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Anaphylaxis in Guinea Pig: Improbability of Release of Serotonin in the Schultz-Dale Reaction.* (23803)

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Much knowledge concerning anaphylaxis has been gained from experimentation using the guinea pig, where it repeatedly has been shown that most symptoms of anaphylaxis are referable to release of histamine. The methods often employed antihistaminic drugs, but as pointed out by Dale(1), and Hawkins and Rosa(2), these drugs have inconsistently blocked the reaction; and their specificity is often suspect. Also, biological assay methods to measure histamine could conceivably be detecting other similarly reactive substances. These findings, together with failure to incriminate histamine in the rat and the persistence of the Schultz-Dale reaction in guinea pig tissues made refractory to histamine(3) cast doubt on the histamine theory of anaphylaxis. Other substances which might be involved in addition to histamine have been discussed by Dragstedt(4). Recent work on another species, the mouse, has demonstrated the improbability of involvement of histamine in the anaphylactic reaction; and the probability of the release of 5-hydroxytryptamine (serotonin). Evidence supporting the serotonin hypothesis includes prevention of the Schultz-Dale reaction in the presence of the highly specific serotonin antagonist, lysergic acid diethylamide (LSD),† and the demonstrably antiserotonic drug, reserpine(6). Further, Weiser(7) has shown almost 100% inhibition of generalized anaphylactic symptoms with reserpine; and Fox *et al.* with LSD(8). Conflicting reports have appeared recently regarding the role of serotonin in guinea pig anaphylaxis. Herxheimer(9) has shown that, although the guinea pig responds to an aerosol of serotonin in a manner similar to its response to histamine, and desensitization to an antigen is accompanied by a parallel tolerance to serotonin, LSD does not alter the anaphylactic reaction obtained after inhalation of antigen. During the course of the present work, however, Geiger *et al.*(10) reported that the Schultz-Dale reaction and the reaction to serotonin, but not the reaction to histamine, could be inhibited with the drugs yohimbine, bufotenine and gramine.

The purpose of this work was to determine whether or not the Schultz-Dale reaction in the sensitized guinea pig could be prevented with the serotonin antagonists LSD and brom lysergic acid diethylamide (BOL)(11). Following the publication of Geiger's work, an attempt was made to confirm his findings.

Procedure. Fully grown guinea pigs of both sexes from various commercial sources were used. They were injected intra-

* This investigation was supported by grant from Continuing Research Fund, University of Colorado Medical School.

† Munoz(5) has claimed that LSD does not specifically block serotonin, but this claim was not substantiated by any reference. We have been unable to uncover any evidence that LSD in the concentration used in these studies antagonizes the action of any other known physiological substance on smooth muscle.

abdominally with 1 ml of a 10% protein solution—either whole dried egg white‡ or bovine serum albumin§—approximately 3 weeks before being tested. At time of testing, animals were sacrificed by blow on head; the uterus or ileum was removed immediately, washed with Tyrode's solution, and placed in this solution at room temperature until used. Whole sections of ileum or uterus, 1-1.5 cm long, were cut and attached to an ink-writing kymograph lever in an aerated 50-ml water bath maintained at 37-38°C. After obtaining the pattern of normal contractions, the antigen—either crystalline ovalbumin‖ (EA) or bovine serum albumin (BSA)— was added 15 μg/ml of bath fluid. This amount of antigen had previously been shown to be optimal for provoking maximal contraction in a sensitized guinea pig tissue, and almost invariably rendered the tissue desensitized upon the second injection of antigen.

When it was definitely established that the tissue was sensitized, fresh strips were set up. Routinely, each strip was tested for its reactivity to serotonin¶ (0.1 μg/ml)**, and to histamine†† (0.1 μg/ml) prior to addition of antigen; a second addition of antigen was made to test for desensitization. In experiments testing drug inhibition of anaphylaxis, reactivity to serotonin and to histamine was determined; then the antagonistic drug was added for various periods of time. After addition of antigen to test for inhibition of anaphylaxis, a second addition of antigen was made to test for desensitization; lastly, reactions to standard amounts of serotonin and histamine were recorded. In any case where tissue failed to react after addition of an inhibitory drug, the potential reactivity of the tissue was tested by addition of 0.1 μg/ml of acetylcholine.

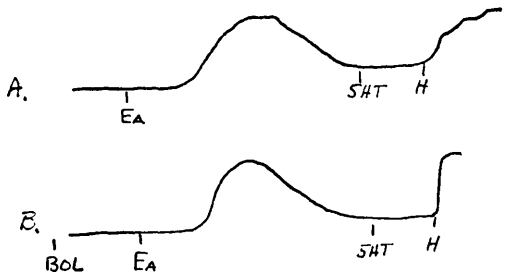

FIG. 1. Persistence of anaphylactic contraction of egg sensitive guinea pig ileum in the presence of BOL.* A—no drug present. B—BOL, 250 γ/l for 15 min.

Results. Experiments with LSD and BOL‡‡ included the use of 6 uteri from egg-sensitized female guinea pigs; the ilia from 6 egg-sensitized male guinea pigs; and the ileum from one male guinea pig sensitized with BSA. Following the routine as outlined previously, the reaction to antigen was tested in the presence of LSD in concentrations of 3 and 30 μg/L for 5 minutes; and in the presence of BOL in concentrations of 5, 50, 250 and 500 μg/L for 5 to 30 minutes. The LSD was allowed to remain in the bath during the addition of antigen; as recommended by Sollers *et al.*(11)§§, the BOL was washed out by replacing the fluid in the bath three times prior to the addition of antigen. All concentrations of BOL and LSD demonstrably inhibited serotonin. In no case was the reaction to antigen or to histamine modified. A typical response is represented in Fig. 1.

Experiments with yohimbine, bufotenine, and gramine.‖‖ To make these experiments more nearly comparable to Geiger's(12),

‡ Albumin, Egg Impalp. Powder, J. T. Baker Chem. Co.

§ Armour and Co. powder.

‖ Armour and Co.

¶ Serotonin creatinine sulfate, Nutritional Biochemicals Corp., Cleveland. Concentration calculated on weight as 5-hydroxytryptamine.

** Occasionally, a strip of tissue was found which did not respond to serotonin at this concentration, or at twice this concentration. These strips did, however, contract on the addition of antigen and of histamine.

†† Histamine phosphate, Parke Davis and Co.

* This tissue did not respond to serotonin.

‡‡ We are indebted to Dr. Harry Althouse, Sandoz Pharmaceuticals, San Francisco, for generous supplies of LSD, BOL, and bufotenine.

§§ These authors report that LSD does not antagonize serotonin in similar preparations in the guinea pig. For this reason, even though serotonin was demonstrably inhibited in these studies, more emphasis was placed on the work with BOL.

‖‖ We are indebted to Dr. W. B. Geiger for yohimbine (Amend Drug and Chemical Co. preparation); and gramine, from the Bios Labs.

the routine technic was modified by omitting magnesium from the Tyrode's solution; and the crystalline egg albumin was dialyzed against Tyrode's solution for 24 hours prior to use. Geiger allowed 15 minutes for contact of tissue with the inhibitory drug; both 15 and 30 minutes were used in this study. Variations from Geiger's technic included the use of histamine in a concentration of 0.1 μg/ml instead of 2 μg/ml; and antigen 15 μg/ml instead of 0.2 μg/ml. The small amount of histamine was used because it invariably provoked maximal contraction of the tissue; the larger amount of antigen was used because it was necessary to provoke maximal contraction of the tissue, and to lead to desensitization of the tissue.

Geiger found that the 3 drugs, in concentration of 20 μg/ml, prevented stimulation by antigen and by serotonin, but not by histamine. The 3 drugs, in concentrations of 20, 40 and sometimes 80 μg/ml were used in this study. Ilia from 3 animals sensitive to BSA and 2 sensitive to EA were tested in various concentrations of yohimbine; gramine was tested on ilia from two sensitized with BSA and two with EA; and bufotenine, on three animals sensitized to EA. Yohimbine in concentrations of 20, 40 and 80 μg/ml blocked serotonin contractions completely, with anaphylactic and histamine contractions being progressively diminished until made extinct at higher concentration of the drug. A typical response is presented in Fig. 2.

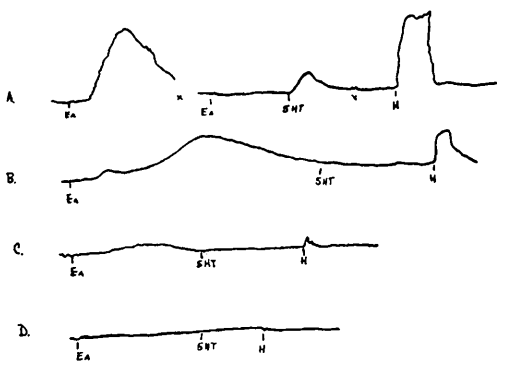

FIG. 2. Parallel effect of various conc. of yohimbine on the response of egg sensitive guinea pig ileum to antigen and to histamine.*

Bufotenine reacted similarly to yohimbine, inhibiting the serotonin contractions at 20 and 40 μg/ml, diminishing the histamine and anaphylactic contractions at 20 μg/ml, and abolishing them at 40 μg/ml.

Concentrations of gramine inhibiting the Schultz-Dale reaction inhibited both the serotonin and histamine reactions.

These reactions were consistently demonstrated in all tissues with all drugs tested.

Miscellaneous experiments. Additional experiments done to illustrate release of serotonin from sensitized guinea pig tissue were of 2 kinds: (1) These experiments employed the general technic of Campbell(13). With normal mouse uterus connected to the kymograph lever, an attempt was made to cause this highly serotonin-sensitive tissue to contract by adding to the bath: (a) perfusion fluid from EA-sensitized guinea pig lung perfused with antigen; or (b) whole sensitized guinea pig uterus, ileum or lung, plus antigen. Some of these experiments were conducted with Marsilid¶¶ 200 μg/ml in Tyrode's solution, to prevent the possible enzymatic destruction of serotonin(14). In no case was a contraction of the mouse uterus obtained. (2) Strips of sensitized guinea pig uterus were made insensitive to histamine by contact with high concentrations of this drug for long periods of time, then tested for reactivity to antigen. An attempt was made to block the persisting anaphylactic contraction with known inhibitory concentrations of BOL and LSD. In no case was the reaction even diminished.

Discussion. Using the Schultz-Dale technic as used previously to demonstrate the complete inhibition of *in vitro* anaphylaxis in the sensitized mouse uterus by the potent and highly specific serotonin antagonists, LSD and BOL, it was impossible to block the reaction in the sensitized guinea pig uterus or ileum. When the less specific serotonin antagonists yohimbine, gramine and bufotenine were used, and in much greater concentration, it was possible to block the reaction to serotonin, but the persisting reaction to antigen was entirely comparable to that of histamine. As the con-

* A, no yohimbine; B, 20 γ/ml; C, 40 γ/ml; D, 80 γ/ml. Ea = 15 γ/ml dialyzed crystalline Ea. 5Ht = 0.1 γ/ml serotonin. H = 0.1 γ/ml histamine.

¶¶ We wish to thank Dr. R. J. Floody, Hoffmann-LaRoche, Inc., Nutley, N. J., for the Marsilid (1-isonicotinyl-2-isopropylhydrazine) used.

centration of the inhibitory drugs was increased to the degree that complete inhibition of anaphylaxis occurred, there was complete inhibition of the reaction to histamine. These results are not in agreement with those of Geiger *et al.*(10), who demonstrated the blocking of the anaphylactic and serotonin contractions, but not the histamine contraction, with these drugs. They used much larger concentrations of histamine and less of antigen to provoke the reaction. It is possible that the histamine dosage used by Geiger was sufficient to overcome the blockade with the inhibitory substances; and that his antigen concentration might not have been sufficient to permit maximum reaction of the tissue.

No evidence for the release of serotonin was obtained by allowing various tissues from sensitized guinea pigs to be in contact with antigen in the presence of the highly serotonin-sensitive mouse uterus. Weissbach *et al.*(15) have reported that the serotonin content is low, and the monoamine oxidase activity high in the guinea pig lung. The low content might account for the failure to demonstrate serotonin release from the sensitized lung even in the presence of an amineoxidase inhibitor. The serotonin content of the small intestine, however, is greater(16), and had serotonin been released from the quantity of ileum used in these studies, it should have been sufficient to provoke a contraction of the mouse uterus, especially with the enzyme inhibitor present.

The persistence of the positive reaction to antigen in the guinea pig ileum or uterus poisoned to histamine is well known(3), and has been used as evidence against the histamine theory of anaphylaxis. Inasmuch as this reaction could not be modified with LSD or BOL, no argument can be made that this could be due to serotonin release. Thus the contention that the reaction is due to the release of intrinsic histamine which is not affected by poisoning with extrinsic histamine, cannot be challenged on these grounds.

The results obtained in this study tend to rule out the participation of serotonin in the anaphylactic contraction of smooth muscle in this species. The opposite situations prevailing in the mouse and in the guinea pig, as demonstrated by use of the Schultz-Dale technic, make even more pertinent the thoughtful admonition of Dragstedt(4): "Nor, . . . is there good reason why the . . . tissue metabolites associated with the phenomenon of anaphylaxis should have parallel degrees of importance in more than one animal species."

Summary. 1. The Schultz-Dale reaction in egg white or BSA sensitized guinea pig uterus or ileum persisted in the presence of serotonin antagonists LSD and BOL. 2. Anaphylactic contraction of these tissues could be partially or completely blocked with varying concentrations of yohimbine, gramine and bufotenine, but the reaction to histamine was affected similarly. 3. It was impossible to demonstrate the release of serotonin from sensitized guinea pig lung, uterus or ileum in the presence of highly serotonin sensitive mouse uterus. 4. Persisting anaphylactic contraction of histamine-poisoned guinea pig uterus was not diminished in the presence of BOL or LSD.

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