EFFECTS OF CHLORPROMAZINE, TETRABENAZINE AND AZACYCLONOL ON BRAIN SEROTONIN LEVEL OF SHOCKED RATS

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Received for publication December 16, 1963

Since the presence of serotonin in the brain was first reported by Twarog and Page (1), and Amin, Crawford and Gaddum (2), serotonin has been approached by many investigators. A number of informations concerning the effects of tranquilizing agents, especially reserpine and its related compounds, on serotonin level in brain have been reported. Brain serotonin is especially associated with reserpine as shown by the evidence that the central actions of reserpine are mediated through serotonin.

In the preceding papers (3, 4), authors reported that the effects of a certain tranquilizing agents on rat brain catecholamine levels in shocked state were investigated, and resulted that some of them, i.e., chlorpromazine, tetrabenazine and azacyclonol showed the effects to inhibit the elevation of catecholamine levels caused by electroshock.

In the present study, the effects of chlorpromazine, tetrabenazine and azacyclonol on rat brain serotonin level in shocked state same conditions as that in the case of catecholamine levels, were investigated.

MATERIALS AND METHODS

The techniques of estimation of serotonin was essentially same as that described by Bogdanski (5). Experimental animals were inbred Donryu albino rats weighing 220-310 g fed with standard Oriental rat food (Oriental Yeast Co.).

Animals were subjected to fasting for 18-24 hours before the experiment, and as tranquilizing agents, chlorpromazine, azacyclonol and tetrabenazine which showed inhibiting effect on the increase of brain noradrenaline and dopamine levels in shocked state were employed. Chlorpromazine, azacyclonol and tetrabenazine were administered subcutaneously in a single dose of 8, 10, and 10 mg/kg respectively, one hour prior to shock.

Animals pretreated with drugs were subjected to the electroshock as described in the previous report (3). After shock, 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 two volumes of 0.1 N hydrochloric acid and the homogenates were followed as the method described by Mead and Finger (6). After homogenization they were kept by surrounding with ice, and sodium carbonate was added to the tube with careful mixing with a glass stirring rod until the pH become 10.0, checking its pH with indicator. Five milliliter of borate buffer solution (pH 10.0) which was previously saturated with both n-butanol and solid sodium chloride, with water up to 15 ml and 15 ml of n-butanol were added. Each tube was capped with a tightly fitting glass cap and wide rubber band. When the series were complete, the tubes were shaken well by hand for 10 minutes and then followed by centrifugation at 2,000× r.p.m. for 15 minutes, which resulted in the two liquid phases being separated by a firm layer of tissue residue, from which the butanol phase was removed and subsequently washed once with the equal volume of borate buffer. Centrifugation was performed again as above, and an aliquot of the washed butanol phase was added to centrifuging tube containing 1.5 ml of 0.1 N hydrochloric acid and 20 ml of n-heptane. These tubes were shaken well for 10 minutes and centrifuged for 15 minutes at 2,000× r.p.m. and followed essentially the method of Wiegand et al. (7). One milliliter aliquots were removed from water layer and placed over a water washed column of Dowex 50W-X4 (200-400 mesh) Na⁺ form. All solutions were passed through the column at atmospheric pressure. The resin was washed with 1 ml of water, and eluted with 5 ml of sodium hydroxide solution, effluents were collected in a tube containing 1.0 ml of 2 M acetate buffer, pH 5.0, and fluorescence of serotonin in effluent was read by Farrand Spectrophotofluorometer at 310 m, for the activation and 340 m, for the fluorescence wave lengths, respectively.

**RESULTS**

1) Effects of electroshock on the brain serotonin level

The conditions which induced electroshock is same as that in the case of catecholamines. Under these environment, the animals responded severe stimulated behaviors, that is, squalling

<table>
<thead>
<tr>
<th>Condition</th>
<th>Serotonin (μg/g)</th>
<th>Mean effect</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiological</td>
<td>0.60±0.037(10)</td>
<td>---</td>
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</tr>
<tr>
<td>Shocked</td>
<td>0.48±0.052(11)</td>
<td>-0.13±0.074</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

*Mean±standard error of the mean.
Figures in parentheses are the number of animals employed.

**TABLE 2. Effects of chlorpromazine, azacyclonol and tetrabenazine on rat brain serotonin level in shocked state.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Serotonin (μg/g)</th>
<th>Mean effect</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control**</td>
<td>0.53±0.084(4)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>CP</td>
<td>0.28±0.060(4)</td>
<td>-0.24±0.09</td>
<td>N.S.</td>
</tr>
<tr>
<td>TB</td>
<td>0.18±0.068(4)</td>
<td>-0.35±0.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>AZ</td>
<td>0.41±0.071(4)</td>
<td>-0.12±0.13</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

CP : Chlorpromazine 8 mg/kg s.c.
TB : Tetrabenazine 10 mg/kg s.c.
AZ : Azacyclonol 10 mg/kg s.c.
*Mean standard error of the mean.
Figures in parentheses are the number of animals employed.
**Electroshock alone.
and jumping, and serotonin level tended decrease compared with non-shocked animals as shown in Table 1.

2) Effects of chlorpromazine, azacyclonol and tetrabenazine on brain serotonin level in shocked state

Tetrabenazine showed decreasing effect on shocked brain serotonin, while chlorpromazine and azacyclonol were found to be non-effective (Table 2).

DISCUSSION

It is well known that reserpine prevents serotonin uptake by blocking an active transport system (8), and a certain metabolic inhibitors poison the process (9). Pletscher et al. (10) showed that tetrabenazine, in the central nervous system, also release serotonin. In spite of the effects of certain tranquilizing agents on brain serotonin level in physiological state are well established, only several studies concerning the effect of electroshock on brain serotonin level have been reported (11-15). For example, Garattini et al. (12) showed the increase of brain serotonin level which has two peaks, namely, at the immediately after shock and 36 hours later, in supramaximal electroshock that was 110V, 0.2 sec once. Recently, Pfeifer et al. studied the effect of electroshock by using bitemporal electrodes on brain serotonin and noradrenaline levels and resulted the decreasing effect of brain serotonin and no change of noradrenaline in adrenalectomized rats, in contrast, the increase of serotonin and no change of noradrenaline were obtained in the intact animals.

On the other hand, the interaction of serotonin and noradrenaline can be seen in a few ways (16): 1) they both depend on the same enzyme for their formation, 2) they exert their pharmacological actions competitively each other. These two points suggest that the amount of each amine presented in brain may, in part, depend on the amount of the other. Consequently, it is reasonable to assume that the increase of brain noradrenaline level in electroshock causes decrease of its serotonin level in our experiments.

The alteration of serotonin level in shocked state extremely depends on the experimental conditions, that is, a variation of voltage, current and the duration of subjecting time. In the shocking of the experiment on the change of serotonin level in the shocked state, two kinds of methods may be considered, 1) more severe experiments using electrodes as employed by Garattini et al. and Pfeifer et al. (14), and 2) comparative mild experiment, which makes animal to access toward stressed state as employed by authors.

The present study concerning both the effects of actions of electroshock and pretreatment of some tranquilizing drugs has demonstrated that electroshock, under these conditions employed here, causes the decrease of serotonin level in rat brain, in addition, the pretreatment of tranquilizing drugs such as tetrabenazine and chlorpromazine decrease serotonin more markedly.

Despite the effective action of chlorpromazine, tetrabenazine and azacyclonol on catecholamine levels, in this experiment, only tetrabenazine induced significant effect
on brain serotonin level. In the case of chlorpromazone, brain serotonin level was decreased apparently, however, it is not significant statistically.

Concerning the mechanism of decrease of serotonin level induced by electroshock, a few speculations are postulated as follows: As shown by Brodie et al. (17, 18), the two biological amines, i.e., brain noradrenaline and serotonin should exert effects of an opposite nature at their sites of action. To some extent, it is logical to consider that the brain serotonin level is decreased when catecholamine levels are increased by electroshock. As the mechanism of the action of tranquilizing agents on serotonin level is more complex, additional studies are needed.

On the other hand, adrenal-pituitary system may participate in the shocked state, that is, shock causes increase of ACTH activity. Consequently, corticosterone level in adrenals is elevated, resulting TPO activity to become higher. The further approaches concerning the mechanism on the decrease of brain serotonin level in the shocked state are now studied in the adrenalectomized rats in our laboratory.

Finally, the two experimental results, that is, the decrease of both serotonin level caused by electroshock and that caused by the administration of either tetrabenazine or chlorpromazone seem to have independent mechanisms each other.

SUMMARY

The effects of both electroshock and tranquilizing agents on rat brain serotonin were investigated and following results were obtained:

1. The brain serotonin was induced to decrease by the electroshock. This result is contradictory with that reported by a few investigators.

2. Administration of tetrabenazine made brain serotonin level to more markedly decrease compared with that in shock alone.

3. Chlorpromazone had some effect on electroshock, for brain serotonin level makes it to decrease, however, statistically not significant.

4. Azacyclonol showed no effect on on brain serotonin level in shocked state.

5. It is likely that the decrease of serotonin level in electroshock alone and by pre-treatments of shock with tetrabenazine and chlorpromazone have independent mechanisms each other.

REFERENCES

1) Twarog, B.M. AND Page, I.H. : Amer. J. Physiol. 126, 596 (1953)
4) Satoh, T., Iwamoto, T. AND Tokumitsu, Y. : Ibid. 14, 63 (1964)