THE EFFECTS OF PSYCHOPHARMACOLOGIC DRUGS ON EXPERIMENTAL AUDIOPHOBIC SEIZURE AND BRAIN NEUROHORMONE LEVELS

by

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Donald Chin

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## TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. GENERAL PROCEDURES</td>
<td>4</td>
</tr>
<tr>
<td>III. THE EFFECTS OF CERTAIN PSYCHO-PHARMACOLOGIC DRUGS ON AUDIOPHAGEIC SEIZURE AND BODY TEMPERATURE</td>
<td></td>
</tr>
<tr>
<td>A. The Effect of Reserpine on Audiogenic Seizure and Body Temperature</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>10</td>
</tr>
<tr>
<td>Methods</td>
<td>12</td>
</tr>
<tr>
<td>Results</td>
<td>13</td>
</tr>
<tr>
<td>Discussion</td>
<td>16</td>
</tr>
<tr>
<td>B. The Effect of Iproniazid on Audiogenic Seizure and Body Temperature</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>19</td>
</tr>
<tr>
<td>Methods</td>
<td>20</td>
</tr>
<tr>
<td>Results</td>
<td>21</td>
</tr>
<tr>
<td>Discussion</td>
<td>23</td>
</tr>
<tr>
<td>C. The Effect of Iproniazid-Reserpine Drug Combination on Audiogenic Seizure and Body Temperature</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>24</td>
</tr>
<tr>
<td>Methods</td>
<td>25</td>
</tr>
<tr>
<td>Results</td>
<td>26</td>
</tr>
<tr>
<td>Discussion</td>
<td>28</td>
</tr>
</tbody>
</table>
### TABLE OF CONTENTS (cont'd)

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.</td>
<td>The Effect of Reserpine-Iproniazid Drug Combination on Audiogenic Seizure and Body Temperature</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Introduction</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Methods</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Results</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Discussion</td>
<td>32</td>
</tr>
<tr>
<td>E.</td>
<td>The Effect of Chlordiazepoxide on Audiogenic Seizure and Body Temperature</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Introduction</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Methods</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Results</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Discussion</td>
<td>40</td>
</tr>
<tr>
<td>IV.</td>
<td>THE EFFECT OF CERTAIN PSYCHOPHARMACOLOGIC DRUGS ON THE BRAIN NEUROHORMONE LEVELS OF 5-HYDROXYTRYPTAMINE AND NOREPIERINEPHRINE</td>
<td></td>
</tr>
<tr>
<td>A.</td>
<td>Introduction</td>
<td>42</td>
</tr>
<tr>
<td>B.</td>
<td>Methods</td>
<td>44</td>
</tr>
<tr>
<td>C.</td>
<td>Results</td>
<td>52</td>
</tr>
<tr>
<td>D.</td>
<td>Discussion</td>
<td>54</td>
</tr>
<tr>
<td>V.</td>
<td>GENERAL DISCUSSION</td>
<td>61</td>
</tr>
<tr>
<td>VI.</td>
<td>SUMMARY AND CONCLUSIONS</td>
<td>67</td>
</tr>
<tr>
<td>VII.</td>
<td>LITERATURE CITED</td>
<td>70</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of Reserpine on Audiogenic Seizure</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Effect of Reserpine on Body Temperature 4 Hours Following Drug Treatment</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Effect of Iproniazid on Audiogenic Seizure</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>Effect of Iproniazid on Body Temperature 15 Hours Following Drug Treatment</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>Effect of the Drug Combination, Iproniazid Followed in 2 Hours by Reserpine, on Audiogenic Seizure</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>Effect of the Drug Combination, Iproniazid Followed in 2 Hours by Reserpine, on Body Temperature 6 Hours Following Iproniazid Injection</td>
<td>27</td>
</tr>
<tr>
<td>7</td>
<td>Effect of the Drug Combination, Reserpine Followed in 2 Hours by Iproniazid, on Audiogenic Seizure</td>
<td>33</td>
</tr>
<tr>
<td>8</td>
<td>Effect of the Drug Combination, Reserpine Followed in 2 Hours by Iproniazid, on Body Temperature 5 Hours Following Reserpine Injection</td>
<td>33</td>
</tr>
<tr>
<td>9</td>
<td>Effect of Chlordiazepoxide on Audiogenic Seizure, Including Clonic Convulsions and Running Movements</td>
<td>38</td>
</tr>
<tr>
<td>10</td>
<td>Effect of Chlordiazepoxide on Body Temperature 1 Hour Following Drug Treatment</td>
<td>39</td>
</tr>
<tr>
<td>11</td>
<td>The Effects of Certain Psychopharmacologic Drugs on Brain Concentrations of 5-Hydroxytryptamine and Norepinephrine</td>
<td>53</td>
</tr>
</tbody>
</table>
**LIST OF TABLES (cont'd)**

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>62</td>
</tr>
<tr>
<td><strong>A Summary of the Effects of Certain Psychopharmacologic Drugs on Audio-genic Seizure-Susceptible Rats</strong></td>
<td></td>
</tr>
</tbody>
</table>

**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td><strong>Sound Chamber</strong></td>
<td></td>
</tr>
</tbody>
</table>

vii
I. GENERAL INTRODUCTION

Sound-induced seizures in various animals have been reported for a number of years. Donaldson in 1924 was probably the first to describe "the almost maniacal running and jumping" of white rats when a bundle of keys was jingled in front of the cage. Since 1939, "audiogenic seizure" has become the most widely used and accepted term for this sound-induced behavior. The audiogenic seizure, although still a scientific enigma, has become regarded by many research workers as an epileptiform-type convulsion caused by the conflict-producing situation of auditory stress. Investigators such as Morgan and Auer and Smith hypothesized that the convulsion is a result of excessive auditory stimulation which causes over-excitation of the auditory areas, with the resultant spread of impulses to adjacent motor areas. Investigations published on audiogenic seizure have received two excellent reviews by Finger (1924 to 1947) and Bevan (1947 to 1954).

Audiogenic seizure-susceptible animals have been employed, not infrequently, in the study of therapeutic agents. One of the first uses of these animals was for the evaluation
of anticonvulsant drugs.\textsuperscript{7,8} Since the advent of psycho-
pharmacologic drugs, some investigators have also employed
audiogenic seizure-susceptible mice and rats in the study of
these agents.\textsuperscript{9-13,38} Some of the above workers believe that
the convulsion or so-called "fright response" of these animals
may be analagous to the exaggerated and abnormal responses
of psychiatric patients to auditory stimulation, as reported
by Malmo and co-workers.\textsuperscript{14,15} The results reported by
various investigators concerning the effect of psycho-
pharmacologic drugs on sound-sensitive animals are often
conflicting.\textsuperscript{9-11,13,38} It has been suggested that these
variations in results may be due to such factors as difference
in route of administration, time intervals for tests, and
strains of animals. In view of the conflicting reports, it
would appear that further studies in this area are warranted.

Several groups of investigators\textsuperscript{16-19} have examined
the effects of certain psychopharmacologic compounds on body
temperature and the neurohormones of the brain. These work-
ers seem to agree that hypothermia, sedation, or hypnosis
and brain neurohormone levels are interrelated. Additional
studies involving the effects of psychopharmacologic drugs on
electroshock seizure and a correlation of these effects with brain neurohormone levels have been reported. However, a correlative study on the effects of psychopharmacologic drugs on neurohormones and audiogenic seizure susceptibility has not been reported.

Because of the conflicting reports in the literature with respect to the action of certain psychopharmacologic drugs on audiogenic seizure, the present studies were initiated in an attempt to clarify some of the discrepancies. Since some psychopharmacologic agents have the ability to alter brain neurohormone levels and since Prockop and co-workers reported a correlation between these neurohormone levels and electroshock seizure, it was thought important to ascertain the effect of certain psychopharmacologic drugs on brain neurohormone concentrations and on audiogenic seizure. In view of the reports of Maier and co-workers that hypothermia tends to antagonize audiogenic seizure, the effects of these psychopharmacologic drugs on body temperature were also determined.
II. GENERAL PROCEDURES

Animals

Early in 1959 male and female albino rats, Sprague-Dawley strain, from the College of Pharmacy animal colony were screened for audiogenic seizure (AGS)*-susceptibility in a sound chamber described below. The initial few animals which displayed convulsions were mated and the offspring were inbred. Since many generations of inbreeding indicated that only approximately 40% of the offspring were AGS sensitive, a constant screening process was conducted to develop and perpetuate a colony of audiogenic rats. The AGS pattern of these rats usually consists of a period of wild and rapid running within the test chamber, followed by convulsions. The convulsions displayed by these animals as the result of sound stimulation are almost invariably clonic in nature. Maximal audiogenic seizure (MAS), as characterized by tonic hind-leg extension, occurred only rarely.

*The designation AGS as used henceforth refers to the pattern of audiogenic seizure activity which consists of wild and rapid running which terminates in clonic convolution.
Audiogenic seizure-susceptible rats were maintained on a diet of Purina Fox Chow pellets or meal and allowed free access to food and water except during the short periods of time when they were removed from their cages for drug treatment and testing. The audiogenic animals employed in these studies weighed between 90 and 500 g and were of mixed sexes. Most of the animals received no prior drug treatment. In those cases where animals had been employed in a previous drug study, an interval of approximately 3 weeks was allowed to elapse before the animals were used again.

McGrael and Frings and Frings have reported that the following sound (pure tone) frequencies were effective for the production of seizure: 8,000, 10,000, 12,000, and 25,000 cps in mice and 9,500 cps in rats. However, in this laboratory, pure tone, in various sound frequencies (20 to 20,000 cps) and various sound pressures (maximum of 127 db), was found ineffective for producing seizure in AGS-sensitive rats. On the other hand, these same animals convulsed when they were exposed to sound produced by doorbells at a sound pressure of 108±14 db. The relative effectiveness of different types of sound to produce seizures has been
investigated by Suter and co-workers\textsuperscript{26} and Frings and Frings\textsuperscript{27}. These investigators reported that noise or white sound (bells, jingling keys, etc.) is more effective than pure tone as a stimulus for producing AGS. Frings and Frings\textsuperscript{27} attributed the superiority of noise to its "acoustic property of complexity."

On the basis of preliminary studies in this laboratory, a sound chamber capable of delivering white sound was constructed as described in the caption for Figure 1. This sound chamber was used in all the studies reported herein concerning the effects of psychopharmacologic drugs on AGS-susceptibility.

\textbf{Test Procedures}

Because there is a variation in the consistency of AGS-sensitivity in some AGS-susceptible animals\textsuperscript{28,29,30}, it was necessary to establish a criterion for the selection of candidate test and control animals for the present studies. A rat was considered suitable for use if it had convulsed within a 100-second period of sound stimulation during each of three trials, spaced at 48-hour intervals. The candidate animals were then randomly divided into test and control groups. Test animals were all injected by the intraperitoneal (i.p.) route with the appropriate drugs. Control animals in
The sound chamber is composed of two enclosures, one inside the other. The outer one consists of a double-walled box (20"x20"x26") made of plywood and insulated with 1 inch of cotton for soundproofing; the inner wall of the box is lined with acoustic tile. The inner enclosure is a cylinder of galvanized tin (16"x20"), the bottom of which is covered with wire mesh and is supported on metal legs so that it stands approximately 4 inches above the acoustic tile. The chamber lid has mounted in it 2 doorbells* (4 and 6 inches in diameter), a 5-watt incandescent light bulb, and a plexiglass window (6"x11-1/2"), consisting of two pieces of plexiglass separated by a 1-inch air space.

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*Edwards Company, Inc. (Model 55), Norwalk, Connecticut
initial studies were injected intraperitoneally with the requisite volume of 0.9% sodium chloride solution. However, it was observed that saline administration had no influence on AGS; therefore, the injection of control animals was discontinued in subsequent determinations. These findings in saline-treated control animals are consistent with those of Suter and co-workers and Bielec.

At appropriate time intervals an individual test or control rat was placed in the test chamber and subjected to sound stimulation. A test was terminated when (a) 3 minutes had elapsed and the animal did not exhibit running or convulsion, (b) 3 minutes had elapsed but the animal exhibited only running, or (c) the rat exhibited running movements followed by clonic and/or tonic convulsions.

The effect of each drug treatment on AGS was compared with sound-induced responses in corresponding control animals. The results were calculated in terms of per cent incidence of seizures, and Student's "t" test of significance was employed to evaluate the difference observed between test and control animals.

In the temperature studies a telethermometer (manufactured by Yellow Springs Instrument Company, model
which utilizes a thermistor probe, was employed to measure the rectal temperature of the animals. For a given study, the temperature of the test animals was determined before drug treatment and at an appropriate period after drug treatment. The temperature of the control animals was determined concurrently with that of the test animals during these measurements. The results of the temperature determinations were then calculated and expressed in terms of temperature change between the readings before and after drug treatment. Test for significance between the values obtained for the control animals and the test animals was determined by means of Student's *t* test.
III. THE EFFECTS OF CERTAIN PSYCHOPHARMACOLOGIC DRUGS ON AUDIOGENIC SEIZURE AND BODY TEMPERATURE

A. The Effects of Reserpine on Audiogenic Seizure and Body Temperature

Introduction

Reserpine is an alkaloid obtained from the root of the Indian plant, Rauwolfia serpentina, which has been used clinically for the treatment of hypertension and mental disorders. Various pharmacologic effects of reserpine on experimental animals, in addition to its effect on AGS, have been reported since the drug first came to the attention of investigators in the Western World almost a decade ago. In general, reserpine causes a decrease in alertness, spontaneous motor activity, body temperature, rate and depth of respiration, blood pressure, and heart rate. It also potentiates hypnotic drugs and possesses a parasympathetic-like effect.

Plotnikoff and Green\textsuperscript{11} and Plotnikoff\textsuperscript{33} who used audiogenic seizure-susceptible mice in the study of reserpine,
reported a graded reduction in the running phase of the "fright response" following oral doses of 10 to 200 mg/kg and a slight reduction in the number of convulsions. Larger oral doses (300-400 mg/kg) intensified the "fright response" as indicated by an increase in the incidence of convulsions and catalepsy.

Plotnikoff further reported that the intraperitoneal (i.p.) injection of reserpine, 5, 15, 25, and 100 mg/kg, protected 30, 56, 97, and 100% of the mice, respectively, from sound-induced seizure. In contrast, Fink and Swinyard reported that reserpine is devoid of a protective effect against maximal audiogenic seizure (MAS). In fact, they found that seizure susceptibility was enhanced as evidenced by the occurrence of death following MAS in reserpine-treated mice. It is also of interest to note that these and other investigators reported that reserpine intensifies electroshock seizure and chemoshock seizure.

In view of the conflicting reports concerning the effect of reserpine on sound-induced seizure in mice, it was

*The "fright response" was graded by Plotnikoff and Green in order of severity as follows: running, jumping, circling, convulsions, and immobility (catalepsy)
considered desirable to determine the effect of this drug on AGS in rats. Such an investigation may be helpful in clarifying the existing controversy.

Methods

Seventy-two AGS-susceptible rats of mixed sexes weighing between 100 and 435 g were randomly divided into 12 groups of 6 rats each. Six groups of these rats served as test animals and were administered reserpine, 5 mg of the base/kg as a 0.58% solution of the lyophilized phosphate salt* or as a 0.5% solution of the base** The remaining groups of rats served as control animals and were untreated.

Three groups of test animals were subjected to sound stimulation 4 and 24 hours following drug administration. The remaining 3 groups of test animals were also tested at these times, but in addition were tested 48 hours after

*Reserpine (Serpasil) Phosphate Lyophilized, Ciba Pharmaceutical Products Co., Summit, N. J.

**Prepared by dissolving 100 mg of the free base in a few drops of glacial acetic acid and adding 2.5 ml. of propylene glycol, 2.5 ml. of ethanol, and 15 ml. of water
injection. Corresponding groups of control animals were concomitantly subjected to sound stimulation with the test animals. Body temperature was determined immediately following drug treatment and also immediately prior to sound stimulation at the 4-hour test time. Several days following the last audiogenic stimulation of the above animals, the 6 control groups were again randomly divided into 3 control and 3 test groups. Audiogenic seizure tests were conducted at the 4, 24, and 48-hour time periods and temperature readings made as specified above. Thus, this study was comprised of a total of 9 test and 9 control determinations, 3 pairs of the determinations made at the 4- and 24-hour test periods and 6 pairs at the 4-, 24-, and 48-hour test periods.

Results

The appearance of the reserpine-treated rat was obviously different from the alert, curious, untreated rat. Within an hour or 2 following the injection of reserpine, the test animals appeared sedated, became less active and less resistant to handling, and displayed ptosis of the eyelids, diarrhea, and an arched-back stance.

Four and 24 hours following drug treatment, 61.1
and 55.5% of the test animals, respectively, displayed MAS upon audiogenic stimulation, whereas none of the control animals exhibited MAS (see Table 1). Forty-eight hours after reserpine the incidence of MAS was still approximately 6% in the test animals and 0% in the control animals. Typically, in reserpine-treated rats that displayed MAS, sedation was abruptly replaced by running movements after approximately 10 to 20 seconds of sound stimulation. The running phase persisted for approximately 20 seconds and was followed by convulsive seizures. Maximal audiogenic seizure occurred within 10 to 100 seconds after the beginning of stimulation, but the usual latency for MAS was between 30 and 50 seconds.

The results of the temperature studies, 4 hours after drug administration, are presented in Table 2. It can be seen from this table that the mean body temperature of the test animals was decreased .88°C, whereas the mean body temperature of the control animals was increased .10°C. The difference between these 2 values (.98°C) is statistically significant (P < .001).
Table 1
EFFECT OF RESERPINE ON AUDIOGENIC SEIZURE

<table>
<thead>
<tr>
<th>Time of Audiogenic Stimulation</th>
<th>Per Cent MAS (tonic hind-leg extension) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 hours</td>
<td>Test Animals: 61.11 ± 4.83</td>
</tr>
<tr>
<td></td>
<td>Control Animals: 0</td>
</tr>
<tr>
<td>24 hours</td>
<td>Test Animals: 55.51 ± 8.75</td>
</tr>
<tr>
<td></td>
<td>Control Animals: 0</td>
</tr>
<tr>
<td>48 hours</td>
<td>Test Animals: 5.55 ± 3.60</td>
</tr>
<tr>
<td></td>
<td>Control Animals: 0</td>
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Table 2
EFFECT OF RESERPINE ON BODY TEMPERATURE 4 HOURS FOLLOWING DRUG TREATMENT

Mean Change in Body Temperature

<table>
<thead>
<tr>
<th>Degrees Centigrade ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Rats</td>
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<tr>
<td>Test Rats</td>
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</table>

Difference: -.98° *P < .001*
**Discussion**

The data obtained in this study indicate that reserpine caused an intensification of AGS. This effect was evidenced by the high percentages of MAS in the sound-stimulated test animals 4 and 24 hours following drug treatment. Forty-eight hours following drug treatment the AGS-enhancing effect of this drug was greatly diminished but was still present (see Table 1).

The report of Plotnikoff\(^3\) that reserpine, administered intraperitoneally, inhibits both the running and the convulsion is not substantiated by the data presented in Table 1. Indeed these results indicate that reserpine intensifies AGS. Fink and Swinyard,\(^1\) who also observed that reserpine intensifies AGS, attribute the variance between their finding and that of Plotnikoff to a difference in strain of animals and test procedures. It should be noted that Fink and Swinyard employed highly inbred mice which exhibit a high incidence of MAS, whereas Plotnikoff used non-inbred mice which display relatively few MAS. The results of the present investigation suggest that the incidence of MAS in an animal strain may not be an important factor, since the
animals employed in this study characteristically do not exhibit MAS, but did so under the influence of reserpine. However, the possibility exists that differences in species of animals may account for the variance in the finding of the present work with that reported by Plotnikoff.\textsuperscript{33,38} In accordance with the view of Fink and Swinyard,\textsuperscript{13} it is also felt that differences in test procedure used may be involved. In the present investigation, as in the study of Fink and Swinyard,\textsuperscript{13} animals were stimulated individually in the sound chamber, whereas Plotnikoff tested animals in groups of 5.

In one of his publications, Plotnikoff\textsuperscript{38} implies that differences in the route of drug administration and times of testing are critical factors which may account for the conflicting reports concerning the action of reserpine on AGS. Difference in route of drug administration does not appear to be a significant point, since the same method of injection (i.p.) was employed in the work conducted in this laboratory and by Plotnikoff. Neither does the time of testing appear to be a critical factor. Plotnikoff\textsuperscript{38} states that the actual peak time of activity of reserpine against AGS is approximately 24 hours. He appears to intimate that testing at an
earlier time period might produce varying results of a
different nature. Although a difference in time of testing
may yield quantitatively different results, it is unlikely that
this factor could give rise to qualitatively different effects.
Thus it can be seen from Table 1 that reserpine intensifies
AGS 4 hours after drug treatment, a result which concurs
with that reported by Fink and Swinyard,\textsuperscript{13} and that it
continues to intensify AGS at the 24-hour testing time, a
result which contradicts that of Plotnikoff.\textsuperscript{33} Indeed, the
AGS-intensifying effect of reserpine is as great at 4 hours
after drug treatment as it is 24 hours after drug treatment
(see Table 1).

The results of the body temperature study indicate
a slight but significant decrease in the temperature of the
rats 4 hours after treatment with reserpine. Although the
decrease in body temperature in the reserpine-treated rats
was significant, it is of interest to note that the change is
not as great as that observed in AGS-susceptible mice
treated similarly with reserpine in this laboratory.\textsuperscript{28,39} In
the case of the latter species a decrease in body temperature
ranging from 3\textdegree{} to 10\textdegree{} C was observed.
B. The Effect of Iproniazid on Audiogenic Seizure and Body Temperature

Introduction

Iproniazid* is a synthetic drug that has been used clinically for slightly over 10 years. This drug has been found to be a therapeutic agent with a broad spectrum of pharmacological activity.\textsuperscript{40} It was first used to treat tuberculosis, and the chance observation that it produces euphoria led to its clinical use in the treatment of mental depression.\textsuperscript{41}

Prockop and co-workers,\textsuperscript{20} in evaluating the anticonvulsant properties of monoamine oxidase (MAO) inhibitors against maximal electroshock seizure (MES) in rats, found that iproniazid exerted a pronounced anticonvulsant effect. They expressed protection against MES in terms of increased latency for hind-leg extension and also in terms of reduction in the number of animals that exhibited hind-leg extension. By either criteria, the anticonvulsant property of iproniazid was very pronounced; they reported that the maximum anticonvulsant effect occurs approximately 15 hours after i.p. injection.

\*Iproniazid (Marsilid), Roche Laboratories, Inc., Nutley, New Jersey
Since Prockop and associates\textsuperscript{20} have demonstrated the anticonvulsant activity of iproniazid against MES, it was considered of importance to investigate the effect of this drug against AGS.

\textbf{Methods}

Ninety-six AGS-susceptible rats of mixed sexes weighing between 100 and 435 g were randomly divided into 16 groups of 6 rats each. Eight groups of these rats served as test animals and were administered iproniazid, 100 mg of the base/kg, as a 7.7\% solution of the phosphate salt. The remaining 8 groups of rats served as control animals and were untreated. The test animals were subjected to sound stimulation 15, 48, and 72 hours following injection. The control animals were tested concomitantly with the iproniazid-treated animals at these time periods.

Body temperature was determined in a separate group of 12 AGS-susceptible rats. Six of these rats served as test animals and were administered iproniazid as indicated above. The remaining 6 of these rats received no treatment and served as control animals. The body temperature of the test animals was determined immediately before and 15 hours
after the injection of iproniazid. The temperature of the control animals was determined at the same times as the test animals.

**Results**

The appearance of the test rat was somewhat different from that of the untreated animal. Approximately 6 to 8 hours following the iproniazid injection, the iproniazid-treated rat exhibited exophthalmia, appeared hyperactive, and was more resistant to handling. These effects persisted for approximately 24 hours. Fifteen hours following drug treatment, 8.3% of the test and 60.3% of the control animals displayed clonic convulsions upon audiogenic stimulation (see Table 3). The difference between these results is highly significant ($P < .001$). However, 48 and 72 hours after iproniazid, the incidence of AGS in the test animals (56.1 and 43.8%, respectively) was no longer significantly different ($P > .05$) from that of the control animals (66.6 and 52%, respectively).

The results of the temperature studies, 15 hours after drug administration, are presented in Table 2. It can be seen that the mean body temperature of the test animals was decreased .08° C, and the mean body temperature of
### Table 3

**EFFECT OF IPRONIAZID ON AUDIOGENIC SEIZURE**

<table>
<thead>
<tr>
<th>Time of Audogenic Stimulation</th>
<th>Per Cent Clonic Convulsion ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test Animals</td>
</tr>
<tr>
<td>15 hours</td>
<td>8.3 ± 5.5</td>
</tr>
<tr>
<td>48 hours</td>
<td>56.1 ± 3.3</td>
</tr>
<tr>
<td>72 hours</td>
<td>43.8 ± 8.3</td>
</tr>
</tbody>
</table>

### Table 4

**EFFECT OF IPRONIAZID ON BODY TEMPERATURE 15 HOURS FOLLOWING DRUG TREATMENT**

Mean Change in Body Temperature

<table>
<thead>
<tr>
<th>Degrees Centigrade ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Rats</td>
</tr>
<tr>
<td>Test Rats</td>
</tr>
<tr>
<td>Difference</td>
</tr>
</tbody>
</table>


the control animals was decreased \(0.02^\circ\) C. The difference between these 2 values is not significant \((P > 0.05)\).

**Discussion**

The data obtained in these studies indicate that iproniazid, in a dose of 100 mg/kg, has a pronounced anticonvulsant effect against AGS in rats. This finding parallels the report of Frockop and colleagues,\(^2\) who reported that iproniazid protects rats against MES. It is interesting to note that although Randall and Bagdon\(^4\) reported that one of the actions of iproniazid (MAO inhibition) persists for about 20 days, the present studies showed that the anticonvulsive property of the drug has essentially disappeared 48 hours following drug treatment. The present study also indicates that iproniazid exerts no effect on body temperature 15 hours after injection, the time when the drug showed a prominent anticonvulsant action.
C. The Effects of Iproniazid–Reserpine Drug Combination on Audiogenic Seizure and Body Temperature

Introduction

The effects of iproniazid–reserpine drug-combination treatment on experimental seizure and body temperature have been reported by various investigators. Brodie and Shore observed that the appearance of the rabbit treated with only iproniazid was no different from that of the untreated control animal. These investigators also reported that the sedative action of reserpine is not only prevented by pretreatment with iproniazid, but that excitement instead of sedation occurred when 100 mg/kg of iproniazid was administered intravenously 2 hours prior to 5 mg/kg of reserpine. In addition, Chessin and co-workers also reported that rabbits, mice, rats, and guinea pigs treated with the iproniazid–reserpine drug combination exhibited a marked excitation instead of depression.

Prockop and co-workers, who evaluated the anticonvulsant properties of MAO inhibitors against MES, reported a pronounced anticonvulsant effect in rats treated with iproniazid and then reserpine 2 hours later. With respect to
body temperature, Brodie and Shore and Davidson and co-workers observed that reserpine-induced hypothermia is reduced or antagonized by pretreatment with iproniazid. In fact, Brodie and Shore indicated that an increase in body temperature may follow this drug-combination treatment.

In view of the effect of the iproniazid-reserpine drug combination on MES, it was thought desirable to investigate the effects of this treatment on sound-induced seizure in AGS-susceptible rats.

Methods

Seventy-two AGS-susceptible rats of mixed sexes weighing between 95 and 430 g were randomly divided into 12 groups of 6 rats each. Six groups of rats served as test animals and were treated with iproniazid, 100 mg of the base/kg as a 7.7% solution of the phosphate salt and 2 hours later with reserpine, 5 mg of the base/kg as a 0.58% solution of the lyophilized phosphate salt. The remaining 6 groups of rats were untreated and served as control animals. The test animals were subjected to sound stimulation 6, 24, and 72 hours following injection of the initial drug. The control animals were tested concomitantly with the test animals.
Body temperature was determined in a separate group of 12 AGS-susceptible rats. Six of these rats served as test animals and were administered the drug combination as indicated above. The remaining 6 rats received no treatment and served as control animals. Body temperature of the test animals was determined before the drug-combination treatment and 6 hours following the injection of iproniazid (4 hours following reserpine). The temperature of the control animals was determined at the same times as the test animals.

Results

The appearance of the rat treated with the iproniazid-reserpine drug combination was not noticeably different from that of the untreated rat. Six hours following the iproniazid administration (4 hours after reserpine), 27.9% of the test and 89.2% of the control animals displayed clonic convulsions when subjected to sound stimulation (see Table 5). Twenty-four hours following the injection of iproniazid (22 hours following reserpine), 22.16% of the test and 75% of the control animals displayed AGS. Seventy-two hours following the injection of iproniazid (70 hours following reserpine), the difference in AGS-susceptibility between the
Table 5

EFFECT OF THE DRUG COMBINATION, IPRONIAZID FOLLOWED IN 2 HOURS BY RESERPINE, ON AUDIOGENIC SEIZURE

<table>
<thead>
<tr>
<th>Time of Audio-genic Stimulation*</th>
<th>Per Cent Clonic Convulsions ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test Animals</td>
</tr>
<tr>
<td>6 hours</td>
<td>27.9 ± 10.2</td>
</tr>
<tr>
<td>24 hours</td>
<td>22.16 ± 10.3</td>
</tr>
<tr>
<td>72 hours</td>
<td>61.0 ± 3.5</td>
</tr>
</tbody>
</table>

*time following iproniazid injection

Table 6

EFFECT OF THE DRUG COMBINATION, IPRONIAZID FOLLOWED IN 2 HOURS BY RESERPINE, ON BODY TEMPERATURE 6 HOURS FOLLOWING IPRONIAZID INJECTION

<table>
<thead>
<tr>
<th>Mean Change in Body Temperature</th>
<th>Degrees Centigrade ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Rats</td>
<td>+ .63 ± .21</td>
</tr>
<tr>
<td>Test Rats</td>
<td>-1.07 ± .36</td>
</tr>
<tr>
<td>Difference</td>
<td>-1.70</td>
</tr>
</tbody>
</table>
test and the control rats was not statistically significant ($P > .05$).

The effect of this drug combination on body temperature (6 hours following iproniazid and 4 hours following reserpine) is presented in Table 6. It can be seen from this table that the mean body temperature of the test animals decreased $1.07^\circ C$, whereas the mean body temperature of the control animals increased $0.63^\circ C$. The difference between these 2 values is significant ($P < .01$).

Discussion

The results presented in Table 5 indicate that the iproniazid-reserpine drug combination exerts an anticonvulsant action, as evidenced by a decrease in the incidence of AGS 6 and 24 hours following injection of the initial drug. Seventy-two hours following injection of the initial drug, the anticonvulsant effect of this drug combination is essentially gone. It would appear that the anticonvulsant effect of this drug-combination treatment is essentially due to the action of iproniazid. It is interesting to note that although reserpine facilitates AGS (see Section III, A), it failed to exert such an apparent effect in animals pretreated with iproniazid.
These findings substantiate those of Prockop and co-workers\textsuperscript{20} who reported that this drug-combination treatment exerts a pronounced anticonvulsant effect against MES in rats.

Although Davidson and associates\textsuperscript{44} stated that pre-treatment with iproniazid abolishes reserpine-induced hypothermia, and Brodie and Shore\textsuperscript{16} indicated that this drug-combination treatment produces hyperthermia, the results of the present study involving body temperature do not substantiate those of either group of workers cited. However, it should be pointed out that Brodie and Shore\textsuperscript{18} employed rabbits, and Davidson and co-workers\textsuperscript{44} employed mice in their investigations, whereas AGS-susceptible rats were used in the present study.
D. The Effects of Reserpine-Iproniazid Drug Combination on Audiogenic Seizure and Body Temperature

Introduction

The effect of reserpine-iproniazid drug-combination treatment on experimental seizures has been reported by Prockop and co-workers. These investigators observed that rats treated with reserpine 2 hours before iproniazid showed an enhancement of electroshock seizure similar to that produced by reserpine alone (See Section III, A). These workers measured intensification of seizure in terms of decreased latency of hind-leg extension and also in terms of the increased number of animals which exhibited hind-leg extension. By either criteria the facilitation of maximal electroshock seizure (MES) by the drug-combination treatment was very pronounced 5 hours after reserpine and 3 hours after iproniazid. In light of the above-cited work, it was thought desirable to investigate the effects of this drug-combination treatment on sound-induced seizure in AGS-susceptible rats.

Methods

Seventy-two AGS-susceptible rats of mixed sexes
weighing between 130 and 430 g were randomly divided into 12 groups of 6 rats each. Six groups of rats served as test animals and were treated with reserpine, 5 mg of the base/kg as a 0.58% solution of the lyophilized phosphate salt, and 2 hours later with iproniazid, 100 mg of the base/kg as a 7.7% solution of the phosphate salt. The remaining 6 groups of rats served as control animals and were untreated. The test animals were subjected to sound stimulation 5, 24, and 48 hours following injection of the initial drug. The control animals were tested concomitantly with the test animals.

Body temperature was determined in a separate group of 12 AGS-susceptible rats. Six of these rats served as test animals and were administered reserpine-iproniazid drug combination as indicated above. Six of these rats received no treatment and served as control animals. The body temperature of the test animals was determined before the drug-combination treatment and 5 hours following the injection of reserpine (3 hours after iproniazid). The temperature of the control animals was determined at the same times as the test animals.

**Results**

The appearance of the rat which received the reserpine-iproniazid drug combination was, in general, similar
to the rat which received reserpine alone, with the exception
that diarrhea, ptosis, and sedation were less intense.

Five and 24 hours following the injection of reserpine
(3 and 22 hours following iproniazid) in this drug-combination
study, 33.3% and 8.3% of the test animals, respectively,
displayed maximal audiogenic seizure (MAS) when subjected to
sound stimulation, whereas none of the control animals
exhibited MAS (see Table 7). Neither the test nor the
control animals displayed MAS 48 hours after reserpine (46
hours after iproniazid).

The effect of this drug-combination treatment on
body temperature (5 hours following reserpine and 3 hours
following iproniazid) is presented in Table 8. It can be seen
from this table that the mean body temperature of the test
animals decreased 3.03° C, whereas the mean body tempera-
ture of the control animals decreased 30° C. The difference
between these 2 values is highly significant (P < .001).

Discussion

The data obtained in this study indicate that the
reserpine-iproniazid drug-combination treatment caused an
intensification of AGS. This effect was evidenced by the
Table 7

EFFECT OF THE DRUG COMBINATION, RESERPINE FOLLOWED IN 2 HOURS BY IPRONIAZID, ON EXPERIMENTAL AUDIOGENIC SEIZURE

<table>
<thead>
<tr>
<th>Time of Audio-genic Stimulation*</th>
<th>Per Cent MAS (tonic hind-leg extension) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test Animals</td>
</tr>
<tr>
<td>5 hours</td>
<td>33.3 ± 11.3</td>
</tr>
<tr>
<td>24 hours</td>
<td>8.33 ± 5.7</td>
</tr>
<tr>
<td>48 hours</td>
<td>0</td>
</tr>
</tbody>
</table>

*time following reserpine injection

Table 8

EFFECT OF THE DRUG COMBINATION, RESERPINE FOLLOWED IN 2 HOURS BY IPRONIAZID, ON BODY TEMPERATURE 5 HOURS FOLLOWING RESERPINE INJECTION

Mean Change in Body Temperature

<table>
<thead>
<tr>
<th>Degrees Centigrade ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Animals</td>
</tr>
<tr>
<td>Test Animals</td>
</tr>
<tr>
<td>Difference</td>
</tr>
</tbody>
</table>

P < .001
appearance of MAS 5 and 24 hours following the injection of the initial drug. Forty-eight hours following injection of the initial drug, the AGS-enhancing effect of this drug combination is completely gone (see Table 7). The data presented in Table 7 are parallel to those of Prockop and co-workers who reported that treatment with reserpine (5 mg/kg) followed by iproniazid (100 mg/kg) enhances MES. It would appear that the AGS-enhancing effect of this drug-combination treatment is essentially due to the action of reserpine. It is interesting to note that although iproniazid has a pronounced anticonvulsant action (See Section III, B), it failed to exert such an apparent effect in animals pretreated with reserpine.

It can be seen from Table 8 that the drug-combination treatment (5 hours after reserpine and 3 hours after iproniazid) causes a highly significant \( P < .001 \) decrease in the body temperature. In view of the fact that iproniazid has no effect on body temperature of AGS-susceptible rats (see Table 3), it is of interest to note that the change in body temperature produced by this drug combination is greater than that produced by reserpine alone (see Table 2).
Introduction

Chlordiazepoxide* is a relatively new synthetic drug which is employed in psychiatry. Randall and co-workers reported that this drug has a calming effect on vicious or agitated monkeys and on laboratory animals such as mice, rats, cats, and dogs. These investigators also demonstrated that it possessed anticonvulsant activity as manifested by its ability to prevent electroshock and pentylenetetrazole-induced convulsions in mice. The anticonvulsant action of chlordiazepoxide has also been reported by Chussid and co-workers, who worked with monkeys in which epilepsy was induced by experimental brain damage.

Since chlordiazepoxide has an anticonvulsant action against electroshock and chemoshock seizures, it was considered important to determine the effect of this drug on AGS in rats.

*Chlordiazepoxide (Librium), Roche Laboratories, Inc., Nutley, N. J.
Methods

Eighty-four AGS-susceptible rats of mixed sexes weighing between 120 and 270 g were randomly divided into 14 groups of 6 rats each. Seven groups of these rats served as test animals and were administered chlordiazepoxide HCl, 10 mg/kg,* as a 1% solution. The remaining groups of rats served as control animals and were untreated. The test animals were subjected to sound stimulation 1, 12, and 24 hours following injection of the drug. The control animals were tested for AGS concomitantly with the test animals.

Body temperature was determined in a separate group of 12 AGS-susceptible rats. Six of these rats served as test animals and were administered chlordiazepoxide as indicated above. Six of these rats received no treatment and served as control animals. The body temperature of the test animals was determined immediately before and 1 hour after drug administration. The body temperature of the

*This dosage was determined to produce no neurological deficit by means of the method described by Dunham and Miya.47 This procedure was necessary since no information concerning the intraperitoneal dosage of chlordiazepoxide was available in the literature.
control animals was determined at the same times as the test animals.

Results

Within 3 to 5 minutes following the injection of the drug, the test animal appeared somewhat sedated, but retained its motor coordination. This effect persisted for a period of approximately 15 minutes. One hour after the injection of chlordiazepoxide none of the test animals displayed AGS, whereas 83.3% of the control animals exhibited AGS (see Table 9). Twelve hours following drug treatment 38.9% of the test and 72.3% of the control animals displayed AGS. Twenty-four hours following drug treatment 41.7% of the test and 38.9% of the control animals exhibited AGS. Chlordiazepoxide also exerted a prominent blocking action against the running movements. Table 9 shows that 1 and 12 hours following drug treatment 90.3% and 83.3%, respectively, of the control animals exhibited running, whereas at these times only 2.4% and 35.7%, respectively, of the test animals exhibited running. At the 24-hour test time, 50% of the test and 55.5% of the control animals displayed running.

The effect of chlordiazepoxide on body temperature is presented in Table 10. It can be seen from this table
Table 9

EFFECT OF CHLORDIAZEPoxide ON AUDIOGENIC SEIZURE, INCLUDING CLONIC CONVULSION AND RUNNING MOVEMENT

<table>
<thead>
<tr>
<th>Time of Audiogenic Stimulation</th>
<th>Per Cent Clonic Convulsion ± S.E.</th>
<th>Per Cent Running Movement ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test Animals</td>
<td>Control Animals</td>
</tr>
<tr>
<td>1 hour</td>
<td>0</td>
<td>83.3 ± 7.3</td>
</tr>
<tr>
<td>12 hours</td>
<td>38.9 ± 10.2</td>
<td>72.3 ± 8.2</td>
</tr>
<tr>
<td>24 hours</td>
<td>41.7 ± 3.7</td>
<td>38.9 ± 12.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90.3 ± 4.4</td>
</tr>
<tr>
<td>12 hours</td>
<td>35.7 ± 1.1</td>
<td>83.3 ± 2.4</td>
</tr>
<tr>
<td>24 hours</td>
<td>50.0 ± 5.5</td>
<td>55.5 ± 11.8</td>
</tr>
</tbody>
</table>
Table 10

EFFECT OF CHLORDIAZEPoxide
ON BODY TEMPERATURE 1 HOUR
FOLLOWING DRUG TREATMENT

<table>
<thead>
<tr>
<th></th>
<th>Mean Change in Body Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Degrees Centigrade ± S.E.</td>
</tr>
<tr>
<td>Control Rats</td>
<td>-0.52 ± 0.31</td>
</tr>
<tr>
<td>Test Rats</td>
<td>-0.47 ± 0.15</td>
</tr>
</tbody>
</table>
| Difference           | -0.05                           | P > 0.05
that the mean body temperature of the test animals decreased 0.47°C, whereas the mean body temperature of the control animals decreased 0.52°C. The difference between these 2 values is not significant (P > .05).

Discussion

The results presented in Table 9 indicate that chlordiazepoxide exerts an anticonvulsant action, as evidenced by complete protection against sound-induced convulsion 1 hour following injection of this drug and by a significant decrease in incidence of these convulsions 12 hours after treatment. It would seem that the anticonvulsant properties of chlordiazepoxide are very pronounced, since the drug blocks not only the clonic convulsion seen in AGS, but also the running phase of the seizure. This view is supported by the report of Conrad, who stated that the running phase of audiogenic seizure is even more difficult to suppress than clonus.

It can be seen in Table 9 that at the 24-hour test period the responses to sound stimulation (clonic convulsion and running movement) of the control animals were considerably less than that observed during the 1- and 12-hour test
periods. Because of these pronounced changes, a comparison of the test values and the control values obtained at the 24-hour test period is of doubtful validity. It has been reported that the incidence of seizure tends to decrease when sound stimulation is repeated at short intervals.

The present study indicates that chlordiazepoxide exerts no significant effect on body temperature 1 hour following treatment.
A. Introduction

In 1954 Woolley and Shaw\textsuperscript{49} postulated that some mental disorders may arise from a deficiency or excess in the 5-hydroxytryptamine (5-HT) content of the brain. At about the same time, Vogt\textsuperscript{50} suggested that norepinephrine (NE) may play a role in the function of the central nervous system, possibly as a "humoral transmitter." Brodie and Shore\textsuperscript{18} at the National Institutes of Health in Bethesda, Maryland, proposed a hypothesis that implicates the brain amines 5-HT and NE as chemical mediators in the brain. The Bethesda group\textsuperscript{18,51,52,53} further suggested that the major portion of brain 5-HT and NE exists in a bound form and is thus biologically inactive and unavailable to enzymatic catabolism.

In the past few years the effects of certain psychopharmacologic agents on brain 5-HT and NE have received a great deal of attention. The effect of reserpine
on the brain levels of 5-HT and NE has been reported by Brodie and co-workers\textsuperscript{18,51} and Holzbauer and Vogt.\textsuperscript{54} The studies of these investigators showed that reserpine causes a reduction in the brain concentrations of 5-HT and NE. Brodie and co-workers\textsuperscript{55,56} have stated that reserpine releases these neurohormones from tissue-binding sites in the brain and in this unbound state they are subject to enzymatic destruction.

There are certain enzymes that destroy these unbound neurohormones, thus preventing their action. One of these enzymes is the tissue catalyst, monoamine oxidase (MAO), which is considered to be the chief enzyme responsible for the metabolism of 5-HT and NE in the rat brain.\textsuperscript{57,58} A class of psychopharmacological agents, referred to as MAO inhibitors, prevents the catabolism of 5-HT and NE by inhibiting the action of MAO. These drugs, such as iproniazid, allow the brain neurohormones to accumulate above normal levels.

Prockop and co-workers\textsuperscript{20} have investigated the effects of reserpine and iproniazid on electroshock seizure and correlated the effects of these drugs on the brain levels of 5-HT and NE and electroshock convulsion.
Since some psychopharmacologic agents have the ability to alter the brain concentrations of 5-HT and NE, and since Prockop and associates\textsuperscript{20} reported a correlation between these neurohormone levels and changes in electroshock seizure, it was thought important to ascertain the effects of certain psychopharmacologic drugs on neurohormone concentrations in order to determine whether a correlation exists between brain amines (5-HT and NE) and AGS susceptibility.

B. Methods

Animal Treatment

Ninety-three AGS-susceptible rats of mixed sexes, weighing between 180 and 420 g, were employed in this study. Drug treatment, as shown in Table 11 (see page 53), was the same as previously stated in the individual AGS experiments in Section III.

Determination of Neurohormone Levels

The extraction procedure reported by Mead and Finger,\textsuperscript{59} which permits the simultaneous determination of brain 5-HT and NE, was used in this laboratory for the estimation of neurohormone levels. This technique is a modification of the Bogdanski method\textsuperscript{60} for determining brain 5-HT concentration and of the Shore and Olin method\textsuperscript{61} for determining brain
NE concentration.

In performing this assay 10 glass-stoppered bottles of 120 ml capacity were employed. Sixty ml of n-butanol* and approximately 8 g of sodium chloride (reagent grade) were introduced into 5 of these bottles. Seventy ml of heptane (99%, pure grade, Phillips Petroleum Co.) and 5 ml of 0.01 N HCl were introduced into the remaining 5 bottles. At appropriate times control animals or test animals were killed by decapitation and the brains were removed as rapidly as possible, rinsed with deionized water** and blotted with filter paper. The 3 brains that comprised each sample were then weighed and homogenized in a motor-driven all-glass Duall tissue grinder (Kontes Glass Co., Vineland, N. J.) with 3 times their volume of 0.01 N HCl. Three 6 ml aliquots of the brain homogenate were transferred to 3 of the bottles which contained n-butanol and sodium chloride. The first of

*Reagent grade n-butanol (Fischer Scientific Co.) was washed with 0.01 N HCl and then the aqueous phase removed. Solid NaCl was added in excess and the butanol was shaken until no more of the NaCl went into solution. The latter procedure served to remove water dissolved in the butanol.

**Distilled water processed through a Crystalab Deeminizer, Crystal Research Laboratories, Hartford, Conn.
the above-mentioned aliquots served as the "test tissue" (bottle No. 1). The second aliquot (bottle No. 2), following the addition of 1 ml of the standard solution* served as the "internal standard." The third aliquot served as a "tissue blank" (bottle No. 3). The n-butanol and sodium chloride in bottle No. 4 served as the "reagent blank." One ml of the standard solution was added to the n-butanol and sodium chloride in bottle No. 5 and served as the "standard."

Sufficient 0.01 N HCl was added to those bottles as necessary to adjust their contents to equal volumes.

Bottles No. 1 through 4 were shaken for 1 hour on a mechanical shaking machine. Bottle No. 5, the "standard," was shaken for only 10 minutes. The bottles were then centrifuged (International Equipment Centrifuge, Size 2) for 5 minutes at 1400 rpm. A 40-ml aliquot of the butanol phase was transferred from each of these bottles to the 5 glass-stoppered bottles which contained the acid-heptane mixture.

*The standard solution contains 1 mcg/ml of 5-HT and 1 mcg/ml of NE in 0.01 N HCl. This solution was prepared daily from a stock solution of 5-hydroxytryptamine creatinine sulfate, 100 mcg/ml in 0.01 N HCl (calculated as the base) and from a stock solution of norepinephrine bitartrate, 100 mcg/ml, in 0.01 N HCl (calculated as the base). The stock solutions of 5-HT and NE were stored in the refrigerator.
Each butanol-acid-heptane mixture was subsequently shaken for 10 minutes and then centrifuged for 5 minutes at 1400 rpm. The butanol-heptane mixture in each bottle was removed and discarded by aspiration, leaving only the aqueous phase. A 3-ml aliquot of the latter from each bottle was used for the NE assay, and a 1-ml aliquot for the 5-HT assay, as described below. It should be mentioned that all the neurohormone determinations in these studies were performed within a period of approximately 3 weeks (May 18 to June 7, 1961).

**Norepinephrine Determination**

Each 3-ml aliquot of the final aqueous phase was transferred to a 16x150 mm test tube which contained 1 ml of acetate buffer.* After the addition of 0.2 ml of sodium thiosulfate solution** to the tissue blank aliquot, 0.1 ml of ethanolic iodine solution*** was added to all of the aliquots.

---

*Acetate buffer pH 5, was prepared by combining 2 volumes of 2M sodium acetate with 1 volume of 2M acetic acid.

**Sodium thiosulfate solution was prepared by dissolving 1.24 g of sodium thiosulfate in water in a quantity to make 100 ml.

***Ethanolic iodine solution 0.1N, was prepared by dissolving 1.27 grams of iodine in 100 ml of ethanol.
The iodine serves to oxidize the NE to a highly fluorescent trihydroxyindole derivative. It will be noted that in the case of the tissue-blank aliquot the action of the iodine was prevented by the previous addition of sodium thiosulfate. Thus, the oxidation of NE in the aliquot does not take place. This procedure is used to account for fluorescence of substances in the brain other than catecholamines. Six minutes later, except in the case of the tissue blank, the excess iodine reagent was neutralized by the addition of 0.2 ml of sodium thiosulfate solution to each of the remaining 3-ml aliquots. One ml of alkaline ascorbate solution* was added immediately following the thiosulfate solution to protect the final product from further oxidative degradation. The contents of the test tubes were thoroughly mixed following the addition of each reagent. The test tubes were placed under a fluorescent light (located approximately 6 inches above the tops of the tubes) for 45 minutes. After the 45-minute

*Alkaline ascorbate solution was prepared immediately before use; 50 mg of ascorbic acid was dissolved in 10 ml of deionized water and then combined with 5 ml of 10N sodium hydroxide solution.
time interval a small portion (approximately 1 ml) of the solution from each test tube was introduced into a quartz cuvette which was placed in the cell chamber of an Aminco-Bowman Spectrophotofluorometer. The solution in each cuvette was activated at a wave-length of 400 millimicrons and the resulting fluorescence read as per cent transmission at a wave-length of 510 millimicrons.

A sample calculation for norepinephrine concentration in brain tissue is presented below.

### SAMPLE CALCULATION OF NOREPINEPHRINE (NE) IN BRAIN TISSUE

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Microphotometer Reading</th>
<th>Corrected Reading (Minus Appropriate Blank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Blank</td>
<td>0.227</td>
<td>--</td>
</tr>
<tr>
<td>Tissue Blank</td>
<td>0.252</td>
<td>--</td>
</tr>
<tr>
<td>Test Tissue</td>
<td>1.725</td>
<td>1.473</td>
</tr>
<tr>
<td>Standard</td>
<td>2.205</td>
<td>1.978</td>
</tr>
<tr>
<td>Internal Standard</td>
<td>3.320</td>
<td>3.068</td>
</tr>
</tbody>
</table>

**Calculation of Norepinephrine Concentration**

\[
\text{Corrected Tissue Reading} = \frac{1.473}{1.978} = 0.744 \text{ mcg} \quad \text{(Total weight of NE in tissue sample)}
\]

\[
\text{Total Weight of Norepinephrine in Tissue Sample} = \frac{0.744}{1.5} = 0.496 \text{ mcg NE per g tissue}
\]
Calculation of Per Cent Recovery

\[
\frac{Corrected\ Internal\ Standard\ Reading}{Corrected\ Test\ Tissue\ +\ Corrected\ Standard\ Readings} = \frac{3.068}{1.473 + 1.978} = 88.9\%\ recovery
\]

5-Hydroxytryptamine Determination

Each 1-ml aliquot of the final aqueous phase was introduced into a quartz cuvette which contained 0.3 ml of concentrated HCl (12N). The mixture was shaken and the cuvette was then placed in the cell chamber of the spectrofluorometer. The solution was activated at a wavelength of 295 millimicrons and the resulting fluorescence read as per cent transmission at a wave-length of 545 millimicrons. The tissue-blank aliquot is not employed in the determination of 5-HT.

A sample calculation for 5-HT concentration in brain tissue is presented below.
SAMPLE CALCULATION OF
5-HYDROXYTRYPTAMINE (5-HT)
IN BRAIN TISSUE

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Microphotometer Reading</th>
<th>Corrected Reading (Minus Reagent Blank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Blank</td>
<td>0.055</td>
<td>--</td>
</tr>
<tr>
<td>Test Tissue</td>
<td>0.310</td>
<td>0.255</td>
</tr>
<tr>
<td>Standard</td>
<td>0.379</td>
<td>0.324</td>
</tr>
<tr>
<td>Internal Standard</td>
<td>0.615</td>
<td>0.560</td>
</tr>
</tbody>
</table>

Calculation of 5-Hydroxytryptamine Concentration

\[
\frac{\text{Corrected Tissue Reading}}{\text{Corrected Standard Reading}} = \frac{0.255}{0.324} = 0.787 \text{ mcg (Total weight of 5-HT in tissue sample)}
\]

\[
\frac{\text{Total Weight of 5-HT in Tissue Sample}}{\text{Weight of Tissue Sample}} = \frac{0.787}{1.5} = 0.52 \text{ mcg 5-HT/g tissue}
\]

Calculation of Per Cent Recovery

\[
\frac{\text{Corrected Internal Standard Reading}}{\text{Corrected Test Tissue + Corrected Standard Readings}} = \frac{0.560}{0.255 + 0.324} = 96.7\% \text{ recovery}
\]

Treatment of Glassware

The glassware used throughout these determinations (with the exception of the pipettes) was scrubbed thoroughly with Tig Liquid Cleaner (Diversy Corp., Chicago, Ill.), rinsed in tap water, and immersed in a diluted HCl solution for a minimum of 2 hours. Following the acid treatment,
each item of glassware was rinsed with tap, distilled, and then deionized water and dried in a hot-air oven. Pipettes were submerged in dichromate cleaning solution for approximately 2 hours, then rinsed once or twice in hot tap water, and then in cold tap water for approximately 2 hours. Finally, they were rinsed with distilled and deionized water and then dried in a hot-air oven.

C. Results

The results of the neurohormone studies are presented in Table 11. It can be seen from this table that 4 hours after reserpine treatment there was a significant decrease in the brain levels of 5-HT and NE. Conversely, 15 hours following the injection of iproniazid there was a significant increase in both the brain levels of 5-HT and NE. The reserpine-iproniazid drug-combination treatment caused a decrease in brain levels of both neurohormones. On the other hand, the iproniazid-reserpine drug-combination treatment caused an increase in the brain concentration of 5-HT but a decrease in the brain concentration of NE. Chlordiazepoxide had no significant effect on the concentration of either 5-HT or NE.
Table 11
THE EFFECTS OF CERTAIN PSYCHOPHARMACOLOGIC DRUGS ON BRAIN CONCENTRATIONS OF 5-HYDROXYTRYPTAMINE AND NOREPINEPHRINE

<table>
<thead>
<tr>
<th>Treatment: Drug, Dosage, Time of Sacrifice (hours)</th>
<th>Samples*</th>
<th>Neurohormones mcg/g of tissue ± Standard Error</th>
<th>5-Hydroxytryptamine</th>
<th>P</th>
<th>Nor-epinephrine</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td></td>
<td>0.52±0.013</td>
<td>.47±0.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reserpine, 5 mg/kg (4)</td>
<td>4</td>
<td></td>
<td>0.18±0.027</td>
<td>.06±0.010 &lt;.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iproniazid, 100 mg/kg (15)</td>
<td>4</td>
<td></td>
<td>1.29±0.034</td>
<td>.88±0.023 &lt;.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reserpine, 5 mg/kg (5) - Iproniazid, 100 mg/kg (3)</td>
<td>4</td>
<td></td>
<td>0.40±0.012</td>
<td>.18±0.071 &lt;.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iproniazid, 100 mg/kg (6) - Reserpine, 5 mg/kg (4)</td>
<td>4**</td>
<td></td>
<td>0.88±0.050</td>
<td>.39±0.054 &lt;.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlordiazepoxide, 10 mg/kg (1)</td>
<td>4</td>
<td></td>
<td>0.53±0.081</td>
<td>.50±0.016 &gt;.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Each sample was comprised of three whole brains.

**In this treatment 4 samples were employed for determination of 5-HT and 6 samples for NE.
D. Discussion

Biochemical concepts of mental illness have placed a great deal of emphasis on the possible roles of neurohormones in brain function. Consequently, the 2 brain neurohormones, 5-HT and NE, are currently undergoing widespread investigation. This study reports the effects of certain psychopharmacologic drugs on 5-HT and NE in AGS-susceptible rats.

In untreated AGS-susceptible rats the brain levels of 5-HT and NE were determined fluorometrically to be 0.52 mcg ± S.E. 0.013 and 0.47 ± S.E. 0.024, respectively (see Table 11). These values compare favorably with those reported by other workers such as: Brodie and co-workers; Moore and Brody; Prockop and co-workers; and investigators in this laboratory, who employed non-AGS-susceptible rats.

The effects of reserpine on the neurohormone levels of 5-HT and NE in the AGS-susceptible rats were found to be pronounced (see Table 11). Four hours after the injection of reserpine, the brain 5-HT level had decreased 64.8%, and the NE level had decreased 86.9%. The magnitude of these changes agrees well with those observed by Prockop and associates and Brodie and associates who reported the effect of reserpine on brain neurohormone concentrations in
non-AGS-susceptible rats and other experimental animals.

Brodie and associates at the National Institutes of Health, Bethesda, Maryland,\textsuperscript{51,52} were the first investigators to report that reserpine reduced the brain 5-HT concentration. The Bethesda group\textsuperscript{51,53,65} demonstrated that the decrease in the concentration of this neurohormone is due to its liberation by reserpine from tissue-binding sites in the brain and, thus, as "free" 5-HT it becomes physiologically active and vulnerable to enzymatic destruction by monoamine oxidase (MAO). These workers also suggested that the synthesis of 5-HT continues in the brain of the reserpine-treated animals and, as a result, there remains a persistent low concentration of free 5-HT. Since free 5-HT is rapidly metabolized, the total 5-HT (bound plus free) level is decreased.

Subsequently, Holzbauer and Vogt\textsuperscript{54} reported that reserpine causes a similar reduction in the brain level of NE. This finding was confirmed by Kärki and Paasonen\textsuperscript{66} and Brodie and associates.\textsuperscript{56} The latter workers suggested that NE, like 5-HT, is normally bound in brain tissue and is released by reserpine by similar mechanisms.

The effect of iproniazid on the brain concentration of the neurohormones, 5-HT and NE, in AGS-susceptible
rats appears to be directly opposite that of reserpine. Fifteen hours following the injection of iproniazid, the 5-HT and NE levels were increased 114.8% and 86.5%, respectively. Since MAO has been determined to be the chief enzyme responsible for the metabolism of 5-HT and NE in rat brain, it follows that the increase in the levels of these neurohormones reported herein is due to the inhibition of MAO by iproniazid. The effects of iproniazid on the brain levels of 5-HT and NE observed in AGS-susceptible rats agree favorably with those reported by Udenfriend, Prockop and co-workers, and Brodie and co-workers, who employed non-AGS-susceptible rats and other experimental animals in their investigations.

The combinations of reserpine and iproniazid produced varying changes in the brain concentrations of the neurohormones 5-HT and NE, depending on the sequence of injections of the drugs.

Animals treated with reserpine 2 hours prior to iproniazid were found to have a 22.5% and 63.0% reduction in the neurohormone levels of 5-HT and NE, respectively. However, this decrease was not as pronounced as that of animals treated with reserpine alone (see Table 11). The results of this drug-combination study are qualitatively
similar to those reported by Prockop and co-workers, in that both 5-HT and NE levels were decreased; however, quantitatively, the results differ somewhat. These workers reported greater degrees of changes, approximately a 60.5% and 75.5% decrease in the levels of brain 5-HT and NE, respectively. It would appear from the results of the present study that following the administration of reserpine, the majority of 5-HT and NE was released from binding sites in the brain and metabolized by MAO. It also appears that the injection of iproniazid subsequent to reserpine prevented the further destruction, by MAO, of the 5-HT and NE. Thus, it is suggested that the concentrations of the neurohormones determined in the reserpine-iproniazid drug-combination study were present in the brain as the free form rather than as the bound form.

In contrast with the previous drug-combination study, the injection of iproniazid 2 hours prior to reserpine caused a 68.3% increase (rather than a 22.5% decrease) in the 5-HT level and a 16.9% decrease (rather than a 63% decrease) in the NE level. In light of the fact that iproniazid inhibits MAO, it would appear that when reserpine releases these neurohormones from the bound form, there is little or no
destruction of the free 5-HT and NE. In addition, it is suggested that, as in the case of the previous drug-combination study, the concentrations of 5-HT and NE are present in the brain as the free form rather than as the bound form. Since NE in brain tissue is bound, released, and metabolized in a manner similar to 5-HT, one would expect that the effect of this drug combination on the brain level of NE would parallel that of 5-HT. However, the results of the present study (increase in 5-HT level but decrease in NE level) do not substantiate this view. This apparent anomalous finding may be due to one or both of the following reasons:

1. Brodie and co-workers reported that following the administration of iproniazid, the brain 5-HT level rises rapidly and reaches a maximal concentration in about 6 hours, whereas the level of NE rises more slowly, reaching a maximal concentration in 17 hours. On the basis of this report, it may be postulated that 6 hours following the administration of iproniazid, NE is still metabolized to some extent by MAO.

2. Although MAO is considered to play a dominant role in the metabolism of NE, there may be other enzymes
involved. For example, there is evidence which indicates that NE may be partially metabolized by the enzyme catechol-ortho-methyl transferase (COMT). Despite the fact that COMT is demonstrated to be 3.6 times less active than MAO in the rat brain, Green and Erickson state that under conditions in which MAO activity has been inhibited, it can be expected that a significant amount of NE is metabolized by COMT. Therefore, the possibility exists that alternate pathways of NE metabolism may account for the decrease in the level of brain NE in the present study.

The results of the iproniazid-reserpine combination study presented herein are opposite to those reported by Prockop and co-workers in an investigation involving non-audiogenic-seizure-susceptible rats. These investigators reported approximately a 47% increase in the NE level and a 26% decrease in the 5-HT level, whereas the results obtained in this laboratory indicate a 16.9% decrease in NE and a 68.3% increase in 5-HT. It should be pointed out, however, that in the work of Prockop and co-workers, the neurotransmitter concentrations were determined at a time interval different from that used in this laboratory. However, it is highly probable that the differences in findings are due
to transposition of the values for NE and 5-HT in Figure 3 on page 648 of reference 20. Evidence to support this contention can be obtained by comparing the values for brain NE and 5-HT 6 hours after iproniazid in Figure 1, page 646 (NE: .68 mcg/g and 5-HT: .88 mcg/g), with those in Figure 3, page 648 (NE: .66 mcg/g and 5-HT: .68 mcg/g) in the reference cited above. Apparently, there has been a transposition of the NE and 5-HT data in Figure 3, page 648, of this reference.

Chlordiazepoxide, 1 hour after injection, exerted no significant effects on the brain levels of 5-HT and NE (see Table 11). These results corroborate the recent findings made in the Roche Laboratory, Nutley, N.J., 70 that no significant change in the brain levels of 5-HT and NE occurs in rabbits which receive as much as 100 mg/kg (i.p.) of chlordiazepoxide. Thus, it is assumed from the results of both of these studies that chlordiazepoxide produces its anti-convulsant action through mechanisms or pathways other than those involving 5-HT and NE.
V. GENERAL DISCUSSION

The present studies were conducted in an attempt to determine whether there might exist a correlation in the effect of certain psychopharmacologic drugs on brain levels of 5-HT and NE, AGS susceptibility, and body temperature. The results of these investigations are summarized in Table 12.

On the basis of these results there appears to be an inverse relationship between the brain level of 5-HT and AGS-susceptibility. For example, in the reserpine- and reserpine plus iproniazid-treated animals, a marked reduction of brain 5-HT was associated with intensification of AGS, whereas in the iproniazid- and iproniazid plus reserpine-treated animals, a marked increase of brain 5-HT was associated with a reduction in incidence of AGS. On the other hand, in the chlordiazepoxide-treated animals there was no change in the level of 5-HT despite the fact that there was complete protection against AGS, including abolishment of running movements. It is tempting to postulate that a reduction in the brain concentration of 5-HT tends to intensify AGS and that an increase in the brain concentration of 5-HT tends to protect against AGS.
Table 12

A Summary of the Effects of Certain Psychopharmacologic Drugs on Audiogenic Seizure-Susceptible Rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect on Audiogenic Seizure</th>
<th>Effect on Brain Neurohormones</th>
<th>Effect on Body Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5-HT</td>
<td>NE</td>
</tr>
<tr>
<td>Reserpine (4 hours)</td>
<td>Intensified: produced hind-limb extension</td>
<td><img src="image" alt="Decrease" /></td>
<td><img src="image" alt="Decrease" /></td>
</tr>
<tr>
<td>Iproniazid (15 hours)</td>
<td>Reduced incidence of clonus; running phase not affected</td>
<td><img src="image" alt="Increase" /></td>
<td><img src="image" alt="Increase" /></td>
</tr>
<tr>
<td>Iproniazid (6 hours) &amp;</td>
<td>Reduced incidence of clonus; running phase not affected</td>
<td><img src="image" alt="Increase" /></td>
<td><img src="image" alt="Decrease" /></td>
</tr>
<tr>
<td>Reserpine (4 hours)</td>
<td></td>
<td><img src="image" alt="Decrease" /></td>
<td><img src="image" alt="Decrease" /></td>
</tr>
<tr>
<td>Reserpine (5 hours) &amp;</td>
<td>Intensified: produced hind-limb extension</td>
<td><img src="image" alt="Decrease" /></td>
<td><img src="image" alt="Decrease" /></td>
</tr>
<tr>
<td>Iproniazid (3 hours)</td>
<td></td>
<td><img src="image" alt="Decrease" /></td>
<td><img src="image" alt="Decrease" /></td>
</tr>
<tr>
<td>Chlordiazepoxide (1 hour)</td>
<td>Abolished all seizure components including running phase</td>
<td><img src="image" alt="Increase" /></td>
<td><img src="image" alt="Increase" /></td>
</tr>
</tbody>
</table>

- Significant Increase
- Significant Decrease
- No Significant Change
However, it is apparent that anticonvulsant activity is not always associated with an increase in brain 5-HT concentration. This fact was demonstrated in the present study involving chlordiazepoxide. It is suggested that this drug exerts its anticonvulsant action by a mechanism which does not necessarily concern brain 5-HT. Further, Prockop and associates\textsuperscript{20} have reported that the anticonvulsant drug, diphenylhydantoin (Dilantin), which prevents MES in experimental animals, does not affect brain 5-HT concentration. This finding has recently been confirmed by Plan and associates.\textsuperscript{71}

The results in Table 12 indicate that the relationship between brain NE and AGS is less consistent than that between brain 5-HT and AGS. For example, in the reserpine- and reserpine plus iproniazid-treated animals a reduction of brain NE was associated with intensification of AGS, whereas in the iproniazid-treated animals an increase in brain NE was associated with reduction in the incidence of AGS. However, in the iproniazid plus reserpine-treated animals there was a decrease in the brain level of NE associated with a reduction in the incidence of AGS. Furthermore, in the chlordiazepoxide-treated animals there was no alteration in the brain level of NE associated with the anticonvulsant action of this drug.
It should be recalled that Sulser and Brodie suggested that when reserpine releases stored (bound) amines in the brain, total amine levels, but not necessarily free amine levels, are lowered. Also, it has been previously reasoned that iproniazid increases the total content of brain amines by inhibition of the enzyme MAO. Thus, it is logical to assume that, with the exception of chlordiazepoxide, all drug treatments employed in the present investigations, whether they increase or decrease total levels of 5-HT and NE, tend to increase the brain content of free 5-HT and NE. On the basis of this assumption and in view of the varying responses to sound stimulation displayed by the test animals as reported herein, it is difficult to accept the statement of Prockop and colleagues that it may be the brain content of free amines (presumably 5-HT and NE) rather than total contents of amines that is important in affecting experimental seizures. It is of interest, in passing, to point out that Prockop and co-workers have stated that it is not known whether 5-HT, NE, or indeed, whether either of these neurohormones is the important amine that affects experimental seizures. At any rate, they speculate that a substance released by reserpine and metabolized by MAO is involved.

It can be seen from Table 12 that there does not appear to be any correlation between changes in body
temperature and AGS. For example, in the reserpine- and reserpine plus iproniazid-treated animals, hypothermia was associated with intensification of AGS, whereas in the iproniazid plus reserpine-treated animals hypothermia was associated with a reduction in incidence of AGS. On the other hand, in the iproniazid- and chlordiazepoxide-treated animals, there was no alteration in the body temperature associated with protection against AGS.

Dubnick and co-workers, who treated mice with MAO inhibitors and then exposed them to cold or warm environmental temperatures, reported that the rate of accumulation of brain 5-HT is decreased as the body temperature is lowered. In view of this publication and in view of the fact that changes in neurohormone levels and in body temperature have been determined in the present studies, it is of interest to examine the data to determine whether a correlation might exist between the effects of drug treatment on brain neurohormone levels and body temperature. It can be observed from Table 12 that there is no correlation between these drug-induced effects. For example, in the reserpine- and reserpine plus iproniazid-treated animals, hypothermia was associated with a reduction of brain 5-HT and NE, whereas in the iproniazid plus reserpine-treated animals, hypothermia was associated with a reduction of brain NE but an increase of brain 5-HT. On the other hand,
in the iproniazid-treated animals no alteration of body
temperature was associated with an increase in brain 5-HT
and NE, whereas in the chlordiazepoxide-treated animals no
alteration of body temperature was associated with no
changes in brain 5-HT and NE.
VI. SUMMARY AND CONCLUSIONS

A. Summary

The present study was initiated in an attempt to determine whether a correlation might exist between the effects produced by certain psychopharmacological drugs on the brain levels of 5-HT and NE, AGS-susceptibility, and body temperature. The following summarizes the results of this work:

1. Reserpine intensified convulsion in AGS-susceptible rats. Typically, sound stimulation does not produce MAS in untreated AGS-susceptible rats. However, 4, 24, and 48 hours following injection of reserpine, 61.1, 55.5, and 5.5% of these rats, respectively, displayed MAS. Four hours following injection of this drug in AGS-susceptible rats, brain levels of 5-HT and NE were reduced 64.8 and 86.9%, respectively; body temperature was reduced 0.98°C (P < .001).

2. Iproniazid decreased the incidence of AGS in rats. Fifteen, 48, and 72 hours following injection of iproniazid, 8.3, 56.1, and 43.8% of the test animals, respectively, exhibited convulsion. On the other hand, 60.3, 66.6, and 52.0% of the control animals, respectively, displayed convulsion. Fifteen hours following injection of this
drug, in AGS-susceptible rats, brain levels of 5-HT and NE were increased 114.8 and 86.5%, respectively; body temperature did not change significantly.

3. Iproniazid-reserpine drug-combination treatment decreased the incidence of AGS in rats. Six, 24, and 72 hours following the injection of iproniazid (4, 22, and 70 hours after reserpine), 27.9, 22.2, and 61.0% of the test animals, respectively, exhibited convulsion. On the other hand, 89.2, 75.0, and 72.2% of the control animals, respectively, displayed convulsion. Six hours following injection of iproniazid (4 hours after reserpine) in AGS-susceptible rats, the brain level of 5-HT was increased 68.3% and the brain level of NE was decreased 16.9%; body temperature was reduced 1.7° C (P < .01).

4. Reserpine-iproniazid drug-combination treatment intensified convulsion in AGS-susceptible rats. Five and 24 hours following the injection of reserpine (3 and 22 hours after iproniazid), 33.3 and 8.3% of the test animals displayed MAS, whereas none of the control animals displayed MAS. Five hours following the injection of reserpine (3 hours after iproniazid) in AGS-susceptible rats, brain levels of 5-HT and NE were reduced 22.5 and 63.0%, respectively; body temperature was reduced 2.7° C (P < .001).

5. Chlordiazepoxide markedly decreased the incidence of AGS in rats. One and 12 hours following
injection of chlordiazepoxide, 0 and 38.9% of the test animals, respectively, exhibited convulsion; whereas, 83.3 and 72.3% of the corresponding control animals displayed AGS. Chlordiazepoxide also decreased the incidence of running in AGS-susceptible rats subjected to sound stimulation, an effect not produced by iproniazid or iproniazid-reserpine drug-combination treatment. One and 12 hours following injection of chlordiazepoxide, 2.4 and 35.7% of the test animals, respectively, exhibited running. In contrast, 90.3 and 83.3% of the control animals, respectively, displayed running. One hour following injection of chlordiazepoxide in AGS-susceptible rats, the brain levels of 5-HT and NE and the body temperature did not change significantly.

B. Conclusions

The concentration of 5-HT in the brain of AGS-susceptible rats appears to be inversely related to susceptibility to AGS. However, protection against AGS is not invariably associated with increased levels of brain 5-HT, as suggested by the chlordiazepoxide study. The relationship between the concentration of NE in the brain and AGS susceptibility is inconsistent. There is no correlation between body temperature and AGS susceptibility or between body temperature and brain concentrations of 5-HT and NE.
VII. LITERATURE CITED


THE EFFECTS OF PSYCHOPHARMACOLOGIC DRUGS
ON EXPERIMENTAL AUDIOGENIC SEIZURE
AND BRAIN NEUROHORMONE LEVELS

by

Donald C. Choisser

An abstract of a thesis submitted to
the faculty of the College of Pharmacy
in partial fulfillment of the require-
ments for the degree of

MASTER OF SCIENCE
In the Graduate College
THE UNIVERSITY OF ARIZONA

1961
Audiogenic seizure (AGS) susceptible rats were employed in the evaluation of the effects of certain psychopharmacologic drugs on AGS, body temperature, and brain levels of the neurohormones 5-hydroxytryptamine (5-HT) and norepinephrine (NE).

A strain of AGS-susceptible rats was developed which typically displays clonic convulsions upon exposure to white noise in a sound chamber.

Reserpine, 5 mg/kg, intensified AGS, decreased body temperature, and reduced the brain levels of 5-HT and NE. In contrast, iproniazid, 100 mg/kg, reduced the incidence and intensity of AGS, did not affect body temperature, but markedly increased the brain levels of 5-HT and NE. Drug-combination treatment with reserpine administered 2 hours prior to iproniazid caused enhancement of AGS (as indicated by the appearance of maximal audiogenic seizures), decreased body temperature, and caused a reduction in the brain levels of 5-HT and NE. On the other hand, reversing the order of administration of these two drugs protected against AGS (as indicated by a reduction in incidence of AGS), decreased body temperature, increased 5-HT, and decreased the NE levels. Chlordiazepoxide exhibited a potent anticonvulsant effect, as evidenced by the complete abolishment of
the clonus and running associated with AGS. Body temperature and the brain levels of 5-HT and NE were not significantly altered by chlordiazepoxide.

The results of these studies on the effects of reserpine, iproniazid, and chlordiazepoxide on AGS-susceptible rats indicate: (1) The concentration of 5-HT appears to be inversely related to susceptibility to AGS. However, protection against AGS is not invariably associated with increased levels of brain 5-HT, as suggested by the fact that chlordiazepoxide prevents AGS, including running, but does not significantly alter the brain 5-HT level. The relationship between the concentration of brain NE and AGS-susceptibility is inconsistent. (2) There is no correlation between body temperature and AGS-susceptibility. (3) There is no correlation between body temperature and the brain concentrations of 5-HT and NE.