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STUDIES ON THE ROLE OF SEROTONIN AND MAST CELLS IN ANAPHYLAXIS OF THE MOUSE PRODUCED WITH SOLUBLE ANTIGEN-ANTIBODY COMPLEXES[1]

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The mechanisms responsible for anaphylaxis in the mouse are not known. Histamine, which is thought to be the principal mediator of acute anaphylaxis in such histamine-sensitive animals as the guinea pig, rabbit and dog is of doubtful importance in anaphylaxis of the histamine-resistant mouse (1).

Fink (2) has proposed that serotonin (5-hydroxytryptamine) is the mediator of the Schultz-Dale reaction in the mouse uterus. She observed that the uteri of both sensitized and normal mice are 1000 times more sensitive to 5-hydroxytryptamine (5-HT) than to histamine. Moreover, the sensitivity of the uteri to 5-HT and to the Schultz-Dale reaction was abolished by the 5-HT antagonists, lysergic acid diethylamide (LSD) and reserpine. Fox and co-workers (3) subsequently reported that the "5-HT antagonists," chlorpromazine, promazine, reserpine, and LSD, were effective in preventing anaphylaxis in the actively sensitized mouse.

The above results are of interest in view of the report of Weissbach *et al.* (4) that, whereas guinea pig lung has a low 5-HT content and high monoamine oxidase activity, mouse lung has a high 5-HT content and low monoamine oxidase activity. Since monoamine oxidase could rapidly convert any 5-HT freed by the antigen-antibody (Ag-Ab) reaction to the pharmacologically inactive product 5-hydroxyindoleacetic acid, these observations are consonant with the hypothesis that 5-HT is important in anaphylaxis of the mouse but not of the guinea pig. The hypothesis that 5-HT is released by the Ag-Ab reaction is supported by studies *in vitro* (5) and *in vivo* (6) on the rabbit, showing that the reaction of Ag and Ab in the presence of platelets causes the release of 5-HT.

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In the present investigation several experimental approaches were undertaken to determine the role which 5-HT may play in anaphylaxis of the mouse. These included pretreatment with reserpine, 5-HT antagonists, 5-HT, and 5-hydroxytryptophan (5-HTP), respectively.

MATERIALS AND METHODS

Unless otherwise stated the materials and methods used were similar to those employed in previous investigations (7, 8).

The animals used for most of the work were "colony inbred" white mice of the Swiss-Webster strain. Mice of this strain have been maintained in our laboratory for the past 10 years. They were from 6 to 8 weeks old and weighed from 22 to 28 g. The mice of the inbred DBA/2 strain were recently obtained from the Jackson Memorial Laboratory at Bar Harbor, Maine.

The soluble "Ag-Ab complex" of crystalline bovine serum albumin (BSA) and antibovine serum albumin (antiBSA) used for producing anaphylaxis was prepared from time to time by adding pooled heat-inactivated antiBSA rabbit serum to a solution of BSA containing 8-fold antigen excess (7, 8). The preparation contained 1.520 mg of antibody nitrogen (Ab N) and 5.[illegible] mg of BSA per ml. This "standard" preparation is hereafter commonly referred to as "the complex." The complex and the antiBSA rabbit serum were stored at −20 C. The challenge dose of the complex that was commonly required to produce fatal anaphylaxis in 80% or more of the mice tested was 0.4 ml. The material was administered intravenously into the tail vein using a 27 gauge needle and a tuberculin syringe.

The resulting shock was indistinguishable from typical anaphylaxis as produced in actively sensitized animals by challenge with Ag alone. In mild shock the mouse appears normal for the first 2–5 min after challenge and then starts to scratch the nose and ears. The lips and snout

begin to swell and the ears become flushed. The animal becomes sluggish and shows slight dyspnea and cyanosis which may persist for 10–20 min. In severe shock, the above symptoms are followed within the next 10 min by severe dyspnea, marked cyanosis and prostration. When the animal attempts to walk, the gait is unsteady. In the terminal stages of fatal shock, prostration and paresis characterized by convulsive kicks and characteristic "frog-like" movements are observed (9). Although death usually occurs within 30 min, the interval may vary from 10 min to 1 hr. Anaphylactic death was used as the criterion of the anaphylactic response. Deaths after 1 hr were rare and were not recorded as anaphylactic deaths. Diarrhea was often noted after 15 min and frequently occurred in animals given sublethal doses of the complex.

Ear skin, subcutaneous tissue and mesentery were stained and examined for mast cells. The subcutaneous tissue and mesentery were spread by teasing on microscope slides, air-dried and stained with May-Gruenwald-Geimsa or Wright's stain. The ear skin was immersed for 3 min in Wright's stain, washed in buffer and placed on a microscope slide for examination.

In certain experiments, mice were injected intraperitoneally with 0.1 ml of *Bordetella pertussis* vaccine, containing 48.8×10^9 cells per ml, 4 days before challenge with the complex.[2]

The Chi square test with Yate's correction (10) was used in several instances to determine the significance of data.

RESULTS

Experiments with reserpine. Since it has been shown in various animals that one of the activities of reserpine is that of releasing 5-HT from certain body cells and tissues such as the enterochromaffin cells (11), mast cells (12), and platelets and brain (13), an experiment was conducted to determine the effect of treatment with reserpine on anaphylaxis of the mouse. A dose of 0.25 mg of reserpine was injected subcutaneously into normal mice 18 to 22 hr before challenge with the standard preparation of the complex.[3] At the time of challenge the animals were in moderate sedation. Whereas 21 of the 28 diluent-treated control mice died of anaphylaxis, all of the 28 reserpine-treated (reserpinized) mice survived. Twenty of the latter group showed no evidence of shock and eight showed symptoms of mild shock. Although the protective effect of reserpine may have been due to its 5-HT depleting action, other possible modes of action which were considered included: sedation (14), depletion of body stores of the catechol amines, epinephrine, norepinephrine and dopamine (15), anti-thyroid action (16) and hypothermia (17).

Carlsson and associates (15) reported that the body stores of 5-HT and the catechol amines that are depleted by reserpine appear to be restored by the administration of their respective precursors, 5-HTP and 3,4-dihydroxyphenylalanine (DOPA). In view of these findings, experiments were conducted to determine the role which a deficiency in the body stores of 5-HT and the catechol amines may play in the resistance of the reserpinized animal to anaphylaxis. Various concentrations of DOPA and 5-HTP were prepared in distilled water. Dosage volumes ranging from 0.2 ml to 0.75 ml were administered intraperitoneally 18 to 22 hr following reserpine treatment and 5 to 30 min before challenge with the complex. The reserpinized control mice received similar volumes of distilled water instead of DOPA and 5-HTP. Normal control animals were also included.

The DOPA-treated animals were hyperactive at the time of challenge with the complex. On the other hand, the 5-HTP-treated animals were in moderate sedation due to the effects of reserpine. The results presented in Table I show that, whereas the DOPA-treated animals were refractory to anaphylaxis the 5-HTP-treated animals displayed the same susceptibility to anaphylaxis as control animals. When both 5-HTP and DOPA were administered, the animals were refractory to anaphylaxis.

Protective action of catechol amines. Since the anaphylactic response of the reserpinized mouse was not restored to normal by either DOPA alone or a combination of DOPA and 5-HTP, experiments were conducted to determine the effects of treatment with the catechol amines on anaphylaxis in the normal mouse. The catechol amines, epinephrine, norepinephrine and dopamine, were diluted in saline and administered intraperitoneally in volumes of 0.2 ml either

[2] The pertussis vaccine was generously supplied by Merck and Company, Rahway, New Jersey.

[3] The reserpine diluent consisted of 5% polyethyleneglycol and 5% benzyl alcohol in physiologic saline.

TABLE I

*Influence of treatment with 5-hydroxytryptophan (5-HTP) and 3,4-dihydroxyphenylalanine (DOPA) on the anaphylactic response of reserpinized mice to challenge with soluble antigen-antibody complex**

Material Used for Treatment	Interval between Treatment and Challenge	Anaphylactic Deaths†
	min	
DOPA, 2 mg	15	0/10
DOPA, 6 mg	30	0/10
DOPA, 15 mg	15	0/10
Distilled H_2O (controls)	15	0/30
None (controls)		22/26‡
5-HTP, 2 mg	5	0/10
5-HTP, 2 mg	15	18/30
Distilled H_2O (controls)	15	0/20
None (controls)		20/24‡
DOPA, 2 mg plus 5-HTP, 2 mg	15	0/12
Distilled H_2O (controls)	15	0/12
None (controls)		9/10‡

* All test mice were given 0.25 mg of reserpine by the subcutaneous route 18 to 22 hours before challenge.

† The numerator denotes the number of mice that died of anaphylaxis and the denominator, the number of mice challenged.

‡ Control mice received no treatment of any kind prior to challenge with the complex.

before or after challenge with the complex. The results, presented in Table II, show that prechallenge treatment with any one of these amines rendered the animals markedly refractory to anaphylaxis. The combined results, shown in Tables I and II, were consistent with the concept that the protective effect of reserpine depends primarily on its action of exhausting body stores of 5-HT.

Protective effect of histamine pretreatment on anaphylaxis. Since histamine has been shown to stimulate the adrenal gland (18), the effect of treatment with histamine on the anaphylactic response to soluble Ag-Ab complex was studied. Histamine phosphate was dissolved in 0.2 ml of M/100 Sorensen's phosphate buffer, pH 7.2, containing physiologic saline (PBS) and administered intravenously 20 to 25 min before challenge with the complex. Although the amounts of histamine used were not lethal, it was noted that immediately after administration of the drug the animals showed some hyperactivity followed by prostration. However, at the time of challenge, the mice had recovered and appeared normal. The results, presented in Table III, show that the histamine treatment afforded good protection against anaphylaxis. Perry and Darsie (19) did not note any effect of histamine treatment on anaphylaxis in the mouse. These varying results may have been due to differences in the drug dosages used and the time intervals between treatment and challenge. An alternate possibility is that the difference in results was due to the systems used, namely active anaphylaxis *vs* passive anaphylaxis provoked with soluble Ag-Ab complex.

Susceptibility to histamine and 5-HT following histamine treatment. Because of the observation that the histamine-treated mouse shows increased resistance to anaphylactic shock, the effects of histamine treatment on challenge with either histamine or 5-HT were studied. The

TABLE II

Influence of treatment with epinephrine, norepinephrine and dopamine on the anaphylactic response of the mouse to challenge with soluble antigen-antibody complex

Material Used for Treatment	Interval between Treatment and Challenge	Anaphylactic Deaths
	sec	
Epinephrine:		
0.010 mg	30 (before)	1/28*
0.010 mg	300 (before)	0/10*
0.010 mg	300 (after)	14/20
0.001 mg	30 (before)	13/20
Saline (controls)	30 (before)	23/28
Norepinephrine, 0.10 mg	30 (before)	7/28*
Saline (controls)	30 (before)	22/30
Dopamine, 0.324 mg	30 (before)	7/30*
Saline (controls)	30 (before)	16/26

* These values differ significantly from their respective control values.

TABLE III

Influence of treatment with histamine on the response of mice to challenge with soluble antigen-antibody (Ag-Ab) complex, histamine and serotonin

Material Used for Treatment	Interval between Treatment and Challenge	Challenge Preparation	Anaphylactic Deaths
	min		
Histamine, 1.0 mg	25	Ag-Ab complex	4/10
Histamine, 3.0 mg	25	Ag-Ab complex	1/20*
PBS (controls)	25	Ag-Ab complex	12/20
Histamine, 3.0 mg	25	Histamine, 8 mg	7/10†
PBS (controls)	25	Histamine, 8 mg	7/10†
Histamine, 3.0 mg	25	5-HT, 4 mg	6/20†
PBS (controls)	25	5-HT, 4 mg	9/26†

* This is the only value which differed significantly from the respective control value.
† These deaths occurred at periods between 2–10 min after challenge.
PBS = phosphate buffer, pH 7.2, containing physiologic saline; 5-HT = 5-hydroxytryptamine.

animals were given 3.0 mg of histamine-phosphate intravenously 25 min before intravenous challenge with either histamine or 5-HT. The controls were treated with a similar volume of PBS. The results of this experiment, presented in Table III, show that histamine treatment did not modify the susceptibility of normal mice to further intravenous challenge with histamine or 5-HT. They provide no evidence to indicate what the response of the animals might be to the local liberation of these agents.

Protective action of 5-HT antagonists. Lysergic acid diethylamide, its bromine analogue (BOL) and chlorpromozine have been shown to possess anti-5-HT activity in the mouse (2, 20, 21). Experiments were conducted to determine whether treatment of normal mice with these compounds would protect against anaphylaxis.

LSD, 0.5 mg in a volume of 0.2 ml of saline, was given subcutaneously to 22 mice 2 hr before challenge with the Ag-Ab complex. Six of the animals died from the LSD treatment. The survivors were hyperactive at the time of challenge. A second group of mice received an intravenous dose of 0.1 mg of BOL in a volume of 0.2 ml of saline 90 min before challenge with the complex. Although the mice given BOL also became hyperactive immediately after injection, they appeared normal at the time of challenge. A third group of mice were treated intravenously with 0.125 mg of chlorpromazine 10 min before challenge. This amount of chlorpromazine caused slight sedation. The results of these experiments, presented in Table IV, show that treatment with the 5-HT antagonists, BOL, LSD, and chlorpromazine afforded good protection against anaphylaxis. The protective action afforded by LSD and chlorpromazine was in good agreement with the results obtained by Fox *et al.* (3).

In view of the report of Kelemen (22) that the anti-inflammatory agent sodium salicylate may be a 5-HT antagonist, this compound was used to treat groups of mice before intravenous challenge with either soluble Ag-Ab complex, histamine or 5-HT. In this experiment a subcutaneous dose of 7.5 mg of sodium salicylate in physiologic saline was given 3 to 4 hr before challenge with the various preparations. The control mice received the saline diluent before challenge. The results of this experiment, presented in Table V, show that sodium salicylate afforded significant protection against anaphylaxis but failed to protect against shock with histamine or 5-HT.

Enhancement of anaphylaxis by treatment with 5-HT and 5-HTP. Waalkes and Coburn (23) have recently shown that the intravenous injection of 5-HT results in an increase in the total amount of 5-HT in the lung. In a trial designed to test the hypothesis that an increase in the stores of 5-HT may enhance the susceptibility of mice to anaphylaxis the effect of treatment with 5-HT on the anaphylactic response was

TABLE IV

Influence of treatment with anti-serotonin agents on the anaphylactic response of the mouse to challenge with soluble antigen-antibody complex

Material Used for Treatment	Route Used for Treatment	Interval between Treatment and Challenge	Anaphylactic Deaths
BOL:			
0.025 mg	Intravenous	30 min	7/10
0.025 mg	Intravenous	90 min	8/10
0.10 mg	Intravenous	90 min	11/32*
Saline (controls)	Intravenous	90 min	19/22
LSD, 0.5 mg	Subcutaneous	2 hr	4/16*
Saline (controls)	Subcutaneous	2 hr	11/14
Chlorpromazine, 0.125 mg	Intravenous	10 min	1/12*
Saline (controls)	Intravenous	10 min	8/10

* These values differ significantly from their respective control values.
BOL = bromine analogue of lysergic acid diethylamide (LSD).

determined. Twenty-four mice were treated intravenously with 1.0 mg of 5-HT in 0.2 ml of physiologic saline 30 min before challenge with 0.4 ml of a dilute solution of the complex containing 0.144 mg Ab N and 0.5 mg BSA. This dosage of complex resulted in a low mortality in the control animals that received the saline diluent before challenge. The 5-HT used for treatment produced immediate mild to severe symptoms of "shock" which subsided completely before challenge with the complex. At the time of challenge all of the treated animals appeared to be slightly sedated, and some showed diarrhea. The results presented in Table VI show that treatment with 5-HT increased the susceptibility of the mice to challenge with the complex.

To test the above hypothesis further another experiment was conducted in which the animals were treated with the 5-HT precursor, 5-HTP. The animals were given an intraperitoneal dose of 2.0 mg of 5-HTP in a volume of 0.25 ml of physiologic saline 15 min before challenge with the same diluted preparation of soluble Ag-Ab complex used in the preceding experiment. Since all the mice showed diarrhea and sedation at the time of challenge, it is probable that appreciable amounts of 5-HT had been produced from the injected 5-HTP (24). The results, which are also presented in Table VI, show that treatment with 5-HTP markedly enhanced the susceptibility of the animals to anaphylaxis.

Attempts to protect against anaphylaxis with pyrilamine maleate. Since pyrilamine maleate has been shown to afford good protection against histamine shock in the mouse (25), the anaphylactic response following treatment with this antihistaminic agent was determined. The compound was dissolved in PBS and administered subcutaneously in various dosage levels in a volume of 0.2 ml. Control mice received a similar volume of PBS. The results, presented in Table VII, show that pyrilamine maleate administered 20–40 min before challenge with the complex did not significantly alter the anaphylactic re-

TABLE V

*Influence of treatment with sodium salicylate on the response of mice challenged with soluble antigen-antibody (Ag-Ab) complex, histamine and serotonin**

Material Used for Treatment	Challenge Preparation	Anaphylactic Deaths
Sodium salicylate, 7.5 mg	Ag-Ab complex, 0.4 ml	8/30†
Saline (controls)	Ag-Ab complex, 0.4 ml	20/26
Sodium salicylate, 7.5 mg	Histamine, 8 mg	6/10
Saline (controls)	Histamine, 8 mg	5/10
Sodium salicylate, 7.5 mg	Serotonin, 4 mg	8/16
Saline (controls)	Serotonin, 4 mg	6/16

* The sodium salicylate was administered subcutaneously 3–4 hr before challenge.
† This value differs significantly from the control value.

TABLE VI

*Influence of treatment with 5 hydroxytryptamine (5-HT) and 5 hydroxytryptophan (5-HTP) on anaphylactic response of the mouse to challenge with soluble antigen-antibody complex**

Material Used for Treatment	Interval between Treatment and Challenge	Symptoms at Time of Challenge	Anaphylactic Deaths
	min		
5-HT, 1 mg	30	Diarrhea, slight sedation	20/24†
5-HT, 2.0 mg	15	Diarrhea, slight sedation	19/22†
Saline (controls)	15	None	12/36

* The mice were challenged with a diluted preparation of complex designed to produce a low fatality rate.

† These values differ significantly from the control value.

sponse of the animals. These results differ from those of Cameron (25) who reported that this compound affords good protection against active anaphylaxis in the mouse. Although the reason for the difference in results is not apparent, it should be noted that the mice used in the present experiments were of a different strain than those used by Cameron. In addition, the manner of producing shock was not the same.

Studies with pertussis-vaccinated mice. Since it was observed that the 5-HT-antagonists and the catechol amines exert protective action against anaphylaxis in normal mice, tests were conducted to determine whether these compounds also possess the capacity to protect pertussis-vaccinated animals. The challenge material used was made by diluting the standard preparation of soluble Ag-Ab complex with PBS to give 0.144 mg Ab N and 0.5 BSA in a total volume of 0.4 ml. The results, presented in Table VIII, show that, among the agents tested, epinephrine was the only compound capable of protecting the pertussis-treated mouse against anaphylaxis. This is in marked contrast to the results previously observed in normal, nonvaccinated mice in which it was noted that prechallenge treatment with either reserpine, BOL or dopamine was also effective in preventing anaphylaxis. Since pertussis vaccination alters the physiologic reactivity of the mouse in many other ways than by increasing their susceptibility to anaphylaxis and 5-HT (26), the above results provide no dependable clues to the mechanisms of anaphylaxis in the normal mouse.

Refractoriness to anaphylaxis following sublethal doses of the complex. It is an old observation that animals of various species may become refractory to anaphylaxis with a specific antigen following treatment with sublethal doses of the antigen or following sublethal shock produced with antigen and antibody of an unrelated system (27). In the course of the present investigations it was observed that mice challenged with sublethal doses of soluble complexes of BSA-anti-BSA become highly refractory to further challenge with lethal doses of this complex or to challenge with lethal doses of complexes of a different antigen-antibody system, such as those of bovine γ-globulin-antibovine γ-globulin. The refractory state lasted for 48–72 hr. Refractory states in mice following challenge with soluble antigen-antibody complexes have been previously reported by others (28, 29).

In the present work preliminary attempts were made to abolish the refractory state produced with the complex by the administration of either mouse complement of 5-HTP or a combination of both substances. The development of diarrhea in the animals treated with 5-HTP indicated that the quantities of serotonin induced were appreciable. Nevertheless, neither 5-HTP nor mouse complement nor a combination of both substances abrogated the refractory state.

Studies on mast cells following anaphylaxis.

TABLE VII

Influence of treatment with pyrilamine maleate on the anaphylactic response of the mouse to challenge with soluble antigen-antibody complex

Material Used for Treatment	Interval between Treatment and Challenge	Anaphylactic Deaths
	min	
Pyrilamine mateate, 1.0 mg	20	8/10
PBS (controls)	20	8/10
Pyrilamine maleate, 1.0 mg	40	9/22
PBS (controls)	40	13/21
Pyrilamine maleate, 1.5 mg	40	8/10
PBS (controls)	40	9/10

PBS = phosphate buffer, pH 7.2, containing physiologic saline.

TABLE VIII

*Influence of treatment with various drugs on the anaphylactic response of pertussis-vaccinated mice to challenge with soluble antigen-antibody complex**

Material Used for Treatment	Route Used for Treatment	Interval between Treatment and Challenge	Anaphylactic Deaths
BOL:	Intravenous	45 min	10/10
0.10 mg			
0.10 mg	Intravenous	90 min	9/10
Saline (controls)	Intravenous	90 min	13/14
Reserpine, 0.50 mg	Subcutaneous	18 hr	9/12
Saline (controls)	Subcutaneous	18 hr	7/8
Pyrilamine maleate:			
1.00 mg	Subcutaneous	20 min	9/10
1.50 mg	Subcutaneous	40 min	9/10
PBS (controls)	Subcutaneous	20 min	10/10
Dopamine, 0.67 mg	Intraperitoneal	1 min	9/10
Saline (controls)	Intraperitoneal	1 min	9/10
Epinephrine, 0.01 mg	Intraperitoneal	30 sec	7/31†
Saline (controls)	Intraperitoneal	30 sec	18/18

* The mice were challenged with a diluted preparation of the complex.

† This is the only value which differed significantly from the respective control value.

BOL = bromine analogue of lysergic acid diethylamide; PBS = phosphate buffer, pH 7.2 containing physiologic saline.

Since it has been reported that the mast cells of the mouse and rat contain serotonin as well as histamine (30) and that morphologic changes occur in the mast cells of certain animal species following the Ag-Ab reaction (31–33), experiments were conducted to determine whether morphologic changes occur in the mast cells of the mouse following challenge with soluble Ag-Ab complex.

In the first experiment, fatal anaphylaxis was produced in mice by challenge with the standard preparation of the complex. Immediately following death, the skin of the ears was stripped off, stained with Wright's stain and examined with the light microscope. Skin from the ears of normal control mice killed by severing the cervical spinal cord was also stained and examined in the same manner. Since the limited cell disruption and degranulation observed in these specimens was patchy in distribution and present in preparations from both the test and control groups, careful mast cell counts were not made. Apparently the mere handling of the specimens was responsible for the changes observed.

Mast cells in the subcutaneous tissues prepared by the air-pouch method of Carter and associates (34) were also examined using the May-Gruenwald stain. The test materials injected into the air pouch were soluble Ag-Ab complex, Ag alone and Ab alone. Since there was no difference in the disruption of mast cells in preparations of control and experimental animals no significance could be attached to the results.

Mast cell studies of the mesenteries of normal and pertussis-treated mice conducted according to the method of Riley (35) immediately following anaphylactic death likewise gave no indication that morphologic changes in mast cells accompany anaphylaxis.

In another series of experiments mice were challenged intravenously with soluble complex. Immediately after death the skin of their paws was removed, fixed, sectioned and stained with toluidine blue according to the method of Rowley and Benditt (36). Examination of these sections did not reveal any morphologic alteration in the mast cells.

Anaphylactic susceptibility of different strains of mice. Preliminary trials have shown that mice of the DBA/2 strain are highly refractory to anaphylaxis and exhibit only mild symptoms following injection of three times the standard dose of complex.

Whereas pertussis vaccination failed to increase the susceptibility of DBA/2 mice to ana-

phylaxis produced with the complex, vaccinated animals became highly susceptible to active anaphylaxis.

DISCUSSION

The responses of animals to injected mediators are commonly regarded to be highly meaningful with respect to the mechanisms concerned in anaphylaxis. However, it seems important to recognize that the injection of mediators may in no sense simulate the natural events leading to anaphylaxis. Indeed, the effectiveness of any biologic mediator(s) responsible for anaphylaxis may depend on its liberation in high concentration in tissue loci where it is free to act on target cells, and at the same time remain sheltered from exposure to destructive enzymes and antagonists such as those present in the circulating blood (37).

The similarity of action of histamine treatment and salicylate treatment in protecting against anaphylaxis but not against intravenous challenge with histamine or 5-HT does not necessarily mean that the mechanisms by which these agents act are similar. It is possible that the state of refractoriness observed may have been on a basis similar to that which underlies the refractoriness induced with Ag-Ab complex. All of these treatments could conceivably involve exhaustion of stores of some unknown mediator(s). It is also possible that the protective action of these treatments results from stimulation of the adrenal gland. The refractoriness to anaphylaxis induced in mice by either chronic or acute shuttlebox stress (38) may also be on a similar basis.

The results obtained with pertussis-vaccinated mice fail to provide reliable evidence on the mechanisms of anaphylaxis in the normal mouse because pertussis-vaccination alters the physiologic reactivity of mice in many ways other than by increasing their susceptibility to anaphylaxis and 5-HT. For example, such mice also show increased sensitivity to histamine, cold shock and other forms of stress (26) and display a marked decrease in histaminase activity of the lung (39). For these reasons the failure of the present attempts to protect pertussis-vaccinated mice against anaphylaxis by prechallenge treatment with either reserpine or BOL lacks meaning with respect to the mechanisms of anaphylaxis in the normal mouse.

Morphologic studies on the mast cells of the mouse, which are known to contain appreciable quantities of serotonin (30), gave no indication that these cells are significantly altered during anaphylactic shock produced with soluble Ag-Ab complex. Although this finding is contrary to the finding of Carter *et al.* (34) in their studies on "local anaphylaxis" of the actively sensitized mouse, it is to be noted that Fink and Rothlauf (40) were unable to detect any morphologic change in mast cells of the actively sensitized mouse uterus subjected to the Schultz-Dale reaction. Since the release of mediators from mast cells can take place without apparent morphologic change (41, 42) the present results do not provide certain evidence that 5-HT is not released by challenge with soluble Ag-Ab complex.

Since reserpine produces multiple effects in animals the observation that prechallenge treatment of normal mice with reserpine affords good protection against anaphylaxis does not by itself provide conclusive evidence that 5-HT plays an important role in anaphylaxis of this species. However, the observation that the only agent which restored the anaphylactic sensitivity of the reserpinized mouse was 5-HTP, supports the hypothesis that the resistance of the reserpinized mouse to anaphylaxis results from deficient body stores of 5-HT. The observation that the 5-HT antagonists, LSD, BOL and chlorpromazine protect the mouse against anaphylaxis likewise indicates that 5-HT is an important mediator of the reaction. The increased susceptibility to anaphylaxis induced in normal mice by prechallenge treatment with either 5-HT or 5-HTP is also compatible with the hypothesis that 5-HT serves as an important mediator of anaphylaxis of the mouse.

Although the evidence obtained in the present investigation appears to be compatible with the hypothesis that 5-HT serves as an important mediator of anaphylaxis of the mouse, it is obvious that further information will be necessary to prove this hypothesis.

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SUMMARY

The influence of various treatments on the anaphylactic response of the Swiss-Webster

mouse to challenge with a soluble antigen-antibody complex composed of bovine serum albumin-antibovine serum albumin was studied.

Protection against anaphylaxis was afforded by prechallenge treatment with either reserpine, epinephrine, norepinephrine, dopamine, histamine and sodium salicylate or with the "serotonin antagonists" lysergic acid diethylamide, its bromine analogue and chlorpromazine. The antihistamine agent, pyrilamine maleate, was without effect.

The protective effect of reserpine can be abolished with 5-hydroxytryptophan but not with 3,4-dihydroxyphenylalanine. Histamine or sodium salicylate did not affect the response to intravenous challenge with serotonin or histamine. Prechallenge treatment with 5-hydroxytryptamine or 5-hydroxytryptophan increased the susceptibility of the animals to anaphylaxis.

The highly sensitive pertussis-vaccinated mouse was protected against anaphylaxis by prechallenge treatment with epinephrine but not by prechallenge treatment with reserpine, the bromine analogue of lysergic acid diethylamide or dopamine.

The refractory state induced by prior sublethal doses of antigen-antibody complex was not reversed by the administration of mouse complement or 5-hydroxytryptophan or a combination of both substances.

Mice of the DBA/2 strain show only mild symptoms of anaphylaxis following doses of antigen-antibody complex three times those necessary to produce fatal anaphylaxis in a high percentage of Swiss-Webster mice.

Examination of various tissues following anaphylactic death produced with the complex gave no indication that morphologic changes in mast cells accompany anaphylaxis.

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