Psychopharmacological Studies of Some 1-(Chlorophenyl)-2-aminopropanes II

Effects on Avoidance and Discrimination Behavior

By JOHN E. OWEN, JR.

A series of 1-(chlorophenyl)-2-aminopropanes previously shown to suppress appetitive-controlled behavior were compared with *d*-amphetamine and methamphetamine on an avoidance procedure. Rats trained to press a lever to avoid intermittent electroshocks were used. In alternate 10-minute periods, a rat received a time-out (TO) when no responding on the lever was necessary. The discrimination between the two periods was cued by appropriate visual stimuli. The two lowest doses that suppressed the appetitive-controlled behavior were studied. With the unsubstituted amphetamines, the rats produced high response rates during both the avoidance and TO periods with a reduction in the number of shocks received. On the whole, the chloro-substituted compounds produced relatively small increases in the rats' response rates with little or no change in the number of shocks received. These results indicate that, at the doses used, the chloro-substituted compounds produced less CNS stimulation than did *d*-amphetamine or methamphetamine.

AN OPERANT-BEHAVIOR avoidance procedure originally described by Sidman (1) has been employed to study the effects of drugs of the amphetamine class on conditioned avoidance responding (2–5). Rats, trained to press a lever (respond) to avoid or postpone brief, intermittent electroshocks, produced higher than normal response rates with the drugs in this study. Verhave (4) introduced a time-out contingency (TO) to the Sidman procedure. Rats were trained to avoid electroshock in the presence of a 433-c.p.s. tone stimulus. They were then taught to discriminate between tone-on avoidance periods and tone-off TO periods when no electroshocks would occur and responding was not necessary. As the discrimination developed the rats did not respond in the absence of the tone stimulus. With the amphetamines the animals had a tendency to continue responding during the TO. This effect with the drugs was considered as a breakdown of the tone stimulus control that maintained the acquired discrimination. In a previous study Verhave (3) had shown that, in the absence of established avoidance behavior, methamphetamine did not cause rats to produce high response rates with or without shock.

Rats given drugs of the amphetamine type show stimulated avoidance behavior only in a limited range of doses (3, 6). Doses above certain optimal levels (the level depending on individual variation from rat to rat) cause the animals to produce erratically depressed response rates. In some cases responding ceases entirely and the animal dies at much less than normally toxic doses of the drugs with normally nonlethal electroshock. Weiss, *et al.* (7), in a study of this phenomenon reported that rats and mice exposed to aversive electroshock died at much lower doses of amphetamine than animals that were not shocked. Also, the mode of death appeared different from that seen with lethal doses of the drug.

The investigation reported here utilized a Sidman-type avoidance TO procedure to study the influence of some 1-chlorophenyl-2-aminopropanes and their optical isomers on avoidance and discrimination behavior. These compounds were reported (8) to suppress appetitive-controlled fixed-ratio behavior. This study was undertaken as an extension of Rathbun's observations (9) of these compounds in mouse activity studies. He found that, although the 1-(4-chlorophenyl)-2-aminopropane compounds were more active central nervous system stimulants than the dichloro compounds, all caused little or no increased activity in the mice at dose ranges that induced appetite suppression in rats on food-consumption, weight-loss studies.

EXPERIMENTAL

Materials.—The compounds and doses used are listed in Table I. The compounds were dissolved in distilled water and administered subcutaneously.

Methods.—The subjects were six male rats of the Long-Evans strain, weighing 300–450 Gm. By means of a modification of the procedure described by Sidman (1) they were trained to press a lever to avoid brief intermittent electroshocks.

Two experimental cages were used in this investigation. Each measured 10 × 5 × 9 in. and was designed with four electrically isolated metal walls and a grid floor made of parallel $^3/_{16}$-in. diameter stainless steel rods. A response lever, a

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TABLE I.—DRUGS AND DOSES USED IN AVOIDANCE TESTING

Drug	Doses, mg./Kg. Low	High
d-Amphetamine sulfate	1.0	2.0
d-Methamphetamine HCl	1.0	2.0
dl-1-(4-Chlorophenyl)-2-aminopropane HCl	2.0	3.2
d-1-(4-Chlorophenyl)-2-aminopropane HCl	1.0	2.0
l-1-(4-Chlorophenyl)-2-aminopropane HCl	2.0	3.2
dl-1-(3,4-Dichlorophenyl)-2-aminopropane HCl	2.0	5.0
d-1-(3,4-Dichlorophenyl)-2-aminopropane HCl	3.2	5.0
l-1-(3,4-Dichlorophenyl)-2-aminopropane HCl	2.0	3.2
dl-1-(2,4-Dichlorophenyl)-2-aminopropane HCl	3.2	5.0
d-1-(2,4-Dichlorophenyl)-2-aminopropane HCl	3.2	5.0
l-1-(2,4-Dichlorophenyl)-2-aminopropane HCl	2.0	3.2
dl-1-(3,4-Dichlorophenyl)-2-methylaminopropane HCl	3.2	5.0
d-1-(3,4-Dichlorophenyl)-2-methylaminopropane HCl	3.2	5.0
l-1-(3,4-Dichlorophenyl)-2-methylaminopropane HCl	2.0	3.2

modified telephone-type switch (10), was mounted on one end wall of each cage. A small 24-volt pilot light was mounted in the wall above the lever and another below the level of the grid floor. The cages were contained in light-proof, sound-resistant, ventilated boxes isolated from the control equipment.

Brief electroshocks of 0.2-second duration were given to the rat through the grid floor and walls of the cage at 2-second intervals (SS). A response on the lever postponed the next shock for 20 seconds (RS). Responses within this 20-second interval produced another 20 seconds without shock. Thus, with a steady rate of responding the rat avoided being shocked. The shock was provided by a constant-current generator passing half-wave 60-cycle d.c. at 1 milliamp. The shock was delivered to the cage through a "grid scrambler" that, in a random fashion, rapidly changed the current polarity on the individual grid rods and walls. The rat was thus unable to avoid shock by standing on rods or leaning against a wall of similar polarity.

Appropriate electrical relay circuits and timers were used to control the procedure and record the data on impulse counters and cumulative recorders.

After several training sessions, when the rats began developing stable avoidance behavior, the TO contingency was introduced. During alternate 10-minute periods the light over the lever went out and the light below the cage floor came on, signifying the TO when no shocks would occur. The rats learned to discriminate between the avoidance

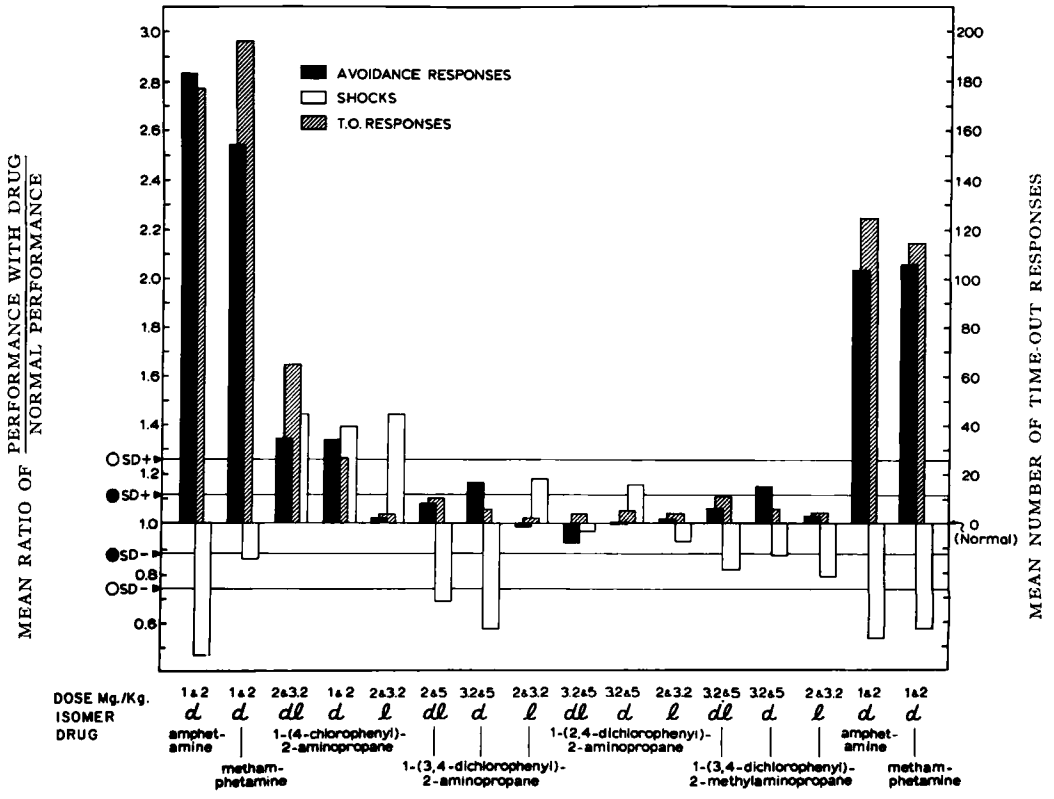

Fig. 1.—Summary of combined dose drug effects of compounds used in this study on avoidance discrimination behavior.

periods and TO by the change in the source of cage illumination. As the rats developed discrimination they no longer responded during the TO. Each avoidance-TO session lasted for 3 hours. When drugs were tested, each rat was given a 1-hour "warm-up" before drug administration. The animal then was returned to the experimental cage for a 3-hour session.

Only the two lowest doses of each compound that suppressed appetitive-controlled fixed-ratio behavior were used (8). *d*-Amphetamine and methamphetamine were tested at the beginning and again at the end of the investigation. The chloro-substituted compounds were run only once at each dose in a random order. Non-drug sessions run at 24 and 48 hours after a drug session failed to show residual drug activities on either avoidance or TO behavior. Thus, a time lapse of at least 48 hours was allowed between sessions for the individual animals.

Data.—The number of avoidance responses, the number of responses during TO, and the number of shocks received were recorded for each hour of each session. The means and standard deviation of avoidance responses and shocks received for the second hour of 11 non-drug sessions that were run during the study were calculated for each rat. Only non-drug sessions were used when at least 48 hours had elapsed since a previous drug session. The avoidance response and shock data from the second hour after drug administration of each drug session were used to calculate ratios of performance with drug to normal performance. Since TO responding normally was nonexistent in non-drug runs, no ratios were determined for this contingency of the procedure. Only the actual number of responses was counted.

Cumulative records of responses were made of each session to provide graphic data of the character and distribution of the responses and shocks.

RESULTS

The results of this investigation have been summarized by combining the data from both doses of each compound—presented in Fig. 1 as means of the ratios of avoidance responding and shocks received and the means of the TO responses. The data were combined because relatively little difference was seen between dose levels of each compound. This was especially true of the chloro-substituted drugs. The normal baseline (ratio of 1.0) and the means of normal standard deviations of the avoidance responses and shocks received have been included in Fig. 1.

At the beginning and at the end of the study with *d*-amphetamine and methamphetamine, the rats showed increased avoidance and TO responding with a decrease in the number of shocks received. With the *dl* and *d*-1-(4-chlorophenyl)-2-aminopropane

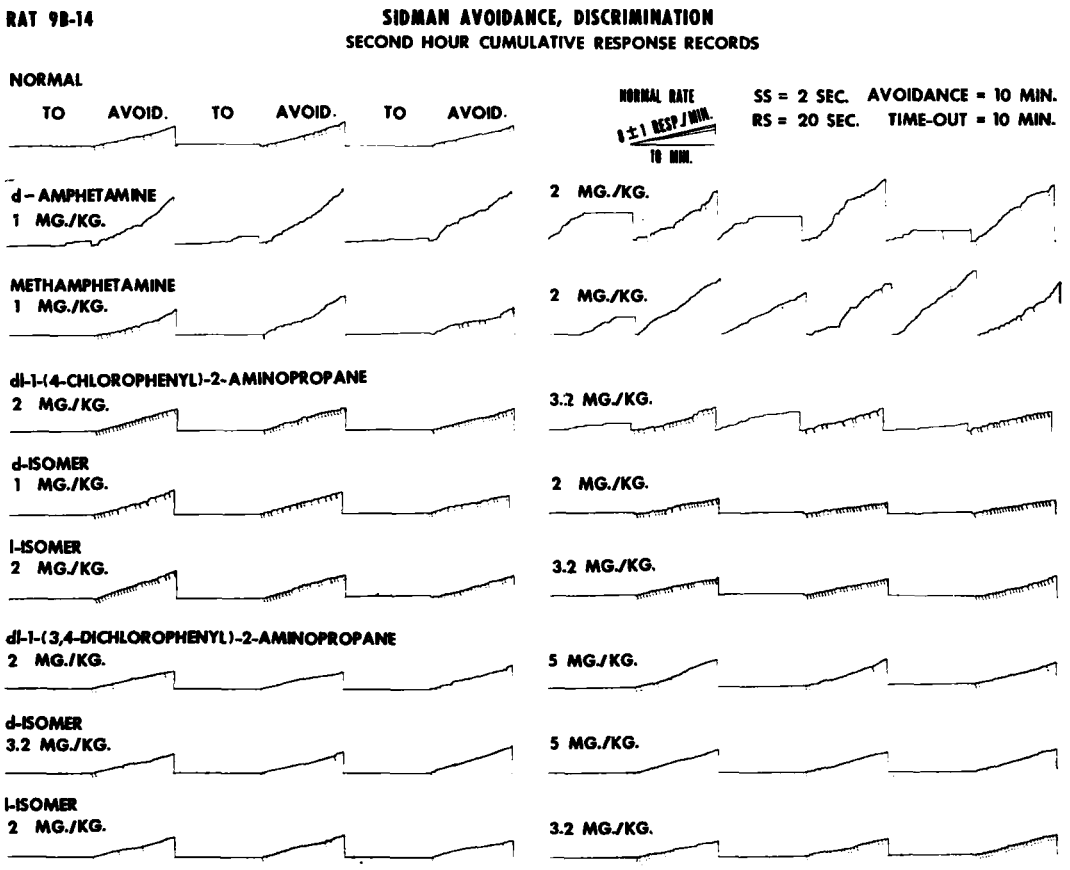

Fig. 2.—Cumulative response records of a rat comparing effects of drugs on avoidance discrimination behavior to normal behavior.

rats showed increased responding during the avoidance and TO periods with an increase in the number of shocks received. The magnitude of the response increases appeared to be one-third to one-half that of the des-chloro-compounds. The *l* isomer showed a marked increase only in the ratios of shocks received. Except for the *dl* and *d*-1-(3,4-dichlorophenyl)-2-aminopropanes, the remainder of the compounds produced ratios that fell well within the limits of normal standard deviation of avoidance responses and shocks, and near the normal of TO responding. The shock ratios of the *dl* and *d*-1-(3,4-dichloro)-2-aminopropane compounds appeared below the lower standard deviation limit.

Figures 2 and 3 show the cumulative response records of one typical rat. Included are examples of records of the second hour of non-drug sessions and records of the second hour after drug administration of *d*-amphetamine and methamphetamine and the 12 chloro-substituted compounds. This rat had a normal avoidance response rate of 8 ± 1 responses per minute, which has been drawn in the figures as the normal rate. The short vertical pen marks on the avoidance segments indicate points when the animal was shocked. Significant TO responding in these records appeared only after the administration of *d*-amphetamine, methamphetamine, and the higher doses of *dl* and *d*-1-(4-chlorophenyl)-2-aminopropane. The increased avoidance responding was apparent with the administration of the amphetamine compounds. The stimulated avoidance responding can be described as erratic and is illustrated with the *d*-amphetamine and methamphetamine and the 3.2 mg./Kg. dose of the *dl*-1-(4-chlorophenyl)-2-aminopropane. The animal received several successive shocks with only one or two responses between shocks and then responded in very rapid bursts of lever pressing. The overall effect produced very irregular appearing cumulative response records.

DISCUSSION

Data from this study have confirmed Verhave's observations (4) that the administration of amphetamine compounds to rats that have well developed avoidance and discrimination behavior causes the animals to show a stimulation of avoidance responding with a loss of ability to discriminate between the two contingencies of the procedure. The *dl* and *d*-1-(4-chlorophenyl)-2-aminopropane, unlike the dichloro-substituted compounds, more nearly resembled *d*-amphetamine and methamphetamine with regard to the rats' avoidance and TO responding. The magnitude of effect was somewhat less. However, qualitatively the *dl* and *d*-4-chloro compounds differed from all of the others since the animals received more shocks in spite of the increased avoidance responding. The *l* isomer of the 4-chloro compounds also showed high shock ratios

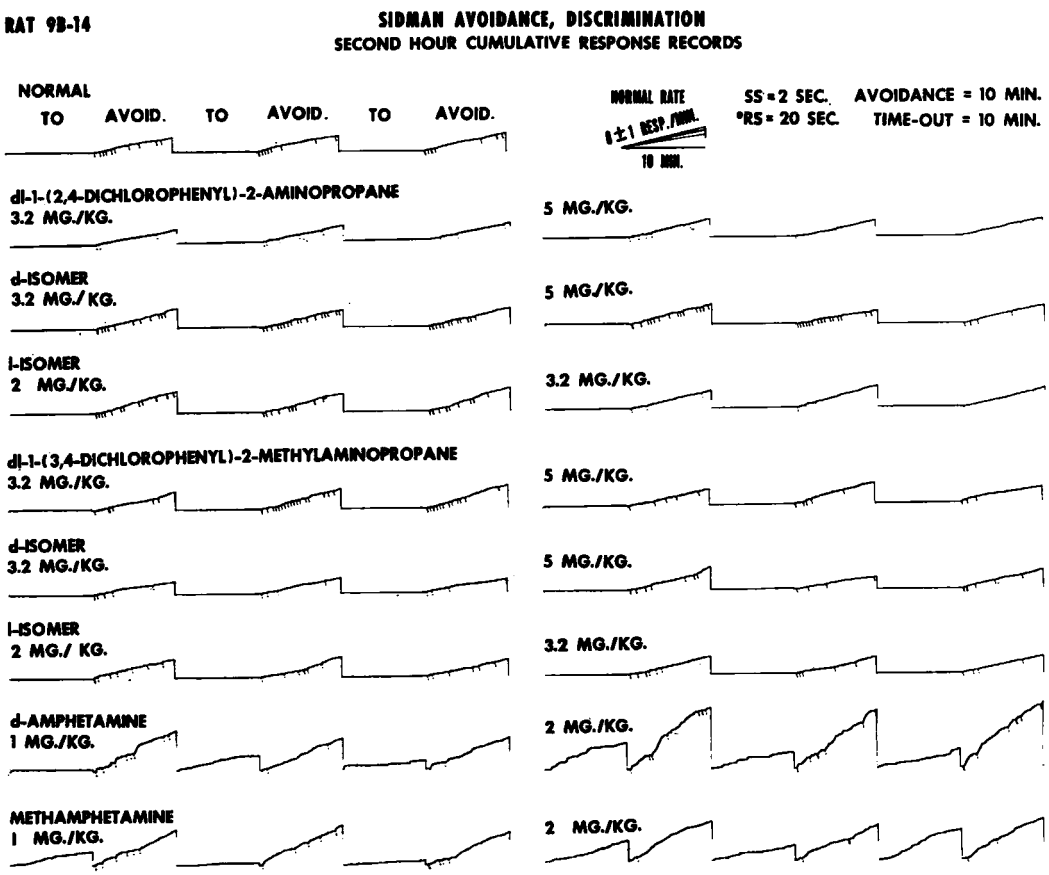

Fig. 3.—Cumulative response records of a rat comparing effects of drugs on avoidance discrimination behavior to normal behavior.

in the rats. Dichloro substitutions on the 2,4 and 3,4 positions of the phenyl ring of 1-phenyl-2-aminopropane structures, at the doses used, inhibited the behavioral stimulation effects seen with the parent structure. The general lack of responding during the TO contingency after administration of the dichloro-substituted compounds showed that the stimulus control of the changing light sources was maintained. The lowered shock ratios seen in the rats with the *dl* and *d*-1-(3,4-dichlorophenyl)-2-aminopropanes could be termed as an increase in efficiency since the avoidance ratios were only slightly raised, *i.e.*, more protection for the same work output.

Although the *d*-amphetamine and methamphetamine response rates were approximately 30% less at the end than at the start of the study, the mean avoidance response-rate ratios and TO responses with the chloro-substituted compounds were well below those of the amphetamine compounds. For the most part, the chloro compound data fell close to or within the normal standard deviation limits. The difference in the amphetamine data could be due to a development of tolerance to the 1-phenyl-2-aminopropane structure or more probably to a gradual strengthening of the behavior over the period of time covered by the investigation to provide a resistance to drug action.

SUMMARY

Some 1 chlorophenyl-2-aminopropanes have been compared with *d*-amphetamine and methamphetamine for effects on avoidance and discrimination behavior in rats. The doses used were the two lowest that caused suppression of an appetitive-controlled behavior.

After *d*-amphetamine, methamphetamine, and *dl* and *d*-1-(4-chlorophenyl)-2-aminopropane, rats showed increased avoidance response rates as well as responding during time-out periods when it was not necessary. This latter effect was considered as a loss of ability to discriminate. The rats when given dichlorophenyl-2-aminopropanes showed little or no change from normal in avoidance or discrimination behavior.

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Alkaloids of *Vinca rosea* Linn. (*Catharanthus roseus* G. Don) XV

Analysis of Vinca Alkaloids by Thin-Layer Chromatography

By NANCY J. CONE, RUTHANNE MILLER, and NORBERT NEUSS

Thin-layer chromatography of various Vinca alkaloids is described. Adsorbents, solvent systems, and their influence on the R_f values are discussed. R_f values for 26 Vinca alkaloids are given.

THE CLINICAL use of vinblastine[1] (2), a member of a new class of oncolytic alkaloids (3) from the ornamental shrub *Vinca rosea* Linn. (*Catharanthus roseus* G. Don), prompts us to publish the results of the use of thin-layer chromatography (TLC) in the identification of this and other alkaloids obtained from this plant in our laboratories (4). The laborious fractionations and repeated chromatographies required in the preparation of several of these alkaloids were greatly facilitated by continuously monitoring fractions with TLC. The technique was also found to be extremely useful in the course of chemical work leading to the structure elucidation of catharanthine (5) and vindoline (6) as well as the dimeric alkaloids vinblastine (VLB) and leurocristine[2] (LCR) (7). Finally, the behavior of compounds on TLC was often found to be a most efficient criterion of purity. This was especially true in the case of several dimeric alkaloids (8).

The advantages of TLC over paper chromatography, however, should not prevent us from mentioning some of the rules of paper chromatographic technique which also apply here. One cannot, for example, use indiscriminantly the results obtained on pure compounds to identify these components in a crude mixture of alkaloids or complex reaction mixtures without using

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Paper XIV in the series is being published elsewhere (1).

[1] Vinblastine sulfate marketed as Velban by Eli Lilly and Co.

[2] The generic name of leurocristine is vincristine.