

## Effect of Epinephrine on Adrenal Ascorbic Acid Following Premedication With Lysergic Acid Diethylamides or 5-Hydroxyindolalkylamines. (24006)

E. COSTA AND G. ZETLER\*

*Thudichum Psychiatric Research Laboratory, Galesburg State Research Hospital, Galesburg, Ill.*

Slight changes in molecular configuration of psychotomimetic drugs may alter their specific activity(1). Thus the interrelations between chemical structure and pharmacological properties of psychotomimetic analogs can be used as pharmacological tools to reveal biochemical mechanisms underlying behavioral changes induced by drugs. New findings have challenged the theories which propose serotonin antagonism as an explanatory clue for the mechanism of d-lysergic acid diethylamide (LSD) induced-psychosis(2). By comparing the pharmacology of d-2-brom LSD (BOL) and LSD it appears evident that increase of central sympathetic tonus following LSD injections fails to occur after BOL(1). It is, therefore, interesting to investigate the influence of various psychotomimetics on the actions of catecholamines on different neuro-

humoral mechanisms. Indolalkylamines are a class of chemicals including such psychotomimetic drugs as bufotenin(3). Lecomte demonstrated that serotonin sensitizes the nictitating membrane of the cat to epinephrine(4). Both bufotenin(5) and serotonin(6) can release epinephrine *in vivo* and the former is the more active compound. In contrast to bufotenin, serotonin does not cause hallucinatory experiences or behavioral changes. Thus, experiments were designed to follow changes in adrenal ascorbic acid content caused by epinephrine in rats premedicated with LSD, BOL, bufotenin and serotonin. In cats premedicated with indolalkylamines the variation of the contractive response of nictitating membrane to injected epinephrine was also investigated.

*Method.* Ascorbic acid was determined according to Roe and Kuether(7) on 350 Wistar rats decapitated 2 hours after intraperitoneal

\* On leave of absence from Dept. of Pharmacology, University of Kiel, West Germany.

TABLE I. Adrenal Ascorbic Acid Content Two Hours following the Injections.

| No. animals | Drug inj. intraper. | Dose ( $\gamma$ kg) | mg ascorbic acid     |              | % variation |
|-------------|---------------------|---------------------|----------------------|--------------|-------------|
|             |                     |                     | 100 g adrenal tissue | Stand. error |             |
| 20          | Saline              | —                   | 423                  | 407-440      | 0           |
| 8           | Epinephrine         | 25                  | 389                  | 361-420      | - 8         |
| 15          | "                   | 50                  | 389                  | 368-412      | - 8         |
| 8           | "                   | 75                  | 246                  | 236-257*     | -42         |
| 10          | Bufotenin†          | 1000                | 330                  | 285-355*     | -22         |
| 6           | "                   | 500                 | 395                  | 338-452      | - 7         |
| 8           | "                   | 250                 | 456                  | 431-481      | + 8         |
| 8           | " + epin.           | 125 + 25            | 305                  | 288-323*     | -28         |
| 7           | " + "               | 30 + 25             | 342                  | 319-367*     | -19         |
| 8           | Serotonin‡          | 1000                | 313                  | 284-341*     | -26         |
| 8           | "                   | 500                 | 338                  | 308-368*     | -20         |
| 8           | "                   | 125                 | 447                  | 433-462      | + 6         |
| 8           | "                   | 62                  | 407                  | 374-445      | - 3         |
| 6           | " + epin.           | 125 + 25            | 244                  | 228-262*     | -42         |
| 8           | " + "               | 30 + 25             | 360                  | 344-377*     | -15         |
| 7           | LSD§                | 200                 | 309                  | 293-327*     | -27         |
| 7           | "                   | 100                 | 381                  | 366-397      | -10         |
| 7           | " + epin.           | 10 + 25             | 313                  | 292-335*     | -26         |
| 7           | BOL§                | 400                 | 430                  | 411-451      | + 2         |
| 8           | " + epin.           | 40 + 25             | 390                  | 358-423      | - 8         |

\*  $P \leq .05$ .† Bufotenin monooxalate  $H_2O$  (California En. for Biochemical Research, Los Angeles, Cal.).

‡ Serotonin creatinine sulfate (Nutritional Biochemicals Corp., Cleveland, O.).

§ BOL-148 and LSD-25 donated by Sandoz Pharmaceuticals, Hanover, N. J.

injections of drugs under investigation. The mean threshold doses evoking a superreactivity of cats nictitating membrane to injected epinephrine was considered the minimal quantity of a drug ( $\gamma$  kg i.v.) causing a clear cut increase of the response of the nictitating membrane to threshold doses of epinephrine injected every two minutes. Furthermore our testing procedure required the increase response to appear 3 times during ten minutes following the injection of the drug. The cats were anesthetized with nembutal (15 mg kg i.v.) and chloralose (40 mg kg i.v.). For statistical analysis of results we used the Student's "t"-test and calculations were made according to Gaddum(8).

**Results.** In experiments reported in the Table both serotonin (0.5 mg kg) and bufotenin (1 mg kg) given intraperitoneally caused a significant depletion of adrenal ascorbic acid. Bufotenin, given in a dose of 0.5 mg kg does not induce a decrease of adrenal ascorbic acid, and serotonin is therefore considered more active than bufotenin.

An ineffective dose of epinephrine (approx-

mately  $\frac{1}{3}$  of the minimal effective dose) had been injected intraperitoneally with drugs under investigation. When  $\frac{1}{8}$  of the ineffective dose of bufotenin and  $\frac{1}{4}$  of an equally ineffective dose of serotonin were injected with the epinephrine (25  $\gamma$ /kg) a significant decrease ( $P < .02$ ) of adrenal ascorbic acid was observed.

A more intense potentiation of the effects of epinephrine on the adrenal ascorbic acid content has been obtained by injecting greater doses of bufotenin and serotonin. A similar potentiation can be seen ( $P < .001$ ) by combining small doses of LSD (10  $\gamma$  kg) with the standard dose of epinephrine. The decrease of the adrenal ascorbic acid content failed to occur after a higher dose (40  $\gamma$ /kg) of BOL associated with 25  $\gamma$ /kg of epinephrine.

Quantitative *in vivo* analysis(9) of the differences in the epinephrine potentiating effect of bufotenin and of serotonin was performed using the contractile response of the nictitating membrane of the cat. Applying this test to 10 cats we found that the threshold dose of

bufotenin which potentiates the effects of epinephrine, was  $4.82 \gamma \text{ kg}$  (3.41-6.81 S.E.) while that of serotonin was  $88.01 \gamma \text{ kg}$  (57.57-134.50). We tried a similar *in vitro* analysis on the isolated ileum and spleen of the rabbit. But here neither bufotenin nor serotonin potentiated epinephrine. Both drugs, injected to cats in doses ranging from 25 to  $150 \gamma \text{ kg}$  failed to facilitate the blood pressure effects of epinephrine. After cholinergic blockage (atropine 200-300  $\gamma \text{ kg}$ ) 25-75  $\gamma \text{ kg}$  of bufotenin did not cause hypotension but hypertension. This hypertension did not occur following adrenalectomy or regitine premedication. While in normal cats bufotenin and serotonin evoked the same kinds of responses (hypotension followed by a small and delayed hypertension) after cholinergic blockage the serotonin effect was antagonized or curtailed while the one of bufotenin was reversed.

*Discussion.* Though LSD is more active than BOL in potentiating the adrenal ascorbic acid depletion evoked by epinephrine the mechanism of this potentiation is not understood. On a quantitative basis an inhibition of enzymes controlling epinephrine metabolism is improbable, in view of the fact that both potentiations can be obtained with very small doses. It is not clear whether, in the case of serotonin, the facilitation of cellular membrane permeability described by Woolley (10) can be proposed as an explanation for the epinephrine potentiation.

We do not believe that pharmacologically-induced hallucinations derive from a single mechanism such as inhibition of serotonin or sensitization toward epinephrine. Perhaps psychotomimetic effects result from more complex interactions. Nevertheless, these experiments demonstrate that epinephrine potentiation might be considered among the neurohumoral effects probably involved in the mechanism of the pharmacologically induced psychosis. The small doses of LSD necessary to potentiate epinephrine and the ineffectiveness of doses of BOL 4 times greater stress the importance of the parallelism between the intensity of the psychotomimetic effects of the 2 drugs in man and that of their interactions with epinephrine in rats.

The following considerations are added to emphasize the specificity of the above mentioned potentiation: though the  $\text{LD}_{50}$  of BOL (20 mg kg i.v., in mice) is smaller than that of LSD (67 mg kg i.v.) (11), the latter, on a weight basis, is more active in causing a depletion of adrenal ascorbic acid.

Thus a non-specific action cannot explain the inferior effectiveness of BOL. The potentiation of epinephrine caused by LSD could take place either by a peripheral mechanism (facilitating the adrenocortical action of ACTH) or by a central one (increasing the release of ACTH by epinephrine). The second hypothesis is more probable because LSD (50  $\gamma \text{ kg}$  i.p. in guinea pigs) (12) fails to potentiate the effects of injected ACTH on the output of corticosteroids from the adrenal cortex. The results with 5-Hydroxyindolalkylamines suggest that epinephrine potentiation correlates psychotomimetic efficiency. The threshold dose of serotonin potentiating the epinephrine effects on nictitating membrane is greater ( $P < .01$ ) than that of bufotenin. This parallelism is less evident in the adrenal ascorbic acid depletion test. Serotonin "per se" reduces more efficiently than bufotenin the adrenal ascorbic acid content; nevertheless, both drugs equally potentiate epinephrine effects on adrenal glands. Finally, in agreement with previous reports (5) our results support the possibility that bufotenin releases catecholamines from adrenal medulla.

*Summary.* 10  $\gamma \text{ kg}$  i.p. of LSD potentiate the adrenal ascorbic acid depletion caused in rats by 25  $\gamma \text{ kg}$  i.p. of epinephrine, 40  $\gamma \text{ kg}$  i.p. of BOL are ineffective in this respect, 30  $\gamma \text{ kg}$  i.p. of both serotonin and bufotenin potentiate the effects of epinephrine (25  $\gamma \text{ kg}$  i.p.). The threshold doses of bufotenin and serotonin enhancing the epinephrine induced contractions of cat's nictitating membrane were  $4.82 \gamma \text{ kg}$  i.v. and  $88.01 \gamma \text{ kg}$  i.v.

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Received February 28, 1958. P.S.E.B.M., 1958, v98.

## Effect of Reserpine Phosphate on Dividing Onion Root-tip Cells. (24007)

M. R. GRIMM, F. C. KULL, AND R. L. MAYER

Research Department, CIBA Pharmaceutical Products, Inc., Summit, N. J.

Goldin *et al.* (1) and Belkin and Hardy (2) have shown that large doses of reserpine, administered to mice carrying lymphoid tumor L 1210 or solid tumor Sarcoma 37, increase survival time and cause tumor regression. Induction of a deeply tranquilized state in animals reported by these authors and noted in our experiments with reserpine, inferred that antitumor activity may be the consequence of prolonged depression rather than an intrinsic and specific antitumor action of the drug. To determine more closely the mode of antitumor action of reserpine, the effects of this alkaloid in various biological systems were investigated. Initial microbiological experiments on *in vitro* activity of reserpine phosphate indicated that it does not act directly on bacteria; no influence on cell multiplication rates was recognized. However, certain colonial modifications and morphological distortions of various gram positive and gram negative cells were observed. Swelling, chain formation, and pigment inhibition were in large measure dependent upon stage of development of the cells concerned. As with many other agents, dividing cells were more vulnerable than resting stages. It was interesting, therefore, to investigate the effect of reserpine on mitosis in higher plants. This was done using root-tip cells of *Allium cepa* var. Silver Skin.

**Methods.** "Silver Skin" resting bulbs were germinated by placing bulb on top of shell vials of 10-20 ml capacity filled daily with freshly distilled water. The vials were coated with thin layer of paraffin to exclude light.

After 3-4 days, bulbs with 15 or more roots were selected for testing. These bulbs were then transferred to similar vials containing 0.125 to 10 mg% reserpine phosphate. The solutions were changed daily for duration of experiment. To obtain as much variation in cell counts as possible, roots from 4 different bulbs were used for each dilution of compounds tested and for controls. Root tips, carefully removed at 24, 48, and 72 hours, were fixed in acetic-alcohol, squashed, and stained with aceto-carmin according to method described by Belling (3). Control bulbs were germinated in water with and without sodium phosphate, smeared, and stained in the same manner as test roots. To reduce deviation and inconsistency, roots were collected at same time each day (1 p.m.)—a time reported by Kellicott (4) to be a period of maximum mitotic division in *Allium* root-tips showing diurnal variation. Totals of at least 1000 cells in the region of cell multiplication were counted for each sample, and the number of cells in mitosis from late prophase to telophase recorded. Countings were made after one, 2, and 3 days in reserpine and after second, third, and fourth day replacement in water. Conclusions reported below are based on determinations of mitotic index and analyses of dividing cell populations in terms of phase percentage. The mitotic index is a ratio of dividing to resting cells.

**Results.** Table I shows mitotic index values from roots exposed 24 hours to various dilutions of reserpine. At concentration of 4 mg%, the compound exerted an irreversible