EFFECTS OF CHLORPROMAZINE, AZACYCLONOL AND 
CHLORDIAZEPoxide ON BRAIN CATEchOLAMINE 
CONTENTS OF STRESSED RATS

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Recently, various problems concerning the mechanism of the action of tranquilizing agents have gradually attracted the attention of many investigators. In the field of psychopharmacology, the depletion of catecholamines and 5-hydroxytryptamine in the brain of laboratory animals after administration of some tranquilizers were studied by many investigators (1–6). In these studies, chlorpromazine and meprobamate were found to have no effect on the depletion of the brain catecholamine contents in the physiological condition, but reserpine and tetrabenazine were found effective (7–15).

On the other hand, authors were unable to find any conclusive data in literatures whether tranquilizers affect on the brain catecholamine contents in abnormal conditions of animals, i.e., stressed state.

In relation to these reports, the effects of anxiety provoked by stress (electric shock) on the brain catecholamine levels of rats and the possibility of inhibiting effects of some tranquilizers on the catecholamine levels were investigated.

MATERIALS AND METHODS

The technique of estimation of catecholamines was essentially the same as that described by Bertler (16). Experimental animals were inbred Wistar strain albino rats weighing 200–280 g fed with standard Oriental rat food (Oriental Yeast Co.). The environmental temperature was kept at 20–25°C throughout these experiments. Animals were subjected to fasting for 18–24 hours before the experiment.

As tranquilizing agents chlorpromazine was administered subcutaneously in a single dose of 8 mg/kg one hour prior to the shock in order to examine whether it affects the catecholamine levels of brain in the state of shock.

The animals with such pretreatments were subjected to the electric shock of 50 V/sec with 10 seconds interval for 4 hours, using the apparatus devised by the authors as shown in Figs. 1–3. Animals responded severely with jumping, defecation and
The present study was designed as shown in Table 1. Animals were sacrificed by decapitation and whole brains were removed and placed in dry ice as soon as possible. A glass homogenizer of Potter-Elvehjem type was used to homogenize the brain in a few milliliter of 0.4 N perchloric acid and the homogenates were added up to 30 ml with the same solution per brain. They were centrifuged in a refrigerated centrifuge for 10 minutes at 9,000×g. The residues were reextracted as before and the second supernatant were added to the first ones. All steps of extraction must have been carried at 0°C. The combined filtrates were concent-

![Fig. 1. The outside of a stress box, 30x30x50 cm.](image1)

![Fig. 2. Inside of the box. Grids intervals: 1 cm.](image2)
rated to about half in volume using lyophilization apparatus and the condensed solutions were brought to pH 4.0 with 5 N potassium carbonate. The resulting precipitation of potassium perchlorate was separated by filtration and washed with a few milliliters of distilled water which was added to the extract.

Purification of the extract was performed using a DOWEX 50 column which was previously washed several times with 2 N hydrochloric acid and treated before use with distilled water, 1 N acetate buffer of pH 6.0 and distilled water in turn. Aliquot of extract was passed through the column and elution was performed with 8 ml of 1 N hydrochloric acid and then rinsed with 20 ml of distilled water. The flow rate should have been kept at 0.25 ml/min when Bertler's apparatus was used.

The catecholamines in elute were determined fluorometrically after oxidation and subsequent rearrangement by adrenolutine and noradrenolutine respectively, according to Euler and Floding technique (17). An aliquot of the effluent which was adjusted to pH 6.0 with 5 N potassium carbonate was taken for assay as a sample. To 1 ml of sample, 1 ml of phosphate buffer of pH 6.5, 0.1 ml of 0.25% zinc sulfate and distilled water were added to give a total volume of 6.9 ml and then oxidation was performed by addition of 0.1 ml of 0.25% potassium ferricyanide. After 2 minutes 1.0 ml of alkaline ascorbic acid solution which is a mixture of 9 parts of 5 N sodium hydroxide and 1 part of 2% ascorbic acid solution was added. After left standing 30 minutes the samples were read by Farrand Spectrophotofluorometer as 410 (adrenaline), 450 (noradrenaline), and 540 μμ respectively for the activation and fluorescence wave lengths. With these values of wave lengths the simultaneous differential estimation of adrenaline and noradrenaline was performed precisely.

In addition to administration of chlorpromazine, other two tranquilizing agents

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Stress*</th>
<th>Drug**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

* Given electrically,
** Chlorpromazine, chlordiazepoxide and azacyclonol.

Fig. 3. Automatic electroshock inducer, which acts 1 second in duration at repeated intervals of 10 seconds.

Table 1. The designs of the experiments.

![Table 1: The designs of the experiments.](image)
which are different in their chemical constructions (Fig. 4), i.e., chlordiazepoxide and azacyclonol, were used and their effects on the brain catecholamine levels in abnormal condition were determined similarly as in the case of chlorpromazine.

Single doses of 10 mg/kg were administered subcutaneously in the latter cases.

FIG. 4. The chemical structures of tranquilizing drugs used.

RESULTS

1) Effects of electric shock on the behavior of animals

The rats treated with electric shock and without drugs responded by squalling, jumping, defecating and urinating. After 30 minutes or so they became immobile during the shocks, generally in a corner of the chamber with an arched back and often with bristling hair and tails raised. Sometimes they remained supporting themselves at the chamber wall during the shocks. These stressed rats appeared to be anxious and apprehensive, while the control rats continued to move about freely in their chambers.

2) Effects of tranquilizing agents on the behavioral responses to the electric shock

Animals after administered 8 mg/kg of chlorpromazine responded even less, some not at all, especially during the last 2 hours during the 4 hours of the shock. These animals appeared relaxed and sleepy, but were not unconscious and could be awakened by being touched. Animals after administered chlordiazepoxide or azacyclonol, in contrast, seemed to have no significant effect.

3) Effects of tranquilizing agents on brain catecholamine levels to electric shock

The results of these experiments are shown in Fig. 5 and in Table 2. The animals administered no drugs showed significant rise in the brain noradrenaline as compared with that of non-stressed animal, but no adrenaline was detected.

On the other hand, the brain noradrenaline of rats administered chlorpromazine were reduced after the shocks, being significantly lower (p<0.001) than those of stressed rats administered no drugs (Table 3), while chlordiazepoxide showed no predominant effects and azacyclonol showed a slightly significant effect (p<0.05).

No adrenaline was detected under all conditions, that is, stress alone, physiological, and stress after the pretreatment with various drugs, i.e., chlorpromazine, chlordiazepoxide and azacyclonol (Table 4).
TABLE 2. Effects of chlorpromazine on brain noradrenaline level.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Experimental condition</th>
<th>Chlorpromazine</th>
<th>Noradrenaline (µg/g)</th>
<th>Adrenaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stressed</td>
<td>None</td>
<td>0.766 ± 0.045** (3)</td>
<td>0 (3)</td>
</tr>
<tr>
<td>2</td>
<td>Stressed</td>
<td>Administered</td>
<td>0.385 ± 0.013 (4)</td>
<td>0 (4)</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>None</td>
<td>0.293 ± 0.027 (4)</td>
<td>0 (4)</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>Administered</td>
<td>0.289 ± 0.024 (4)</td>
<td>0 (4)</td>
</tr>
</tbody>
</table>

* Chlorpromazine (8 mg/kg) was administered subcutaneously 1 hour prior to the beginning of stress.
** Mean ± standard error of the mean.
Figures in parentheses are the number of animals employed.

TABLE 3. Analysis of variance for the data of Table 2.

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Significant level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>51.35</td>
<td>17.11</td>
<td>162.95</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Drug</td>
<td>1</td>
<td>11.53</td>
<td>11.53</td>
<td>109.80</td>
<td>*</td>
</tr>
<tr>
<td>Stress</td>
<td>1</td>
<td>22.97</td>
<td>22.97</td>
<td>217.61</td>
<td>*</td>
</tr>
<tr>
<td>Interaction*</td>
<td>1</td>
<td>16.85</td>
<td>16.85</td>
<td>160.47</td>
<td>*</td>
</tr>
<tr>
<td>Replicates**</td>
<td>3</td>
<td>2.80</td>
<td>0.93</td>
<td>8.85</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.84</td>
<td>0.105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>54.99</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Drug × Stress,
** Replicates by the days in experiment.

DISCUSSION

The entire stress procedure was accompanied with a rise in the noradrenaline and it is possible that the rise in the brain noradrenaline level might have occurred as a result of the tension and apprehension provoked by the recurrence of the stimuli, rather than as a result of the pain caused by the stimuli per se.

The alteration of noradrenaline level in the stressed rats brain, compared with non-treated one, is perhaps due to severe stimulation in the central nervous system and

TABLE 4. Effects of chlorpromazine, chlordiazepoxide and azacyclonol on brain noradrenaline level.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CP</th>
<th>CD</th>
<th>AZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline</td>
<td>0.347 ± 0.033* (6)</td>
<td>0.215 ± 0.026 (4)</td>
<td>0.389 ± 0.055 (4)</td>
<td>0.259 ± 0.020 (6)</td>
</tr>
<tr>
<td>Mean effect</td>
<td>-</td>
<td>-0.177 ± 0.010 (4)</td>
<td>+0.023 ± 0.013 (4)</td>
<td>-0.072 ± 0.028 (6)</td>
</tr>
<tr>
<td>P</td>
<td>-</td>
<td>0.001</td>
<td>N.S.</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Figures in parentheses are the number of animals employed.
* Mean ± standard error of the mean.
CP : Chlorpromazine 8 mg/kg, s.c.
CD : Chlordiazepoxide 10 mg/kg, s.c.
AZ : Azacyclonol 10 mg/kg, s.c.
All drugs were administered 1 hour prior to the beginning of stress.
Azacyclonol showed the inhibitory effect on the rise of noradrenaline level but showed no effect on behavioral change. On the other hand, the results obtained with chlorpromazine indicate that this agent may, depending on the dose and the time interval between drug administration and stress, significantly affect the brain noradrenaline level as well as the behavioral responses in the stressed state.

These findings suggest that the inhibitory activity of tranquilizing agent for increase of noradrenaline level induced by electric shock is not always comparable with the changes in the animal behavior.

It can be presumed that the ability of both chlorpromazine and azacyclonol, which...
were effective to inhibit the increase of brain noradrenaline level induced by electric shock, appeared either to interfere with the incorporation of noradrenaline into so-called "granules", as shown by Gey (18), or to alter the surface activity of the granule membrane, consequently that the affinity of the noradrenaline for the granule might be changed. In contrast, chlordiazepoxide, non-effective for both noradrenaline level and behavior, may be assumed to have other different mechanism of tranquilizing action.

SUMMARY

A few tranquilizing agents were investigated to determine their effects on the brain catecholamine levels after electric shock and the following results were obtained:

1. In the case of stress alone and without the administration of drugs, the brain noradrenaline level was raised significantly as compared with that in the physiological state.

2. Chlorpromazine showed no significant effect on the brain noradrenaline level in normal situations, while it remarkably inhibited the elevation of the level in stressed state.

3. Azacyclonol and chlordiazepoxide showed a little or no inhibition on the elevation of noradrenaline level.

4. No adrenaline was detected in rat brains under all conditions, that is, stress alone, physiological, and stress after the pretreatment with various drugs, i.e., chlorpromazine, chlordiazepoxide and azacyclonol.

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REFERENCES

17) EULER, U.S.v. AND FLODING, I. : Ibid. 33, suppl. 118, 45 (1955)