

Inhibiting effect of desensitization on release of histamine from guinea pig lung by specific antigen

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Guinea pigs passively sensitized by intrathoracic injection of rabbit antiserum were subsequently desensitized by parenteral introduction of sublethal doses of antigen, and the amount of histamine released from the perfused, chopped lungs by specific antigen 18 or 24 hours later was compared with that liberated in parallel tests from lungs of animals not desensitized. The average amount of histamine released from the lungs of 20 desensitized guinea pigs originally sensitized with 50 or 100 μ g antibody nitrogen was approximately 13 per cent of that liberated from the lungs of 20 similarly sensitized controls (0.019 μ g per 100 mg. lung tissue as opposed to 0.147 μ g). Twelve desensitized animals among 24 guinea pigs originally sensitized with 150 to 400 μ g antibody nitrogen released on the average 0.039 μ g per 100 mg. lung tissue as opposed to 0.280 μ g for the 12 controls, or about 14 per cent of the mean for the sensitized lungs. Relatively large amounts of antigen were required for maximum histamine release in this group. The total histamine content of the guinea pig lungs was not reduced by desensitization; on the contrary, both the sensitized and desensitized lungs contained nearly twice the average amount present in normal guinea pig lungs. Measurements of the probable reduction in available specific antibody in the desensitized lungs was not feasible without special techniques. It is thought that the classical specific desensitizing procedures employed may have caused a change in the sensitized lung tissue which subsequently suppressed normal activity of the histamine-releasing enzyme system. The findings gain interest from the fact that a similar reduction in histamine release from leukocytes of allergic human patients is known to follow successful hyposensitization.

The addition of specific antigen under controlled conditions in vitro to chopped lung tissue of adequately sensitized guinea pigs regularly results in the release of histamine and slow-reacting substance (SRS).^{1, 2} Important advances have been made in recent years toward understanding the mechanism of

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the histamine-releasing process in the sensitized animal.^{1, 2} Meanwhile, comparable studies of desensitized guinea pigs exhibiting the specific refractory (antianaphylactic) state, which so characteristically follows recovery of the sensitized animal from a subclinical or sublethal anaphylactic shock, have not been described. Preliminary to further investigation of the desensitized state, the experiments reported here were carried out. The well-established technique for demonstrating the *in vitro* antigenic release of histamine from guinea pig lung³ was extended to passively sensitized animals made refractory by specific desensitization. It was found that histamine release was significantly inhibited when antigen was incubated with the lung tissue of desensitized guinea pigs.

METHODS

Guinea pigs of 300 to 350 grams weight were sensitized passively by the intrathoracic injection of rabbit antiserum prepared against purified crystalline egg albumin (CEA) or crystalline bovine serum albumin (BSA). Commercial 5 times crystallized CEA was further refined by ammonium sulfate treatment and lyophilization before use as antigen, as suggested by Dr. C. D. Dukes. Minimal sensitization of the lung tissue of 42 guinea pigs was obtained by injection of 50 or 100 μ g of antibody nitrogen (AbN) of an anti-CEA serum. The lung tissue of a second group of 24 animals was sensitized more thoroughly by introducing into the thorax 150 to 400 μ g AbN, using anti-BSA serum.

Desensitization was accomplished by the slow injection of a sublethal amount of specific antigen. Usually a first dose (for example, 0.05 mg.) was given intraperitoneally, followed some 20 or 30 minutes later by a second injection (0.01 to 0.03 mg.) intracardially or intravenously. When the doses were correctly calculated, the animals recovered after reactions varying from slight to moderately severe. Desensitizing doses were given 30 or 48 hours after sensitization, and the lungs of these animals, and of control guinea pigs not desensitized, were tested for histamine release at 48 hours, or at 72 hours, respectively.

Techniques used for the rapid excision of the heart and lungs, for perfusion of the lungs with Tyrode's* solution,⁴ and for chopping, washing, and incubating the washed tissue with antigen were essentially those described by Austen and Brocklehurst,³ with minor modifications. A McIlwain tissue chopper⁵ was used. In the routine finally adopted, a minimum of twelve 100 mg. aliquots of the moist, perfused chopped lung tissue were weighed out from each lung, and each aliquot was suspended in 5 ml. Tyrode's solution in a 50 ml. Erlenmeyer flask. The tissue was washed by incubating the flasks in a water bath shaker at 37° C. for 45 minutes. After decanting the supernatant Tyrode's solution, the tissue was again washed briefly; then it was resuspended in fresh, warm Tyrode's solution (pH 7.8). Two of the suspensions were made in 3.0 ml. of Tyrode's solution for duplicate controls without antigen, eight were made in 2.7 ml. for duplicate tests with increasing concentrations of antigen (0.01 mg. per milliliter, 0.1 mg. per milliliter, 0.5 mg. per milliliter, and 1.0 mg. per milliliter),

*Tyrode's solution: NaCl 8.0 Gm., KCl 0.2 Gm., glucose 1.0 Gm., $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.1 Gm., CaCl_2 0.2 Gm., NaH_2PO_4 0.05 Gm., NaHCO_3 0.1 Gm. per liter, pH 7.8. For histamine assay with Schultz-Dale apparatus, atropine was added (0.1 mg. per liter).

and the remaining two were made in 10 ml. of Tyrode's solution for duplicate tests of the boiled tissue for total histamine content. Antigen (0.3 ml.) in the different concentrations was added to the appropriate, well-stirred suspensions, and the flasks were incubated at once in the water bath shaker for 15 minutes at 39 to 40° C. Supernatants were then decanted into stoppered test tubes, and refrigerated until assayed for histamine on the same day. Total histamine content of the duplicate 100 mg. tissue suspensions reserved for this purpose was assayed on the supernatants obtained after the suspensions were boiled for 5 minutes, and then allowed to stand at room temperature for one hour.

Assays for histamine were carried out with a sensitive Schultz-Dale apparatus using normal guinea pig ileum. Contractions were recorded electronically with aid of a Physiograph.* Individual tests of contractility were always separated by an interval of 3 minutes, and each pair of duplicate tests with unknown supernatants from the lung tissue was bracketed, before and after, by a test with a standard histamine dose (0.0072 μ g histamine base per milliliter of Tyrode's solution in the 5 ml. tissue bath). This amount of histamine caused a contraction giving a curve of convenient height (about 5 cm.) on our recorder with which to compare the response of unknown solutions (Fig. 1). The routine assay procedure permitted demonstration of the presence of as little as 0.001 to 0.002 μ g histamine base in 0.5 ml. amounts of the supernatants from lung tissue suspensions.

RESULTS

The desensitizing procedure used, which involved only the production of mild or moderately severe anaphylactic reactions to the specific antigen, was sufficient to produce a marked change in the washed lung tissue of the desensitized guinea pigs, so that incubation with antigen generally failed to release more than minimal amounts of histamine. The degree in which histamine release was inhibited in individual animals could not be correlated with the severity of the reaction provoked by the desensitizing injections. In 14 instances, parallel guinea pigs, sensitized and desensitized in the same manner as the animals whose lungs were tested, were shown to be relatively refractory to systemic anaphylactic shock.

No significant differences were found in histamine release from the lungs of sensitized or desensitized guinea pigs when the tests were performed at 48 or at 72 hours after sensitization (18 or 24 hours after desensitization). Hence, these time differences were disregarded in tabulating the results. The principal findings are summarized in Table I.

The average amount of histamine (0.147 μ g per 100 mg. lung tissue) released by antigen from the lungs of 20 guinea pigs, originally sensitized with 50 or 100 μ g AbN of anti-CEA serum and not desensitized (Group A1, Table I), was more than 7 times greater than the amount liberated from the lungs of the corresponding 20 desensitized guinea pigs (Group A2). Histamine release in the latter was consistently low, averaging 0.019 μ g per 100 mg. lung tissue, or approximately 13 per cent of the mean quantity liberated from the sensitized lungs.

*E and M Instrument Company, Inc., Houston, Texas.

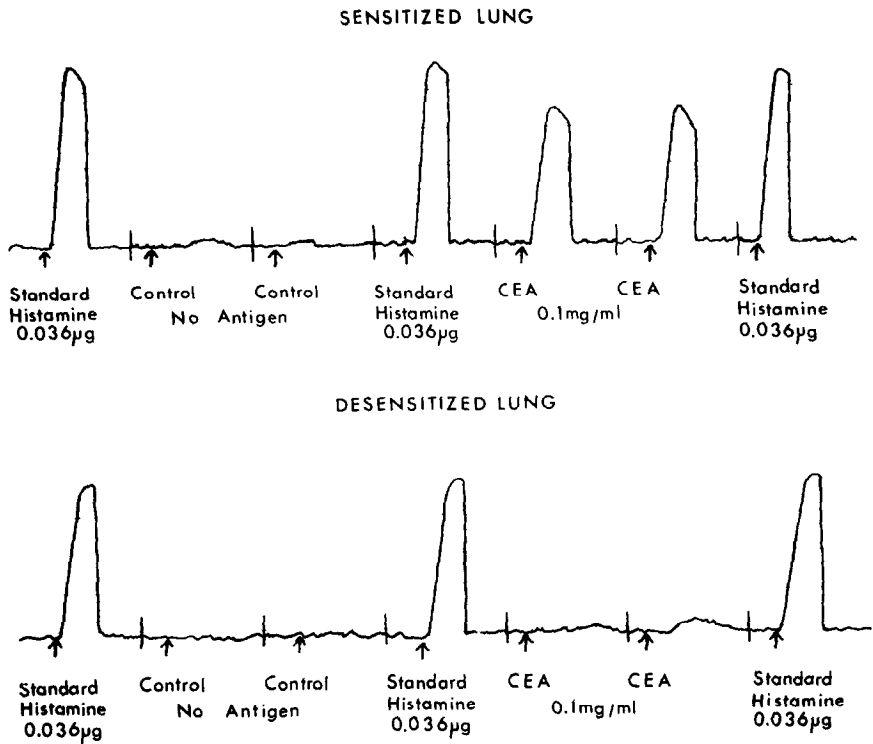


Fig. 1
Histamine release from guinea pig lung. Examples of reactions of normal guinea pig ileum in a Schultz-Dale apparatus to supernatants from perfused, washed, chopped lung tissue of passively sensitized and of desensitized guinea pigs. A 3 minute interval was allowed between separate challenges of the ileum.

Table 1. Histamine released by antigen from perfused, chopped guinea pig lung

Group	Passive sensiti- zing dose (µg AbN)	Guinea pigs		Histamine* released from 100 mg. lung tissue (µg)		% of total histamine released from 100 mg. lung tissue	
		No.	State	Mean ± S.E.	Range	Mean ± S.E.	Range
A1	50 or 100, anti-CEA	20	Sensitized	0.147 ± 0.008	0.102 - 0.260	3.7 ± 0.6†	1.7 - 6.7†
A2	50 or 100, anti-CEA	20	Desensitized	0.019 ± 0.003	0.000 - 0.046	0.3 ± 0.1†	0.0 - 1.1†
B1	150 - 400, anti-BSA	12	Sensitized	0.280 ± 0.053	0.080 - 0.765	8.9 ± 2.1	1.3 - 28.5
B2	150 - 400, anti-BSA	12	Desensitized	0.039 ± 0.010	0.007 - 0.120	1.0 ± 0.2	0.1 - 2.7

*In terms of histamine base.
†Eight animals only.

When the maximum amounts released by any concentration of antigen were averaged, the mean was 0.176 µg for the sensitized and 0.026 µg for the desensitized. On the basis of the percentage of the total histamine released, the lungs of the sensitized animals (Group A1) yielded, on the average, 12 times the amount obtained from Group A2.

In the case of the 24 guinea pigs whose lungs were more fully sensitized by 150 to 400 μ g AbN of anti-BSA serum (Groups B1 and B2), almost twice as much histamine was liberated, on the average, as was measured in the minimally sensitized A1, A2 groups. Nevertheless, the inhibiting effect of applying the same specific desensitizing procedures was equally definite. The average amount of histamine liberated from the 12 sensitized lungs of Group B1 (0.280 μ g per 100 mg. lung tissue) was again more than 7 times greater than the mean quantity liberated from the 12 corresponding desensitized lungs (Group B2), and the percentage of total histamine released was about 9 times greater. The average release of 0.039 μ g histamine in the desensitized lungs was about 14 per cent of the mean recorded with the sensitized tissue.

With the lung suspensions from the minimally sensitized guinea pigs (Groups A1, A2), the amounts of the specific antigen causing maximum histamine release followed no particular pattern. Maximum release occurred as often with 0.01 mg. antigen per milliliter of the suspension as with 1.0 mg. antigen per milliliter. With the more thoroughly sensitized lungs (Groups B1, B2), however, the concentration of antigen used had an apparently significant effect, especially with desensitized tissue. In Group B2 maximum histamine liberation was recorded three times with 0.1 mg. per milliliter antigen, three times with 0.5 mg. per milliliter, and six times with 1.0 mg. per milliliter. In several instances (Table II) no measurable histamine (or almost none) was released from the desensitized lungs by the lesser amounts of antigen, while the greater concentrations (0.5 or 1.0 mg. per milliliter) liberated relatively large amounts. Although, on the average, the inhibiting effect of desensitization in these more fully sensitized guinea pigs was as strong as in those minimally sensitized, the inhibition was at least partially overcome in several instances with the higher concentrations of antigen.

In agreement with observations of other investigators,⁶ the capacity of the lung tissue from different individual guinea pigs to release histamine on incubation with antigen showed marked variation. The six animals of Group B1, for example, all of which were sensitized with 400 μ g AbN of an anti-BSA serum

Table II. *Effect of concentration of antigen on in vitro histamine release (desensitized guinea pigs originally sensitized with 340-400 μ g AbN [anti-BSA Serum])*

Guinea pig No.	Histamine* released per 100 mg. lung tissue (μ g)				Average
	0.01 mg. antigen/ml. suspension	0.1 mg. antigen/ml. suspension	0.5 mg. antigen/ml. suspension	1.0 mg. antigen/ml. suspension	
207	0.012	0.012	0.015	0.018†	0.014
227	0.000	0.012	0.006	0.018†	0.009
201	0.063	0.033	0.051	0.066†	0.053
209	0.000	0.000	0.000	0.108†	0.027
210	0.000	0.000	0.000	0.168†	0.042
213	0.039	0.024	0.069	0.177†	0.077
211	0.022	0.018	0.069†	0.039	0.037
215	0.024	0.096	0.108†	0.000	0.057

*In terms of histamine base.

†Maximum histamine release.

intrathoracically, yielded averages ranging from 0.080 to 0.423 μg histamine per 100 mg. tissue. In the whole group of 12 guinea pigs of Group B1, three released especially large amounts of histamine, averaging 0.410, 0.423, and 0.765 μg , representing 10.1, 16.3, and 28.5 per cent of the available histamine in the respective lung suspensions. The highest average yield among the other nine lungs was 0.276 μg , and the highest average percentage of total histamine released was 8.3 per cent.

A similar, exceptionally high histamine-releasing capacity may explain the results obtained with two desensitized guinea pigs, originally sensitized with 50 and 100 μg AbN of anti-CEA serum. These results have been excluded from Table I. Although both of these animals had typical, moderately severe anaphylactic reactions following desensitizing injections, their lungs, tested 24 hours later, released averages of 0.133 and 0.216 μg histamine per 100 mg. tissue. These values were within the range recorded with other guinea pigs sensitized similarly, but not desensitized. It is impossible to say whether or not these figures represent a reduction in the amount of histamine that these lungs would release had there been no desensitization.

Values for total histamine in 100 mg. samples of chopped, washed lungs from 40 of the guinea pigs included in Table I, and from 16 normal animals never inoculated, are summarized in Table III. The average amount of histamine contained in the lungs from all of the 40 sensitized and desensitized guinea pigs was 4.5 μg per 100 mg. sample, whereas the comparable amount for the 16 normal lungs was 2.7 μg . The latter figure is virtually within the range of values (5 to 25 μg histamine per gram) quoted by Austen and Humphrey² as a characteristic of normal guinea pig lung. The much higher values obtained with the lungs of the experimental animals suggests that the sensitization procedure used may involve the local accumulation or mobilization of extra stores of bound histamine. Another point of special interest was the finding that desensitization was not accompanied by any reduction in available histamine in the lungs.

DISCUSSION

The present studies have demonstrated that passive sensitization of guinea pigs (at least when the antiserum is introduced intrathoracically) results in a

Table III. *Total histamine per 100 mg. guinea pig lung*

Group	Passive sensitizing dose (μg AbN)	Guinea pigs		Total histamine (μg) present in 100 mg. chopped lung	
		No.	State	Mean $\pm$ S.E.	Range
A1	50 or 100, anti-CEA	8	Sensitized	4.7 $\pm$ 0.6	2.6 - 7.4
A2	50 or 100, anti-CEA	8	Desensitized	4.6 $\pm$ 0.6	3.0 - 7.4
B1	150 to 400, anti-BSA	12	Sensitized	4.0 $\pm$ 0.6	1.4 - 8.2
B2	150 to 400, anti-BSA	12	Desensitized	4.7 $\pm$ 0.6	1.7 - 7.6
C	None	16	Normal	2.7 $\pm$ 0.3	0.9 - 5.1

material increase in the stores of bound histamine in the lungs 48 hours later. The importance of the rapid release of this pharmacologically active substance in the production of acute anaphylactic shock in the guinea pig has been well established.^{1, 2} The decreased capacity of the desensitized lung tissue to free histamine on incubation with antigen offers, therefore, a logical explanation, at least in part, for the relative refractoriness of the intact, desensitized animal.

This reduction in histamine yield from the desensitized lungs is a clear indication of interference with the chain of events which normally is set off by challenge of the tissue with antigen. Further experimentation will be necessary, however, before the mechanism of desensitization can be established. It may be assumed a priori that the desensitized (or hyposensitized) state may be the result of loss or reduction of some essential element required for the usual allergic response expected from the fully sensitized individual; or it may be due to the addition to the tissues of new, inhibitory elements; or to both of these *in vivo* changes occurring simultaneously. Broder and Schild⁷ list at least four apparent factors that may be involved in desensitization: (1) saturation of available antibody, (2) exhaustion of the enzyme system which is activated by the antigen-antibody reaction, (3) exhaustion of the stores of pharmacological materials and their precursors, or (4) a decreased responsiveness of the end organ(s) to the released mediator materials.

The probable reduction in available specific antibody is usually regarded as of prime importance. It should be noted, however, that the only antibodies involved in these present studies are those which are fixed to the perfused lung 48 hours after intrathoracic injection of antiserum, and remain there after thorough washing of the tissue. Quantitative measurement of these antibodies was not feasible without the use of special techniques beyond the scope of the present study. It may be significant that there were enough antibodies in the desensitized lungs of Group B2 guinea pigs (Table II) to permit release of considerable quantities of histamine when sufficient concentration of antigen was used. At the same time, the amount yielded by lesser amounts of antigen was materially reduced in comparison with that obtained from the lungs of fully sensitized animals. Additional experiments, especially designed to investigate the specificity of the desensitizing process and to quantitate the probable antibody loss, will be required to define the role of reduced specific antibody.

Points 3 and 4 do not apply in the present experiments; there is no lack of histamine in the desensitized lungs available for release by antigen, and the responsiveness of the "end organ" (lung tissue) to free histamine is not in question.

There remains the possibility that in the lungs of the desensitized guinea pigs we encountered a change which interfered with the functioning of the histamine-releasing enzyme system. The responsible enzyme in antigenic histamine release from guinea pig lung has been described as an esterase having a chymotrypsin-like substrate specificity.^{2, 8} It may have the characteristic of a phospholipase A.⁹ Enzymatic histamine release by antigen occurs *in vitro* only when conditions are precisely suitable,^{1, 2, 10} and it is readily inhibited by a variety of chemicals, as well as by lack of calcium, low temperature, and other circumstances which prevent normal activity of the essential enzyme system. Moreover, the reaction

is affected markedly by compounds normally present in mammalian tissue. Enhancement of histamine release, for example, is produced by certain dibasic aliphatic acids, such as succinic or maleic, whereas monobasic fatty acids are inhibitory.^{2, 3, 10} It seems a reasonable assumption that following desensitizing antigen injections, biochemical changes may occur in the lung itself, or in the body fluids, causing inhibition of the chain of events which, in the sensitized animal, leads to release of significant amounts of histamine. Perhaps inhibitory substances appear which could be demonstrated experimentally.

There is considerable evidence that the same basic mechanism governs the anaphylactic release of histamine from both the guinea pig and the human lung.^{10, 11} Closely similar, if not identical, mechanisms appear to operate also in release of histamine from the whole blood or sensitized leukocytes of allergic patients.^{10, 12} Recent observations on reduction in the quantity of histamine released from the leukocytes of ragweed-sensitive patients, by contact with the allergen after a course of hyposensitization,^{13, 14} offer a striking parallel to these reported here with desensitized guinea pig lungs. The probable presence of a humoral inhibitor in the plasma of patients after hyposensitization has been reported.^{14, 15}

Further studies on possible mechanisms of desensitization are in progress.

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