injections amphetamine was administered in combination with saline as a control for the amphetamine plus cinanserin phase. Since the effects of amphetamine were similar either with or without saline, the two determinations were averaged. Three rats performing on the A-B DRL schedule were then used to determine dose-response functions for cinanserin (duplicate determinations, mixed order) and the remaining three animals received two doses of PCPA methyl ester hydrochloride (single determinations). Combinations of amphetamine and cinanserin (single determinations, mixed order) were then administered to the three rats previously given cinanserin alone. Finally, all six rats were administered PCPA followed by amphetamine on the 3rd, 8th, 12th and either the 19th or 26th day post-PCPA. Saline control injections were administered periodically throughout the experiment, including multiple saline injections during the amphetamine plus cinanserin phase and a saline injection on the 5th day post-PCPA during the amphetamine plus PCPA phase. For the PCPA phases, the control injections consisted of a few drops of Tween 80 suspended in saline which was administered at least five days preceding PCPA administration. All injections were administered intraperitoneally. Amphetamine was administered 10 min before the experimental session, cinanserin 60 min before, and PCPA in a single dose 90 min before.

At least one week intervened between the different drug phases and injections were administered no more than twice per week. Amphetamine, cinanserin and combinations of these drugs were usually administered on Wednesdays and Saturdays, with Tuesdays and Fridays serving as control sessions. For PCPA, at least two weeks and usually four weeks elapsed between injections.

RESULTS

All rats exhibited low stable rates of responding that were maintained throughout the course of the experiment. The unconnected points labelled "C" in the upper and lower panels of Figs. 1 and 2 show that the mean control rates of responding and the range of response rates about the means were similar during the different drug phases for the individual rats.

The top panels of Figs. 1 and 2 show that amphetamine altered the response rates of all rats in a dose-dependent manner. Increased rates of responding were observed at one or more dose levels (0.25–2.0 mg/kg) for five of the six rats performing on either the A-B or the single-lever DRL schedule. For two of the rats (R9 and R7), rate increases were observed over a range of four doses with peak effects occurring at the 1.0 mg/kg dose. These results show that A-B and single-lever DRL responding were similarly sensitive to the response rate increasing effects of amphetamine. The rate of responding was not increased by any dose of amphetamine for one rat (R6). Decreased rates of responding were also observed for the five rats tested with the higher doses of amphetamine. Rate decreases were moderate for

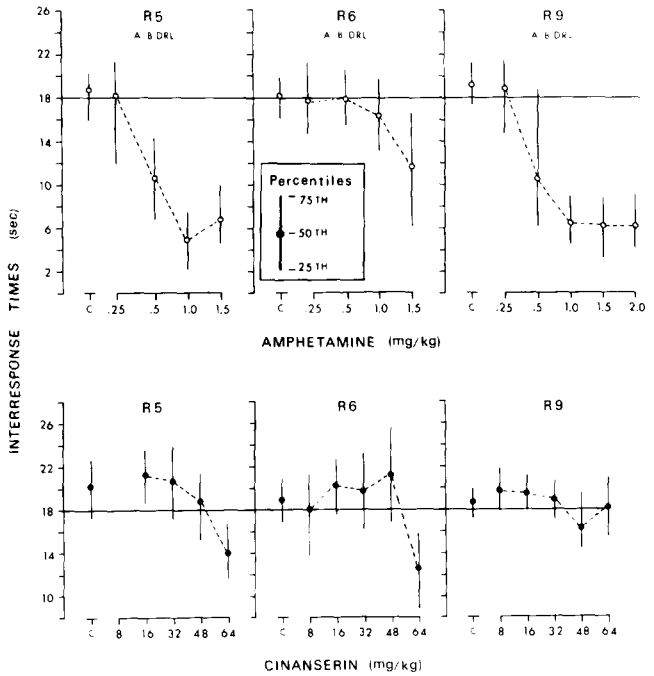

FIG. 3. Effects of amphetamine and cinanserin on A-B DRL interresponse times presented as the semi-interquartile range (middle 50%) of the interresponse time distributions excluding all interresponse times<2 sec. The points labelled C represent the mean control rates of responding of 10 noninjection or saline control days. The horizontal line at 18 sec on the ordinate denotes the DRL criterion value.

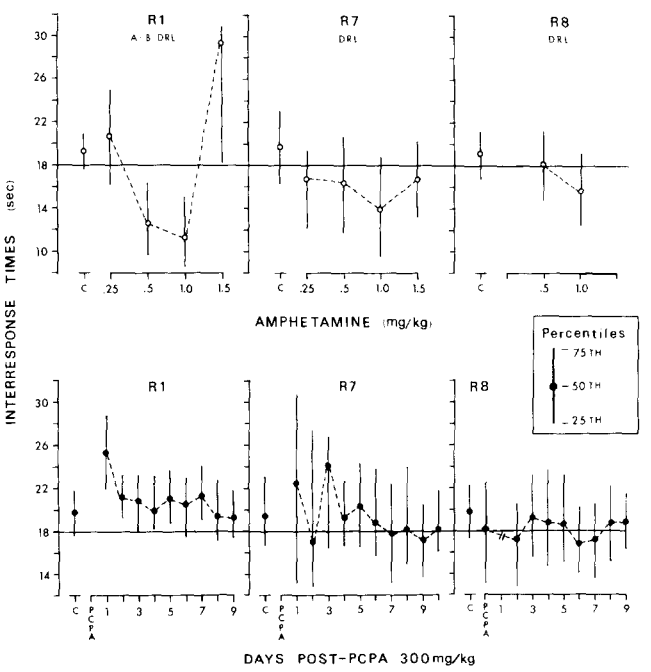

FIG. 4. Effects of amphetamine and PCPA (300 mg/kg) on A-B and single-lever DRL interresponse times presented as the semi-interquartile range (middle 50%) of the interresponse time distributions excluding all interresponse times<2 sec. The points labelled C represent the mean control rate of responding of 10 noninjection or saline control days for amphetamine and 6 days for PCPA. The horizontal line at 18 sec on the ordinate denotes the DRL criterion value.

rats 1 and 5 following 1.5 mg/kg of amphetamine; near total suppression of responding was observed with four rats (R5, R6, R7, R9) following the 2.0–3.0 mg/kg doses.

The bottom panels of Fig. 1 show that cinanserin was generally ineffective in altering A-B DRL response rates for the three rats tested with this drug alone, except for rate decreases which occurred for rats 6 and 9 at the highest doses given (48 and 64 mg/kg). Cinanserin did not increase the response rate of any animal beyond control values over an eight-fold dosage range.

The bottom panels of Fig. 2 show that 300 mg/kg of PCPA severely decreased both the A-B and single-lever DRL response rates for up to 24 hours following administration. A similar though less severe effect was observed following a 200 mg/kg dose (not shown). Baseline rates were recovered by all animals by the second day post-PCPA administration and remained within control ranges over the subsequent eight sessions for rats 1 and 7. For rat 8 the rate of responding was decreased on the 5th day and increased over the 6th through 9th days post-PCPA.

The semi-interquartile ranges (the middle 50%) of the interresponse time distributions which were obtained under control and drug conditions are presented in Figs. 3 and 4. Interresponse times less than 2 sec were excluded in the calculation of the quartiles, since these short IRTs represent "bursts" of responding that have frequently been treated separately from longer IRTs which were used to define temporally spaced responses [5, 22, 42]. Figures 3 and 4 show that the control IRT distributions were centered at or near the 18 sec criterion value; the 50th percentile (median) IRTs were between 18–20 sec in duration with the 25th and 75th percentile ranges extending ± 2–4 sec. The top panels of Figs. 3 and 4 show that amphetamine administration resulted in shifts in the IRT distributions toward shorter values for all six rats at one or more doses. Increases in the semi-interquartile ranges were also sometimes observed. Quartiles were not calculated when the rates of responding were decreased by the drug to less than 0.5 responses per minute. The bottom panels of Fig. 3 show that cinanserin-induced alterations in the A-B DRL interresponse times were minor. Only at the highest dose tested (64 mg/kg) were the overall semi-interquartile ranges decreased for two of the three rats (R5 and R6).

The effects of PCPA on the IRTs are presented in the bottom panels of Fig. 4. There were disruptions in the IRT distributions of all rats either on the day of PCPA administration or for 1 to 3 days post-injection. For rat 1 the disruption was characterized by a shift in the semi-interquartile range toward longer values, while for rat 7 there were primarily increases in the degree of dispersion of the semi-interquartile range. For rat 8, only small increases in the degree of dispersion of the IRT distributions were observed following PCPA administration. For the remainder of the post-PCPA period the IRT distributions were similar to control performances for all rats.

The effects of the combined administration of amphetamine and cinanserin on the A-B DRL response rates are presented in Fig. 5. Drug effects are expressed as a percent change from control values to allow for a direct comparison between the amphetamine, cinanserin, and drug combination phases. Percent change was calculated by divid-

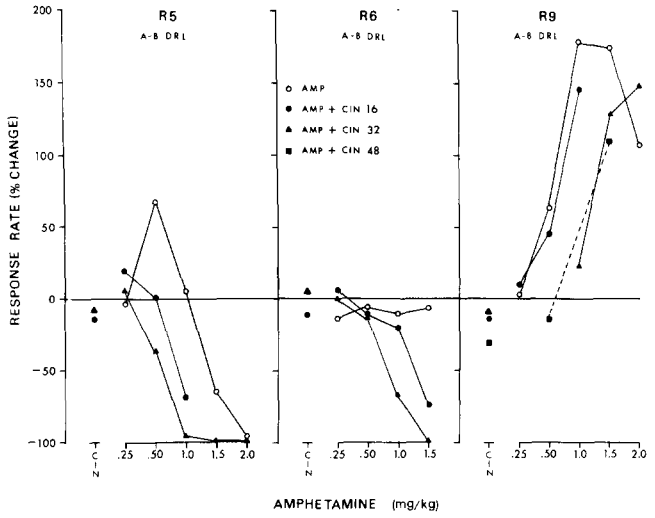

FIG. 5. Effects of amphetamine and cinanserin alone and in combination on A-B DRL rates of responding where a single A-B response sequence was considered as one response. Drug rates are expressed as a percent change from the mean rate of 10 noninjection or saline control sessions. The horizontal line at 0% represents the mean control rate. The unconnected points labelled CIN represent cinanserin alone, the unfilled connected points represent amphetamine alone, and the filled connected points represent amphetamine plus cinanserin combinations. Amphetamine doses are indicated on the abscissa.

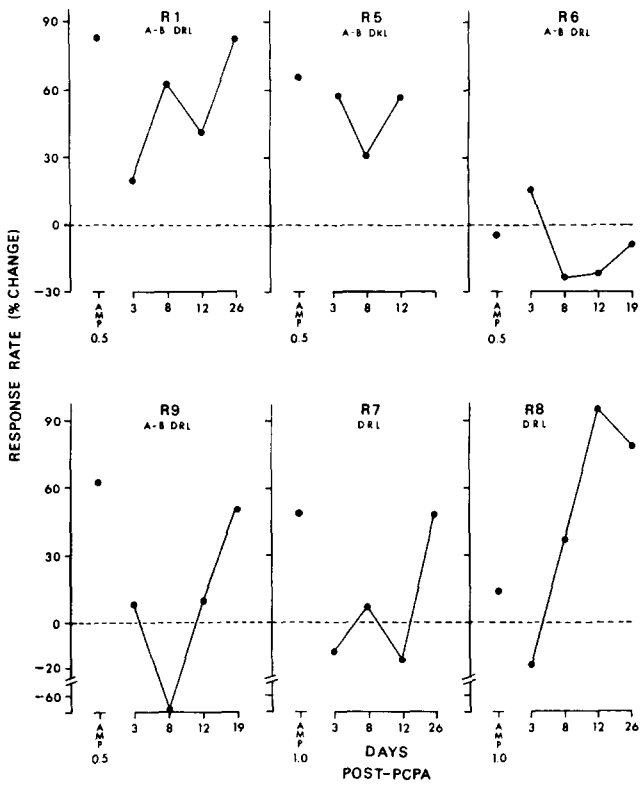

FIG. 6. Effects of a single dose of amphetamine administered alone and following PCPA (300 mg/kg) pretreatment on A-B and single-lever DRL 18 sec response rates. For rats 1, 5, 6 and 9 performing on the A-B DRL schedule, a single A-B response sequence was considered as one response. Drug rates are expressed as a percent change from the mean rate of 6–10 noninjection or saline control sessions. The horizontal line at 0% represents the mean control rate. The unconnected points labelled AMP represent the indicated dose of amphetamine-alone. The connected points represent this dose of amphetamine administered 3, 8, 12 and either 19 or 26 days post-PCPA administration. Rat 5 did not receive the final injection of amphetamine.

ing the difference between the mean control response rate and the mean response rate obtained following drug administration by the mean control rate. Figure 5 shows that, over a range of doses of both drugs, combinations of amphetamine and cinanserin decreased response rates relative to those obtained with amphetamine alone. This effect was observed with each of the three rats tested regardless of whether amphetamine alone increased, decreased, or did not alter the ongoing rate of responding, and was apparent for amphetamine doses of 0.5 mg/kg and above. The one exception to this interaction occurred with rat 9 following the combined administration of 2.0 mg/kg of amphetamine with 32 mg/kg of cinanserin. Figure 5 also shows that the magnitude of these response rate decreasing effects were directly related to the dose of cinanserin, with higher doses decreasing response rates to a greater degree than lower doses. Furthermore, it can be seen that response rates observed following these drug combinations could not be predicted from an algebraic summation of the individual drug effects.

Figure 6 presents the effects of a single dose of amphetamine on response rates when administered alone and following pretreatment with 300 mg/kg of PCPA. Of the six rats tested, four (R1, R5, R7 and R9) showed marked rate increasing effects with the indicated dose of amphetamine alone. For these four rats, pretreatment with PCPA antagonized the amphetamine-induced rate increases on the 3rd, 8th, and 12th days post-PCPA. Individual rats showed this effect on either one (rat 5), two (rat 1), or all three (rats 7 and 9) of these post-PCPA sessions and the occurrence of this effect was not systematically related to the time following PCPA administration. Response rates were similar to nondrug control values during the post-PCPA sessions when amphetamine was not administered, and when saline was administered on the 5th day post-PCPA (not shown). When amphetamine was again administered either 19 or 26 days post-PCPA, rate increasing effects similar to those obtained previously with amphetamine alone were recovered. For the two rats (R6 and R8) that did not show substantial response rate increases with amphetamine alone, pretreatment with PCPA resulted in inconsistent rate changes when amphetamine was subsequently administered (i.e., response rates were either greater than or less than those obtained with amphetamine alone). For rat 8, an altered response to amphetamine was still evident on the 26th day post-PCPA.

Figure 7 shows the effects of amphetamine on the IRT distributions of rats 5, 7 and 9 when administered alone and in combination with cinanserin or PCPA. The drug combination distributions presented were selected from sessions where the amphetamine-induced response rate increases were completely antagonized by cinanserin or PCPA pretreatment. These distributions show, that there was a decreased percentage of short nonreinforced IRTs (0–6 sec) and an increased percentage of longer IRTs, both nonreinforced (6–18 sec) and reinforced (18 sec or longer). The one exception to this finding was for rat 9 following 0.5 mg/kg of amphetamine administered on post-PCPA day 8; inspection of the cumulative record for this session revealed that the

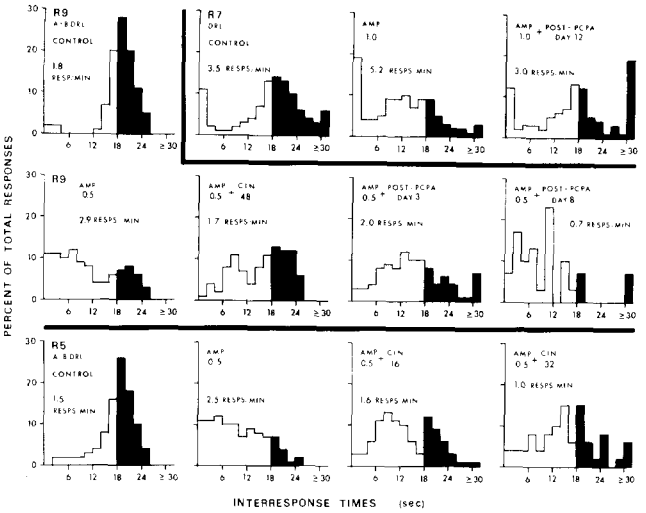

FIG. 7. Effects of amphetamine alone and combinations of amphetamine and cinanserin or PCPA on the interresponse time distributions for rats performing on the A-B (rats 5 and 9) and single-lever (rat 7) DRL 18 sec schedules. The control distributions are the means of 10 noninjection or saline control days. Each interresponse time class interval was 2 sec in duration and the shaded portions represent reinforced interresponse times.

pattern of responding was characterized by a series of short IRTs which alternated with periods of no responding. Some of the drug combination distributions also show a greater degree of dispersion than those for amphetamine alone. Additionally, even though the IRTs were often shifted toward longer values following the drug combinations relative to those obtained with amphetamine alone, the overall distributions were always characterized by a greater percentage of nonreinforced IRTs than was observed during nondrug control sessions.

DISCUSSION

The present results are consistent with the findings of other studies which have demonstrated that amphetamine altered both single-lever and A-B DRL responding by increasing response rates and shortening interresponse times over a range of doses [26, 42, 44, 45, 55]. Since A-B DRL interresponse times were shortened by amphetamine, this confirms previous findings that this procedure provides a sensitive index of alterations in temporal discrimination that is not confounded with alterations in post-reinforcement pausing [33,34].

In contrast to the effects obtained with amphetamine, both cinanserin and PCPA were shown to be relatively ineffective in altering DRL performance. For cinanserin, elevations in the rate of responding beyond control values were not observed, thus providing evidence that this serotonin antagonist does not nonspecifically increase low rates of responding. Winter [56] has also reported that 25 mg/kg of cinanserin did not alter the DRL performance of rats, while other studies have shown that cinanserin did not alter response rates maintained by variable-interval [9,57], fixed-interval [41], or fixed-ratio [41] schedules of reinforcement at doses up to 32 mg/kg. In the present study, only the highest dose of cinanserin (64 mg/kg) altered A-B DRL responding by decreasing response rates and shortening IRTs for two of the three rats. Although these effects were similar to ones observed with certain doses of amphetamine, the overall dose-response functions for these two drugs were quite distinct. The 64 mg/kg dose of cinanserin has been reported to alter fixed-interval performance such that the low rates occurring early in the interval were increased and the higher rates occurring later in the interval were decreased [41]. Although this result appears to be in contrast to the present findings, it demonstrates that a high dose of cinanserin may increase rates of responding depending upon the schedule of reinforcement maintaining the responding.

Although PCPA disrupted both single-lever and A-B DRL performance for 24 to 48 hours following administration, there were only limited alterations in responding over the subsequent eight-day period. These results are consistent with other studies reporting few effects of PCPA on fixed-interval [41] and variable-interval [13, 40, 57, 58] responding. In contrast, fixed-ratio responding was decreased for nine to ten days following PCPA administration [3,41]. This result may reflect an interaction between serotonin depletion and the ongoing rate of responding, since fixed ratio responding, which typically occurs at a high rate, has been reported to be decreased by drugs which increased or did not alter responding occurring at a lower rate [7, 26, 41, 45].

The present results provide little evidence that serotonin plays a functional role in the maintenance of the low-rate spaced responding generated by the DRL schedule. It was shown that cinanserin had few effects on DRL responding at dosage levels which have been demonstrated to antagonize serotonin and other indolamines [8, 56, 57]. For PCPA, there were no consistent alterations in responding during the time following drug administration (post-PCPA days 3 to 10) when serotonin levels have been shown to be maximally decreased [28]. Although DRL responding was disrupted on the day of PCPA administration and for several days thereafter, the time period which coincides with falling serotonin levels following PCPA administration, there are other biochemical changes which also occur at this time that may account for the observed behavioral disruptions; these include high tissue levels of PCPA and phenylalanine [25].

The failure to obtain rate increasing effects with cinanserin or PCPA on DRL performance suggests that these drugs increase low rates of punished responding as a result of a specific interaction with the punishment contingency, and not as a result of general rate-enhancing effects. The punishment attenuating effects of these drugs appear qualitatively distinct from similar effects of drugs such as chlordiazepoxide and pentobarbital. Since these latter drugs have been shown to increase both punished and unpunished rates of responding, their punishment attentuating effects have been attributed, either in whole or in part, to their general rate-enhancing properties [32,59]. Furthermore, since it has previously been suggested that serotonin is a mediating factor in the suppression of responding produced by punishment [13, 14, 16, 40, 49, 58], the present findings suggest that punishment suppressed responding and responding that has been suppressed by the DRL contingency are mediated by different neurochemical mechanisms.

The effects of the drug combinations reported here were consistent in that when amphetamine was administered following pretreatment with cinanserin or PCPA, response rates were frequently reduced relative to those obtained with amphetamine alone. For cinanserin, this was true regardless

of whether amphetamine alone increased, decreased, or did not alter the ongoing control rate of responding. For PCPA, the most pronounced reductions in response rates were found when amphetamine alone markedly increased rates of responding. This apparent difference between cinanserin and PCPA when given in combination with amphetamine may reflect an actual qualitative difference between these drugs, or it may be due to the fact that only a limited range of amphetamine plus PCPA dose combinations were examined. The IRTs revealed that the disruption of the temporal spacing of responses produced by amphetamine was still evident following the drug combinations, even though the rates of responding were often at or below control values. These findings suggest that the effects of cinanserin and PCPA, when given in combination with amphetamine, were to primarily interact with amphetamine-induced changes in the ongoing rate of responding, and not with the amphetamine-induced changes in the spacing of responses.

In contrast to the present results, the catecholamine antagonist chlorpromazine [21] and the catecholamine depletor alpha-methyl-para-tyrosine [44] have been shown to completely antagonize both the response rate increasing and the IRT shortening effects of amphetamine in rats performing on DRL schedules of reinforcement. These reports and others (e.g. [37]) suggest that the behavioral effects of amphetamine are mediated by the central release of the catecholamines. Our results show that a serotonin antagonist and a serotonin depletor also alter the behavioral response to amphetamine, suggesting that serotonin also plays a role in the manifestation of the behavioral effects of this drug. Therefore, it is likely that the present results represents an interaction between drug-induced alterations in both catecholaminergic and serotonergic systems.

Since higher doses of cinanserin decreased response rates to a greater degree than lower doses when given in combination with amphetamine, a direct relationship between the degree of interference with serotonergic systems and the degree to which DRL responding was altered is suggested. The joint effects of PCPA and amphetamine, however, indicate that the extent of the interference with serotonergic activity was not directly related to the absolute levels of serotonin, since the effects of this drug combination were not related to the degree to which serotonin levels were presumably decreased over days. Although biochemical determinations were not done in the present study, Koe and Weissman [28] reported that rat brain serotonin levels were approximately 10, 50 and 80 percent of nondrug control values on the 3rd, 8th and 12th days post-PCPA (316 mg/kg) administration respectively. Serotonin levels that were 100% of control values were not obtained until the 16th day post-PCPA administration. It might be argued that these different degrees of serotonin depletion would result in quantifiably distinct alterations in behavior at their respective post-injection times. The present findings, however, show that when amphetamine was administered on the 3rd, 8th and 12th days following pretreatment with 300 mg/kg of PCPA, the magnitude of the alterations in response rate were not systematically related to the time following PCPA administration, and, therefore, to the presumably different levels of serotonin. That reduced serotonin levels were nevertheless a factor involved in the altered response to amphetamine is indicated by the finding that the response-stimulant effects of amphetamine were recovered when serotonin levels were also presumably recovered (i.e., on either the 19th or 26th day post-PCPA). Another approach in examining the relationship between serotonin levels and the behavioral effects of amphetamine might be to use different doses of PCPA to obtain different degrees of serotonin depletion at similar post-injection times.

An alternative to the proposed catecholaminergic-serotonergic interaction is that amphetamine may exert some of its behavioral effects by acting on serotonergic neurons directly. However, this appears to be unlikely since studies examining the behavioral and neurochemical effects of this drug showed that amphetamine did not result in the central release of serotonin [54] and that amphetamine did not act on serotonin receptor [47]. Although nonbehavioral results have shown that amphetamine is capable of releasing serotonin in various brain regions [2,12] and of altering serotonin metabolism [29, 35, 36, 38, 43], the doses used in these procedures were generally higher than those typically used in behavioral conditioning paradigms. Another possibility is that cinanserin and PCPA interacted with amphetamine independently of their effects on serotonergic systems. Other evidence suggests that this is not the case for PCPA, however, since it has been reported that PCPA does not alter the distribution of amphetamine or its metabolites [23]. Furthermore, direct pharmacological interaction between PCPA and amphetamine appears unlikely since little PCPA is present in brain tissue 72 hours post-injection [25]. Although PCPA itself results in catecholamine depletion which may serve to modify the effects of amphetamine, the magnitude and duration of this effect is minor compared to PCPA's effects on serotonin levels [28]. There are no reports to our knowledge concerning the effects of cinanserin on the absorption, distribution, or metabolism of amphetamine, or of its effects on the functional state of catecholaminergic systems.

It should be noted that some caution is required in interpreting the present results in terms of a central site of drug action. This is because amphetamine [24] and PCPA [28] affect catecholaminergic and serotonergic systems respectively in both the central and peripheral nervous systems. Further, there is conflicting evidence as to the central efficacy of cinanserin as a serotonin antagonist. Cinanserin has been shown to be effective in antagonizing the behavioral effects of the serotonin precursor 5-hydroxytryptophan and of various other indolamines believed to act at central serotonin receptors, while the peripheral serotonin antagonist xylamadine tosylate was ineffective [56,57]. In contrast, the reduction in neuronal activity produced by centrally applied serotonin in various brain areas receiving prominent serotonergic input was not antagonized by cinanserin and other putative antagonists [20]. To unequivocally determine whether the present results are indeed central in nature, the use of a serotonin antagonist and a depletor devoid of central effects and of a peripherally acting sympathomimetic amine with actions similar to those of amphetamine would be required.

The present results are not consistent with those of studies which have shown that serotonin depletion or antagonism potentiated the response rate increasing effects of amphetamine in rats performing on various schedules of reinforcement (e.g. variable interval, fixed interval, fixed ratio) [17, 19, 46]. However, there is evidence which indicates that the particular test situation employed is a critical factor in determining the effects of the different serotonin antagonists [8] and of the different methods of serotonin depletion [30]. Since both cinanserin [53] and PCPA [23,31] have been shown

to potentiate the response stimulant effects of amphetamine in rats, and since DRL responding has been shown to be a sensitive measure of amphetamine potentiation with a variety of other drugs [6,55], it may be that DRL responding does not provide an index of amphetamine potentiation with either cinanserin or PCPA. Whether any of the antiserotonergic manipulations are effective in this way in the DRL situation, however, remains to be determined.

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