RELATIVE EFFICACY OF CERTAIN CENTRAL NERVOUS SYSTEM DEPRESSANTS AGAINST DRUG-INDUCED CONVULSIONS AND MORTALITY

by

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A Thesis Submitted to the Faculty of the COLLEGE OF PHARMACY In Partial Fulfillment of the Requirements for the Degree of MASTER OF SCIENCE In the Graduate College THE UNIVERSITY OF ARIZONA

1969
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SIGNED: Allen P. Davidson

APPROVAL BY THESIS DIRECTOR

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January 17, 1969
ACKNOWLEDGMENTS

The writer wishes to express his sincere gratitude to Dr. Lincoln Chin and Dr. Albert L. Picchioni for their valuable guidance and encouragement throughout the period of this investigation.

Sincere appreciation is also extended to Dean Willis R. Brewer and the faculty and staff of the College of Pharmacy for their generous aid during the course of this study.

Grateful acknowledgment is made to Roche Laboratories, Nutley, New Jersey, whose financial assistance made this investigation possible. Chlordiazepoxide (Librium) and diazepam (Valium) used in this investigation were generously supplied by Roche Laboratories. Chlorpheniramine (Chlortrimeton) was generously provided by Schering Corporation, Bloomfield, New Jersey.
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ABSTRACT

Short acting barbiturates are usually recommended for the control of the central nervous system manifestations of amphetamine and antihistamine intoxication; however, there is a lack of information concerning the antidotal efficacy of the short acting barbiturates. This study was performed in rats and dogs to evaluate the effectiveness of pentobarbital, chlordiazepoxide, chlorpromazine, and diazepam as antagonists in experimental pentylenetetrazol, chlorpheniramine and d-amphetamine intoxication. The results indicate that chlordiazepoxide, diazepam and pentobarbital are most effective in the treatment of pentylenetetrazol intoxication; chlordiazepoxide and diazepam are most effective in the treatment of chlorpheniramine intoxication; and chlorpromazine is most effective in the treatment of d-amphetamine intoxication. The data also suggest that extra-central effects contribute to the lethality of chlorpheniramine and d-amphetamine overdosage.
I. GENERAL INTRODUCTION

Amphetamines and antihistamines are frequently involved in accidental poisonings. Statistics from the National Clearinghouse for Poison Control Centers reveal a rise in the number of intoxications attributable to these drugs during the past few years (Verhulst and Crotty, 1968). Acute toxicity is manifested by a variety of symptoms, among which is strong central nervous system excitation. Treatment for overdosage is symptomatic and emphasis is placed on antagonizing the central nervous system stimulation (Done, 1961; Gleason, Gosselin and Hodge, 1963; Deichman and Gerarde, 1964).

The usual recommended therapy for management of the excitatory effects produced by amphetamines and antihistamines is sedation with a short acting barbiturate (Beckman, 1961; Sicé, 1962; Gleason, Gosselin and Hodge, 1963, Douglas, 1966; Goth, 1966). However, there is a lack of information concerning the effectiveness of short acting barbiturates in controlling convulsions and preventing mortality when used in the treatment of poisonings due to the amphetamines and antihistamines.

In the past few years reports have appeared suggesting the use of chlorpromazine as an antagonist to the central nervous system
stimulation produced by amphetamines and antihistamines. Espelin and Done (1966) have found that chlorpromazine is very effective in the treatment of amphetamine poisoning. Mael and Bester (1963) suggest the use of chlorpromazine to control the central stimulation produced by acute antihistamine toxicity. Since the two benzodiazepine derivatives, chlordiazepoxide and diazepam, have been reported to be effective in the treatment and control of convulsions due to a wide variety of causes (Banziger, 1965; Swinyard and Castellion, 1966; Parsonage and Norris, 1967; Prensky, et al., 1967; Snyder, 1968; Sawyer, Webster, and Schut, 1968), they should also be investigated as potential antidotes for amphetamine and antihistamine intoxication.

Chlorpheniramine is one of the most commonly used antihistaminic drugs and is employed in these studies as the prototype for this class of drugs. Pentylenetetrazol serves in the present studies as a representative of the "pure" central nervous system stimulants. This investigation was initiated to evaluate the effectiveness of chlordiazepoxide, pentobarbital, chlorpromazine and diazepam as antagonists in experimental d-amphetamine, chlorpheniramine, and pentylenetetrazol intoxication. In addition, the results of this research may yield useful information concerning the basic mechanism of death caused by amphetamine and chlorpheniramine.
II. GENERAL PROCEDURES

The following general procedures were employed in all experiments. Adult male Sprague Dawley rats were used as experimental animals. They were allowed free access to food and water except 24 hours prior to testing when the animals were fasted and during testing when both food and water were withheld.

In order to provide baselines for comparison the TDL0's and TD90's of each potential antagonist were determined. These doses of antagonist were then tested against the agonists in the constant infusion studies described below.

1. TDL0 and TD90.

A modification of the rotorod technique described by Dunham and Miya (1957) was used to determine the doses of chlordiazepoxide, pentobarbital, chlorpromazine and diazepam that produce toxicity in 10% and 90% of animals (TDL0 and TD90, respectively).

The method involves use of a wooden rod, 36 inches in length and 1 1/8 inch in diameter, suspended on either end by means of bearings and rotated by a variable speed motor. The rod, set to rotate at a speed of 12 r.p.m., was held on ring stands 24 inches above table top. Dividers were placed every six inches along the rod to prevent the animals from turning around and also to allow the training of more than one animal at a time.
Male Sprague Dawley rats, weighing between 180 and 250 Gm., were trained to "walk the rod". Practice sessions were repeated until the animals, when placed on the rotating rod, could consistently stay on the rod for three one-minute trials.

Toxicity is defined as the inability of the animal to maintain its equilibrium on the rotating rod for one minute in three trial runs after drug treatment.

Test animals were randomized and divided into groups of eight. Three to four doses of each test drug were given intravenously in a volume of 2.5 ml/kg. Two minutes after drug treatment in the case of chlordiazepoxide, diazepam, and pentobarbital and ten minutes after injection of chlorpromazine the trained animals were placed on the rotating rod. At least three dosage increments of each drug which produced neurotoxicity were employed. Three points between 0 and 100% neurotoxicity were established and plotted on logarithmic probability paper according to the method of Litchfield and Wilcoxon (1949) and the TD10 and TD90 were extrapolated from the regression line. The data thus obtained are tabulated below.

<table>
<thead>
<tr>
<th>Drug</th>
<th>TD10</th>
<th>TD90</th>
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</thead>
<tbody>
<tr>
<td>Chlordiazepoxide</td>
<td>8.00 mg/kg</td>
<td>24.50 mg/kg</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>2.40 mg/kg</td>
<td>17.20 mg/kg</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>0.39 mg/kg</td>
<td>2.35 mg/kg</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.06 mg/kg</td>
<td>0.35 mg/kg</td>
</tr>
</tbody>
</table>
2. **Constant Infusion Technique**

Seizure and mortality thresholds were determined for the convulsive drugs (pentylenetetrazol, chlorpheniramine and d-amphetamine) by the technique of Orloff, Williams and Pfeiffer (1949) as modified by McQuarrie and Fingl (1958). The infusion solutions were made up in 0.9% sodium chloride. An optimum rate of infusion was determined for each of the solutions as suggested by Fingl and McQuarrie (1960).

The potential antagonists chlordiazepoxide, pentobarbital, and chlorpromazine were dissolved in normal saline and injected intravenously; diazepam was dissolved in a diluent consisting of 10% ethanol, 40% propylene glycol, and 50% water* for intravenous injection. Two control treatments were included in each series of observations, one consisted of 2.5 ml/kg of normal saline and the other consisted of 2.5 ml/kg of the vehicle for diazepam. With the exception of chlorpromazine, each candidate antagonist was administered 2 minutes before infusion of the agonist. Chlorpromazine was administered 10 minutes before infusion of the agonist.

Each rat was placed in a plexiglass restraining chamber and two needle electrodes were implanted subcutaneously on each side of the rib cage of the animal. Respiration and cardiac activity were recorded on an E & M physiograph and monitored on a Tektronix dual beam oscilloscope. The convulsant solutions were infused via the

*Formula of solvent for commercially available diazepam parenteral solution.
tail vein until two end points were observed. The first end point consisted of 3 seconds of persistent clonus, and the second end point consisted of death. Respiratory arrest as indicated by absence of activity on the physiograph and the oscilloscope was used as the end point for death. The data obtained were evaluated by analysis of co-variance (Finney, 1952) and the results expressed as convulsive and lethality threshold ratios.
III. THE EFFECTS OF VARIOUS CENTRALLY ACTING DRUGS ON DRUG INDUCED TOXICITY

A. The Effects of Chlorziazepoxide, Pentobarbital, Chlorpromazine and Diazepam in Acute Pentylene-tetrazol Intoxication in Rats and Dogs

1. Introduction

Pentylene-tetrazol is employed therapeutically as a central nervous system stimulant. Experimentally, it is used in the evaluation of drugs for potential anticonvulsant activity (Orloff, et al., 1949; Swinyard, Brown and Goodman, 1952; Swinyard and Castellion, 1966) as well as for the assessment of central nervous system excitability (Graham and Bohner, 1957; Hint and Richter, 1958; Richter, 1958; Chin and Swinyard, 1959; Wolf and Stock, 1966).

The action of pentylene-tetrazol is exerted primarily on the central nervous system. Appropriate doses will result in excitement, isolated myoclonic jerks, clonic seizure, superseded by one or more tonic seizures, and death. The convulsions produced resemble those induced by supramaximal brain stimulation in that the movements of the limbs consist of flexion followed by extension (Esplin and Zablocka, 1966).

The mechanism of action of pentylene-tetrazol is not due to blockade of presynaptic or postsynaptic inhibition nor does it appear that its stimulant action is accomplished by depolarization. Its activity is probably due to the enhancement of synaptic recovery by a presynaptic action (Esplin and Zablocka, 1966).
The exact site of action of pentylenetetrazol has not been determined but there is general agreement that the cortex and brain stem are more sensitive to its effects than the spinal cord (Sanders, 1967). Evidence suggests that for maximum activity the entire area lying between cortex and brain stem must be involved (Ten Cate and Swijgman, 1945).

Pentylenetetrazol is employed in this study because it lacks direct peripheral effects and is a standard experimental tool for evaluation of anticonvulsant drugs. A comparison of the effectiveness of the potential antagonists in this section with the results in sections B and C should provide a basis for interpretation of factors contributing to the lethality of the animals.

2. Methods

Administration of pentylenetetrazol by constant infusion. A 1.5% solution of pentylenetetrazol was infused into rats at a rate of .299 ml/minute (44.8 mg/minute) after pretreatment with the candidate antagonists.

Acute administration of pentylenetetrazol to dogs. Male mongrel dogs, weighing between 6 and 12 kg, were fasted for 24 hours. A previously determined lethal dose of pentylenetetrazol (150 mg/kg) was administered by gavage in a constant volume of 3 ml/kg and was followed by a wash of 3 ml/kg of water. Immediately after the administration of pentylenetetrazol, the following drugs were administered via the cephalic vein: chlordiazepoxide, 15 mg/kg; pentobarbital, 20 mg/kg; chlorpromazine, 3 mg/kg; diazepam, 3 mg/kg; normal saline, 0.5 ml/kg (control).
3. Results

**Constant infusion.** The results of this study are presented in Figure 1. Figure 1 shows the effect of the TD10 of all the antagonists and the effect of the TD90 of chlorpromazine on the convulsive and lethality threshold ratios.

Chlordiazepoxide, pentobarbital and diazepam, which were tested at only one dose (TD10), elevated seizure threshold 167%, 43%, and 96% respectively. These changes were accompanied by an elevation of mortality threshold of 66%, 67% and 53% respectively. Chlorpromazine was tested at its TD10 and TD90. The TD10 failed to elevate seizure or mortality thresholds, and the TD90 elevated seizure threshold 17% but did not increase the mortality threshold. The vehicle for diazepam elevated seizure threshold 40%; however, it was not effective in elevating mortality threshold.

**Acute dog studies.** The results of this study are presented in Table 1. All of the antidotes except chlorpromazine provided protection against the convulsive and lethal effects of pentylentonetzol. Chlorpromazine failed to protect any of the animals.

4. Discussion

Rats infused with pentylentonetzol responded with clonic convulsions followed by depression, a return of clonic convulsions, then tonic convulsions and death. Of the compounds tested, only chlordiazepoxide, pentobarbital, and diazepam were effective in increasing both the time for onset of convulsions and time for death.
Figure 1. Effect of TD10 and TD90 of various antagonists on pentylentetrazol threshold ratios. — Open symbols represent convulsive threshold ratios and solid symbols represent lethality threshold ratios. Bracketed lines designate 95% fiducial limits. (*TD90 of chlorpromazine)
Table 1. The effect of various antagonists on lethality of pentylene-tetrazol in dogs

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Antagonist</th>
<th>No. of Dogs</th>
<th>Death</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentylenetetrazol 150 mg/kg, P.O.</td>
<td>None</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Pentylenetetrazol 150 mg/kg, P.O.</td>
<td>Chlordiazepoxide 15 mg/kg, I.V.</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Pentylenetetrazol 150 mg/kg, P.O.</td>
<td>Diazepam 3 mg/kg, I.V.</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Pentylenetetrazol 150 mg/kg, P.O.</td>
<td>Pentobarbital 20 mg/kg, I.V.</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Pentylenetetrazol 150 mg/kg, P.O.</td>
<td>Chlorpromazine 3 mg/kg, I.V.</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
Within 3 minutes after administration of 150 mg/kg of pentylenetetrazol to dogs, the control animals displayed intermittent tonic-clonic convulsions which lasted for approximately one-half hour. The animals then passed into a depressed state which terminated in death within 3 to 4 hours. Because of the quick onset of convulsions, the antidotes were administered immediately after the agonist.

Chlordiazepoxide and diazepam treated animals were all successfully antidoted. The dogs were ataxic after administration of the benzodiazepines, however ataxia was transient and lasted only 10 to 15 minutes. Both chlordiazepoxide and diazepam prevented seizures in the pentylenetetrazol intoxicated animals.

Pentobarbital treated animals became unconscious after injection and during this time displayed running motions with their front paws and twitching. Although somewhat ataxic, the animals were usually awake and able to stand within 3 hours. The pentobarbital antidoted animals all had good recoveries.

Chlorpromazine was ineffective as an antidote for acute pentylenetetrazol poisoning. Animals treated with chlorpromazine convulsed within 3 to 4 minutes after administration of pentylenetetrazol and displayed toxic symptoms similar to those of the control animals. All of these animals died within 3 to 4 hours.
B. The Effects of Chlordiazepoxide, Pentobarbital, Chlorpromazine, and Diazepam in Acute Chlorpheniramine Intoxication in Rats and Dogs

1. Introduction

Acute poisoning with antihistamines is common. There were 930 reported cases in 1967 (Verhulst and Crotty, 1968). The antihistamines are among the drugs most commonly found in medicine cabinets. Twenty to 30 tablets of most commercially available antihistamines represent a lethal or near-lethal dose in children (Douglas, 1966). The central nervous system effects elicited by the antihistamines seem to be the main factors contributing to lethality (Labelle and Tislow, 1954). In a poisoned individual some of the typical signs and symptoms are depression, hyperexcitability, jumpiness, tremors, and intermittent tonic-clonic convulsions. Central nervous system stimulation with convulsions is characteristically seen in the small child (Way and Herbert, 1952; Sice, 1962; Douglas, 1966).

Chlorpheniramine, a propylamine derivative, was selected as a representative of antihistamines because it is commonly found in many of the commercially available preparations. It is one of the most potent oral antihistamines available and in overdosage exerts strong central nervous system effects, particularly stimulation.

A short acting barbiturate commonly used for control of the central nervous system stimulation produced by an antihistamine (Sollman, 1957; Dreisbach, 1963; Goth, 1966) is compared with chlordiazepoxide, chlorpromazine, and diazepam in their ability to elevate convulsive and mortality thresholds.
2. Methods

**Administration of chlorpheniramine by constant infusion.** A 1% solution of chlorpheniramine was infused intravenously into rats at a rate of .821 ml/minute (8.21 mg/minute), after pretreatment with the candidate antagonists.

**Acute administration of chlorpheniramine to dogs.** Male mongrel dogs, weighing between 8 and 12 kg, were fasted for 24 hours. A previously determined lethal dose of chlorpheniramine (60 mg/kg) was administered by gavage in a volume of 3 ml/kg and followed by a wash of 3 ml/kg of water. Thirty minutes after the administration of chlorpheniramine the following drug treatments were administered via the cephalic vein: chlordiazepoxide, 15 mg/kg; chlordiazepoxide, 40 mg/kg; pentobarbital, 20 mg/kg; chlorpromazine, 3 mg/kg; chlorpromazine, 6 mg/kg; diazepam, 3 mg/kg; diazepam, 6 mg/kg; normal saline, 0.5 ml/kg (control).

3. Results

**Constant infusion.** The results of this study are presented in Figures 2 and 3. Figure 2 shows the effect of the TD10 of the antagonists on the convulsive and lethality threshold ratios and Figure 3 shows the effect of the TD90 of the antagonists on convulsive and lethality threshold ratios.

The TD10 of chlordiazepoxide, pentobarbital, and diazepam, elevated chlorpheniramine convulsive threshold 63%, 20% and 35%, respectively, but failed to raise the lethality threshold. The TD10 of chlorpromazine was ineffective in modifying either seizure or mortality.
Figure 2. Effect of TD10 of various antagonists on chlorpheniramine threshold ratios. Open symbols represent convulsive threshold ratios and solid symbols represent lethality threshold ratios. Bracketed lines designate 95% fiducial limits.
Figure 3. Effect of TD90 of various antagonists on chlorpheniramine threshold ratios. Open symbols represent convulsive threshold ratios and solid symbols represent lethality threshold ratios. Bracketed lines designate 95% fiducial limits.
threshold. The TD90 of chlordiazepoxide, pentobarbital, chlorpromazine, and diazepam elevated seizure threshold 112%, 124%, 20% and 76% respectively. These changes were accompanied by an elevation of the mortality threshold of 20%, 28%, 20% and 27% respectively. The diluent for diazepam did not affect seizure or mortality threshold.

**Acute dog studies.** The results of this study are presented in Table 2. The benzodiazepines, chlordiazepoxide and diazepam were the most effective antagonists in preventing death against chlorpheniramine. Chlorpromazine at the higher dosage, antidoted 2 of the 8 dogs. Chlorpromazine, at the lower dose, and pentobarbital, at the only dose tested, were ineffective in preventing death due to acute chlorpheniramine intoxication.

4. **Discussion**

Rats infused with chlorpheniramine progressed through a series of responses, the most clearcut of which are initial clonic convulsions and death. The initial convulsive phase was followed by a depressed period, a return of clonic convulsions after prolonged infusion, tonic convulsions and finally death. Pretreatment of animals with the various antagonists altered the toxicity picture mainly by causing a delay in the onset of initial clonus and time of death. All of the antidotes at the highest dosage tested were capable of significantly delaying appearance of the two main end points.
Table 2. The effect of various antagonists on lethality of chlorpheniramine in dogs

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Antagonist</th>
<th>No. of Dogs</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpheniramine</td>
<td>None</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>60 mg/kg, P.O.</td>
<td>Chlor Diazepamoxide</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>60 mg/kg, P.O.</td>
<td>15 mg/kg, I.V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>Chlor Diazepamoxide</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>60 mg/kg, P.O.</td>
<td>40 mg/kg, I.V.</td>
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<td></td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>Diazepam</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>60 mg/kg, P.O.</td>
<td>3 mg/kg, I.V.</td>
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<td></td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>Diazepam</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>60 mg/kg, P.O.</td>
<td>6 mg/kg, I.V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>Pentobarbital</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>60 mg/kg, P.O.</td>
<td>20 mg/kg, I.V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>Chlorpromazine</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>60 mg/kg, P.O.</td>
<td>3 mg/kg, I.V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>Chlorpromazine</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>60 mg/kg, P.O.</td>
<td>6 mg/kg, I.V.</td>
<td></td>
<td></td>
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</table>
Symptoms of acute chlorpheniramine toxicity in dogs included drowsiness, followed by restlessness, hyperexcitability, repeated bouts of tonic-clonic convulsions, depression and finally death.

Chlordiazepoxide, the most effective antagonist, prevented seizure or reduced the intensity and duration of convulsions in the antidoted animals although preconvulsive activity such as hyperexcitability, twitching and tremors were still prevalent. The two dose levels of chlordiazepoxide produced transient neurotoxic effects following intravenous injection in dogs. The lower dose caused ataxia of 10 to 15 minutes duration; the higher dose caused ataxia of somewhat greater intensity and longer duration and, in addition, unconsciousness in several dogs.

Diazepam seemed as effective as chlordiazepoxide in antagonizing the lethal effects of chlorpheniramine. As with chlordiazepoxide, transient ataxia was seen at both the high and low dose of diazepam and unconsciousness of 1 to 2 hours duration was observed in several of the animals at the higher dosage level. Dogs successfully antidoted with diazepam showed no seizure or displayed central nervous system excitement of decreased intensity compared to control animals. Animals which did not survive demonstrated typical symptoms of chlorpheniramine poisoning prior to death.

Pentobarbital did not increase survival in dogs after the administration of chlorpheniramine. The animals became unconscious within 5 minutes after pentobarbital injection and remained comatose for 2 to 3 hours. After this time period the animals displayed
excitability which progressed to tremors, convulsions, depression and death.

At the lower dose tested chlorpromazine did not alter the toxicity or lethality of chlorpheniramine in dogs; however, at the higher dose there were two survivors. All of the animals, including the dogs that survived, were extremely excited and displayed convulsions.

C. The Effects of Chlordiazepoxide, Pentobarbital, Chlorpromazine and Diazepam in Acute Amphetamine Intoxication in Rats and Dogs

1. Introduction

Therapeutically the amphetamines are commonly used in the treatment of obesity and the management of depressive states. The stimulating effects of amphetamines have led to their hazardous abuse to produce a "thrill" or to increase physical or mental endurance. Data from National Clearinghouse for Poison Control Centers indicate an increase in the number of intoxications due to the amphetamines (Verhulst and Crotty, 1968).

Central nervous system stimulation is one of the most alarming manifestations of acute intoxication from amphetamine and related compounds. Barbiturates have been the traditional drug of choice for the symptomatic treatment of amphetamine poisoning (Dobbs, 1961; Gleason, Gosselin and Hodge, 1963; Deichman and Gerarde, 1964). In view of the favorable reports of Lasagna and McCann (1957) and Espelin and Done (1966) regarding the use of chlorpromazine in amphetamine poisoning, it is desirable to evaluate the treatment of amphetamine intoxication
by other tranquilizing drugs. Hence, chlordiazepoxide and diazepam were compared with chlorpromazine and pentobarbital to determine their ability to elevate convulsion and mortality thresholds in amphetamine toxicity.

Dextroamphetamine differs from the other available amphetamines mainly in relative potency. It was selected as an example of the amphetamines because of its representative activity and common usage.

2. Methods

Administration of d-amphetamine by constant infusion. A 0.5% solution of d-amphetamine was infused intravenously into rats at a rate of 1.15 ml/minute (5.75 mg/minute) after pretreatment with the candidate antagonists.

Acute administration of d-amphetamine to dogs. Male mongrel dogs, weighing between 6 and 12 kg, were fasted for 24 hours. A previously determined lethal dose of d-amphetamine (45 mg/kg) was administered by gavage, in a volume of 3 ml/kg, and followed by a wash of 3 ml/kg of water. Thirty minutes after the administration of d-amphetamine the following drug treatments were administered via the cephalic vein: chlordiazepoxide, 15 mg/kg; chlordiazepoxide, 40 mg/kg; pentobarbital, 20 mg/kg; chlorpromazine, 3 mg/kg; chlorpromazine, 6 mg/kg; diazepam, 3 mg/kg; diazepam, 6 mg/kg; normal saline, 0.5 ml/kg (control).
3. Results

Constant infusion. The results of this study are presented in Figures 4 and 5. Figure 4 shows the effect of the TD10 of the antagonists on the convulsive and lethality threshold ratios and Figure 5 shows the effect of the TD90 of the antagonists on convulsive and lethality threshold ratios.

The TD10 of chlordiazepoxide elevated dextroamphetamine seizure threshold 28%. Although the TD10 of pentobarbital and chlorpromazine appear to elevate the seizure threshold by approximately 30%, the broad variance rendered the data nonsignificant. The TD10 of diazepam and the diluent for diazepam exerted no effect on seizure threshold. All the antagonists at their TD10 failed to raise the mortality threshold. In fact, the TD10 of diazepam and the diluent for diazepam significantly lowered mortality threshold 28% and 24% respectively.

The TD90 of chlordiazepoxide elevated seizure threshold 42% but had no effect on lethality threshold. The TD90 of pentobarbital prevented seizure in 80% of the animals and increased mortality threshold 15%. Chlorpromazine in an equivalent neurotoxic dose, elevated seizure and mortality thresholds 34% and 32% respectively. Diazepam did not affect seizure threshold but lowered mortality threshold 15%.

Acute dog studies. Data from this study is presented in Table 3. The lower and higher doses of chlordiazepoxide protected 2 of 8 and 1 of 8 dogs, respectively, whereas the lower dose of diazepam offered no protection against d-amphetamine and the higher dose
Figure 4. Effect of TD10 of various antagonists on d-amphetamine threshold ratios. Open symbols represent convulsive threshold ratios and solid symbols represent lethality threshold ratios. Bracketed lines designate 95% fiducial limits.
Figure 5. Effect of TD90 of various antagonists on d-amphetamine threshold ratios. Open symbols represent convulsive threshold ratios and solid symbols represent lethality threshold ratios. Bracketed lines designate 95% fiducial limits.
Table 3. The effect of various antagonists on lethality of d-amphetamine in dogs

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Antagonist</th>
<th>No. of Dogs</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Amphetamine</td>
<td>None</td>
<td>8</td>
<td>8 0</td>
</tr>
<tr>
<td>45 mg/kg, P.O.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Amphetamine</td>
<td>Chlordiazepoxide</td>
<td>8</td>
<td>6 2</td>
</tr>
<tr>
<td>45 mg/kg, P.O.</td>
<td>15 mg/kg, I.V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Amphetamine</td>
<td>Chlordiazepoxide</td>
<td>8</td>
<td>7 1</td>
</tr>
<tr>
<td>45 mg/kg, P.O.</td>
<td>40 mg/kg, I.V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Amphetamine</td>
<td>Diazepam</td>
<td>8</td>
<td>8 0</td>
</tr>
<tr>
<td>45 mg/kg, P.O.</td>
<td>3 mg/kg, I.V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Amphetamine</td>
<td>Diazepam</td>
<td>8</td>
<td>7 1</td>
</tr>
<tr>
<td>45 mg/kg, P.O.</td>
<td>6 mg/kg, I.V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Amphetamine</td>
<td>Pentobarbital</td>
<td>8</td>
<td>3 5</td>
</tr>
<tr>
<td>45 mg/kg, P.O.</td>
<td>20 mg/kg, I.V.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Amphetamine</td>
<td>Chlorpromazine</td>
<td>8</td>
<td>0 8</td>
</tr>
<tr>
<td>45 mg/kg, P.O.</td>
<td>3 mg/kg, I.V.</td>
<td></td>
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</tr>
</tbody>
</table>
successfully antidoted only one dog. Chlorpromazine provided protection against the lethal effects of d-amphetamine in all 8 animals tested and pentobarbital, the next most effective antidote, successfully antidoted 5 of 8 dogs.

4. Discussion

The symptoms displayed by rats during constant infusion of d-amphetamine were less intense than those observed in the previous chlorpheniramine study. The initial convulsive phase was followed by depression with no return to clonic seizure. In addition, tonic convolution was usually absent. Diazepam and the vehicle for diazepam not only failed to protect the rats against seizure, but actually hastened death.

Acute amphetamine intoxication in dogs was characterized by aimless head motions, hyperexcitability, twitching and tremors. The dogs lost their ability to stand, displayed clonic convulsions and viscous salivation. Deep depression followed the seizure stage, which terminated in death.

Chlordiazepoxide and diazepam treated animals were not protected from most of the signs of d-amphetamine intoxication. All of the benzodiazepine treated animals exhibited hyperexcitability, some clonic convulsions, viscous secretions and deep depression. Diazepam treated animals seemed to have a more intense seizure episode than those treated with chlordiazepoxide. The animals which did not survive, did not recover from the deep depression and were comatose until death.
Pentobarbital rendered the dogs unconscious but proved to be effective in antidoting 5 out of 8 dogs. The dogs remained comatose for 2 to 3 hours and during this period showed running motions with their front paws and thrashed about their cages. The animals which survived, after regaining consciousness, were in a depressed state, ataxic and exhibited dyspneic breathing. However, within 48 hours they recovered completely.

Chlorpromazine produced the most dramatic results as an antidote to d-amphetamine intoxication. It successfully antidoted all of the poisoned animals, and although the dogs were somewhat excited, convulsions and most of the other signs of intoxication were absent. In addition, the animals were not rendered unconscious and were able to walk without difficulty.
IV. GENERAL DISCUSSION

Chlordiazepoxide, pentobarbital, and diazepam at their TD10 significantly elevated pentylenetetrazol seizure and mortality thresholds in rats. Chlorpromazine, at its TD10, had no effect on pentylenetetrazol seizure or mortality threshold. The parallel fluctuation of the mortality threshold with convulsive threshold suggests that the lethal effects of pentylenetetrazol is directly related to the severity of seizure. This assumption is strengthened by the results obtained from the dog studies. In these studies, pentobarbital, chlordiazepoxide and diazepam prevented seizures and successfully increased survival in dogs which had received a lethal dose of pentylenetetrazol. On the other hand, chlorpromazine failed to control seizure and also failed to prevent death.

Chlorpheniramine seizure threshold in rats was elevated slightly by the TD10 of all the antagonists with the exception of chlorpromazine. However, none of the antidotes, when administered at their TD10, raised the mortality threshold. In contrast, the TD90 of all four test antidotes significantly increased the convulsive threshold over the levels noted when the TD10 of these drugs was utilized; yet the increase in mortality threshold was only 20% to 28%, an antidotal effect not at all comparable to that obtained with the TD10 treatments against pentylenetetrazol lethality. The data from these experiments suggest that although central nervous system
stimulation and convulsions may contribute to lethality they are not necessarily the sole cause of death in chlorpheniramine infused rats. Antihistamines are reported to have a quinidine-like action on the heart (Schallek, 1952) and indeed, McCawley, Weston and David (1951) indicate that in this respect chlorpheniramine is equivalent in potency to quinidine itself. Hence, it is possible that excessive doses of an antihistamine can produce direct toxic effects on the heart. The dog studies also provide suggestive evidence that extra-central effects contribute to the toxicity of chlorpheniramine. Of the potential antidotes tested, chlordiazepoxide and diazepam were the most effective in antagonizing convulsions and increasing survival in dogs administered a lethal dose of chlorpheniramine. In a few dogs, 24 or more hours after administration of chlorpheniramine, at a time when they appeared to be completely recovered from the effects of the antihistamine, they suddenly collapsed and died upon exposure to external stimulation such as a sudden noise. The abrupt manner in which these animals died suggest that a cardiovascular factor may be involved. Numerous investigators report that antihistamines sensitize the cardiovascular system to epinephrine (Loew, MacMillan, and Kaiser, 1946; Yonkman et al., 1946; Sherrod, Loew and Schloemer, 1947; Isaac and Goth, 1967). It is well known that exposure of a sensitized heart to epinephrine could give rise to arrhythmia and cardiac failure (Kuriaki and Uchida, 1955). Hence, an important cause of death during intravenous infusion with chlorpheniramine may be referrable to the quinidine effect. This mechanism of death could account for the poor correlation between
protection against seizure and protection against death in rats infused with chlorpheniramine. On the other hand, a fixed lethal dose of chlorpheniramine may cause death in dogs by a combination of central and cardiac effects; central excitation and convulsions may cause endogenous release of epinephrine which in turn, induces fatal cardiac arrhythmia in sensitized myocardia. Thus, protection against central nervous system excitation may serve secondarily to prevent cardiac arrhythmia in dogs. In the dogs poisoned with chlorpheniramine, chlordiazepoxide and diazepam effectively prevented death in 50% and 37.5% of animals, respectively, whereas pentobarbital completely failed to prevent death. The doses of the benzodiazepines employed caused unconsciousness. The dogs antidoted with the benzodiazepines generally had milder seizures or no seizures at all; most of the animals that succumbed did so in 6 to 12 hours. A few, as mentioned above survived over 24 hours and appeared completely recovered but died suddenly when startled by a sudden noise. In contrast, the dogs antidoted with pentobarbital generally lost consciousness and after sleeping for 2 to 3 hours would then demonstrate symptoms of severe central nervous system stimulation, including convulsions, and ultimately die. Although none of the pentobarbital antidoted dogs survived the lethal dose of chlorpheniramine, a certain degree of protection was observed in that the latency for seizure was delayed until the hypnotic effect was dissipated and the total time required for death was prolonged by several hours. Probably because of their longer lasting anticonvulsant action single doses of
benzodiazepines are more effective than single doses of pentobarbital in protecting dogs against seizures and death caused by chlorpheniramine. In view of the relative effectiveness of chlordiazepoxide, pentobarbital, and diazepam in suppressing chlorpheniramine induced seizures and delaying death it is possible that repeated dosing with these antidotes may enhance their protective effect against death. However, Way and Herbert (1952) cautioned that the degradation products of antihistamines may enhance the actions of pentobarbital and contribute to the overall toxicity.

Data from the infusion studies with pentylenetetrazol and chlorpheniramine indicate that chlorpromazine is not a very effective anticonvulsant. Indeed, chlorpromazine has been reported to lower seizure threshold in a number of experimental and clinical situations (Kopeloff, Chusid and Kopeloff, 1955; Meszaros and O'Rielly, 1956; Cares et al., 1957; Voegele and May, 1957; Tedeschi et al., 1958; Shaw et al., 1959). On the other hand chlorpromazine offered some protection at the higher dose against death from chlorpheniramine.

In view of the reported ability of chlorpromazine to alter uptake and release of catecholamines (Axelrod, Whitby and Hertting, 1960; Gey and Pletcher, 1961), it could be conjectured that chlorpromazine protects against the lethal cardiac effects of antihistamines by lessening the influence of endogenous catecholamines.

In both the rat and dog studies chlorpromazine and pentobarbital were the most effective antidotes for amphetamine intoxication. Chlorpromazine seems to be a highly effective antidote for amphetamine intoxication. Since the amphetamines are believed to exert their
action by releasing catecholamines (Burns and Rand, 1958; Axelrod and Tomchick, 1960) it is conceivable that chlorpromazine exerts an antidotal action by modifying the release and uptake of catecholamines as mentioned above or by blocking adrenergic receptors (Martin, Riehl and Unna, 1960; Janssen, 1967). Pentobarbital which seems to act more as a physiological antagonist demonstrated antidotal activity in both rat and dog studies; however, the results were not as dramatic as those observed with chlorpromazine. Espelin and Done (1968) observed that patients intoxicated with amphetamines respond rather poorly to barbiturates. This study indicates that the benzodiazepines demonstrate little promise as antidotes in acute amphetamine intoxication. There is the possibility that the central nervous system depressants may add to the post convulsive depression that usually follows poisoning by a central nervous system stimulant. Gurdocki, Schuler and Goldstein (1966) showed an enhancement of amphetamine lethality in mice treated with chlordiazepoxide. Data from our infusion studies indicate an enhancement of amphetamine lethality in the animals antidoted with diazepam; however, this increase might be attributable to the diluent for diazepam which also increased lethality.
V. SUMMARY AND CONCLUSIONS

1. Since the medical literature and the present study indicate that chlorpheniramine overdosage can result in central nervous system stimulation with convulsions, direct cardiac toxicity, and myocardial sensitization to epinephrine, the following recommendations may be advanced for the treatment of chlorpheniramine intoxication:
   a. hospitalization of patient
   b. continuous deep sedation, preferably with benzodiazepines
   c. monitoring of electrocardiogram for quinidine-like effects
   d. protection of patient against stress, including noise, in order to avoid adrenal discharge and precipitation of cardiac arrhythmia

2. The results of this study support the clinical findings of Espelin and Done that chlorpromazine is very effective in the treatment of amphetamine intoxication. Observations in dogs imply that chlorpromazine may be considered the antidote of choice in amphetamine overdosage.
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