

Studies of desensitization and cross-desensitization to immunologic and nonimmunologic stimuli that evoke contraction and histamine release in superfused guinea pig trachea

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This study examined the possibility that there is cross-desensitization between immunologic and nonimmunologic stimuli that evoke contraction and histamine release (HR) in the isolated guinea pig trachea. Compound 48/80 and D-tubocurarine were found to cause homologous and heterologous desensitization for both contraction and HR from superfused trachea. Specific antigen challenge of trachea obtained from animals sensitized with either IgG1 (ovalbumin [OA]) or IgE (oxazolone-human serum albumin [OX-HSA]) also resulted in homologous desensitization for both contraction and HR. However, in experiments with animals sensitized with both IgG1 and IgE antibodies, prechallenge with OA resulted in cross-desensitization to OX-HSA, whereas the reverse sequence was ineffective in eliciting this phenomenon. This may be related to the type of desensitization produced by each antigen (specific versus nonspecific) or to heterogeneity of mast cells in the tissue. Prechallenge of the trachea with compound 48/80 or D-tubocurarine failed to alter subsequent effects of antigen after active sensitization with OA or passive sensitization with either IgG1 or IgE antibodies. Small but statistically significant decreases in tracheal responses to D-tubocurarine were observed after antigen prechallenge to activate both IgG1 and IgE antibodies. This is the first study to demonstrate a cross-desensitization between compound 48/80 and D-tubocurarine and the first to examine cross-desensitization with IgG1 and IgE antibodies in the guinea pig trachea. The overall conclusion is that there is no major overlap in the desensitization mechanisms between immunologic and nonimmunologic stimuli in the guinea pig trachea. (J ALLERGY CLIN IMMUNOL 1991;87:655-61.)

Antigen desensitization, a decrease in response to antigen resulting from prior antigen exposure, is a well-described, but poorly understood, phenomenon.¹⁻⁷ Desensitization has also been demonstrated

Abbreviations used

PCA: Passive cutaneous anaphylaxis
OA: Ovalbumin
OX: Oxazolone
HSA: Human serum albumin
HR: Histamine release

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Supported by the National Institutes of Health Grant HL33237.

Received for publication July 18, 1990.

Revised Sept. 27, 1990.

Accepted for publication Oct. 23, 1990.

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1/1/26321

for a variety of nonimmunologic (and noncytolytic) secretagogues, such as compound 48/80 and D-tubocurarine.⁸ Within each class of stimuli, cross-desensitization is possible, but cross-desensitization between immunologic and nonimmunologic stimuli does not usually occur.⁹⁻¹² This finding would be consistent with suggestions that sites responsible for desensitization are related to activation of relatively spe-

cific receptors and are located closer to initial stages of cell activation than at more distal and, presumed common, steps in the secretory pathways.¹³⁻¹⁵

However, Joiner et al.¹⁶ reported that prior exposure of the isolated guinea pig trachea to a large concentration of compound 48/80 resulted in a diminution of subsequent contractions evoked by specific antigen. Compound 48/80 and D-tubocurarine have been demonstrated to have a marked preference for evoking HR from trachea as opposed to peripheral lung tissue obtained from the guinea pig, and it is suggested that this selectivity might reflect a differential distribution of "curare-sensitive" mast cells.¹⁷ Therefore, it appears possible that the reported ability of compound 48/80 to decrease antigen-induced contractions in guinea pig trachea was a consequence of a unique tissue (or mast cell)-specific cross-desensitization. The studies described in this article were designed to examine further this possibility and to explore the potential for a differential degree of cross-desensitization when the challenging antigen was specific for either IgG1 or IgE antibody.¹⁸

METHODS

Animals

Female albino guinea pigs (Bio-Lab Corp., St. Paul, Minn., and Department of Veterinary Science, University of Wisconsin, Madison, Wis.), weighing 300 to 500 gm were used throughout the study. Animals were maintained in the Research Animal Resource Center of the University of Wisconsin. "Principles of Laboratory Animal Care," formulated by the National Society for Medical Research, was the guideline used for animals in this work, and the protocol was approved by the University of Wisconsin Animal Welfare Committee.

Immunologic techniques

Preparation of hapten-protein conjugates, immunization procedures for development of guinea pig IgG1 and IgE antibodies, antibody separation techniques with affinity-column chromatography, and quantitation of serum antibody titers by PCA have been comprehensively described in previous articles.^{18,20}

Sensitization of pulmonary tissue and mediator release and contraction

Guinea pigs were either passively or actively sensitized for specific antigen challenge. Passively sensitized animals received intravenous injections of antibody-rich serum 1 day before being killed for tracheal removal. Passive sensitization was achieved by administration of (1) 0.5 ml/kg of serum rich in IgG1 antibody directed against OA (anti-OA IgG1; PCA titer, 1:2560), and (2) 2.5 to 4.5 ml/kg of protein A-adsorbed serum devoid of all IgG antibodies

and containing IgE antibody directed against OX-*Ascaris* (anti-OX IgE; PCA titer, 1:160 to 1:640). Some animals were passively sensitized with 0.5 ml/kg of serum rich in IgG1 antibody directed against OX-guinea pig albumin (anti-OX IgG1; PCA titer, 1:1600) as previously described.²⁰ Anti-OX IgG1 and anti-OX IgE were not administered to the same animal. Doses of the sera for passive sensitization were selected on the basis of preliminary experiments in which the doses were found to produce optimum sensitization for guinea pig tracheal contractions with 10^{-4} mg/ml of specific antigen, OA, or OX-HSA. Animals used in experiments involving only compound 48/80 and D-tubocurarine as challenges were not actively or passively sensitized to the antigens used in this study.

After the guinea pigs were stunned by a sharp blow to the back of the head, the trachea were excised, trimmed of excess tissue, and cut in a spiral fashion. Tracheal spirals were suspended in air-filled, water-jacketed tissue chambers and superfused²¹ with a physiologic salt solution of the following composition (millimoles per liter): NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 0.5; NaH₂PO₄, 1; NaHCO₃, 25; and glucose, 11. The solution was gassed with a mixture of oxygen (95%) and carbon dioxide (5%) and pumped from a heated (37.5° C) reservoir through a tygon tubing to a water-jacketed coiled glass tube (heat exchanger) with a Gilson Minipuls II peristaltic pump (Gilson Medical Electronics, Middleton, Wis.). From the heat exchanger, the physiologic salt solution superfused the tissues at a rate of 2.1 ml/min. The tracheal spirals were maintained at initial tensions of 5 g for a 90- to 120-minute equilibration period before challenge. Changes in tension were recorded via force transducers (FT, 03) on a Grass model 7 polygraph (Grass Instrument Co., Quincy, Mass.) or a Gilson physiologic recorder.

Challenges were conducted by dissolving specific antigen or secretagogue in the superfusion solution. Usually, superfusate samples were collected continuously at the base of the superfusion chamber for 90-second periods beginning 90 seconds before (spontaneous release) and continuing to the end of each challenge. Tracheal tension was monitored throughout the experiment. Each challenge consisted of continuous superfusion with antigen or secretagogue for a total of 15 minutes (10 collection periods). Each tissue received two (or sometimes three) challenges with an intervening period between challenges of 15 minutes of superfusion with antigen- and secretagogue-free physiologic salt solution. This was adequate time for tension and superfusate histamine content to return to basal levels. At the end of the second (or third) challenge, trachea were superfused with carbachol, 10^{-4} mol/L, to elicit the maximum contractile response from each tissue. Responses to antigen or secretagogue were expressed as a percentage of the response elicited by carbachol.

At the end of each experiment, the trachea were weighed and placed in 3 ml of 0.4 N perchloric acid, and the tubes were placed in a boiling water bath for 15 minutes before homogenization. After centrifugation, the supernatant was

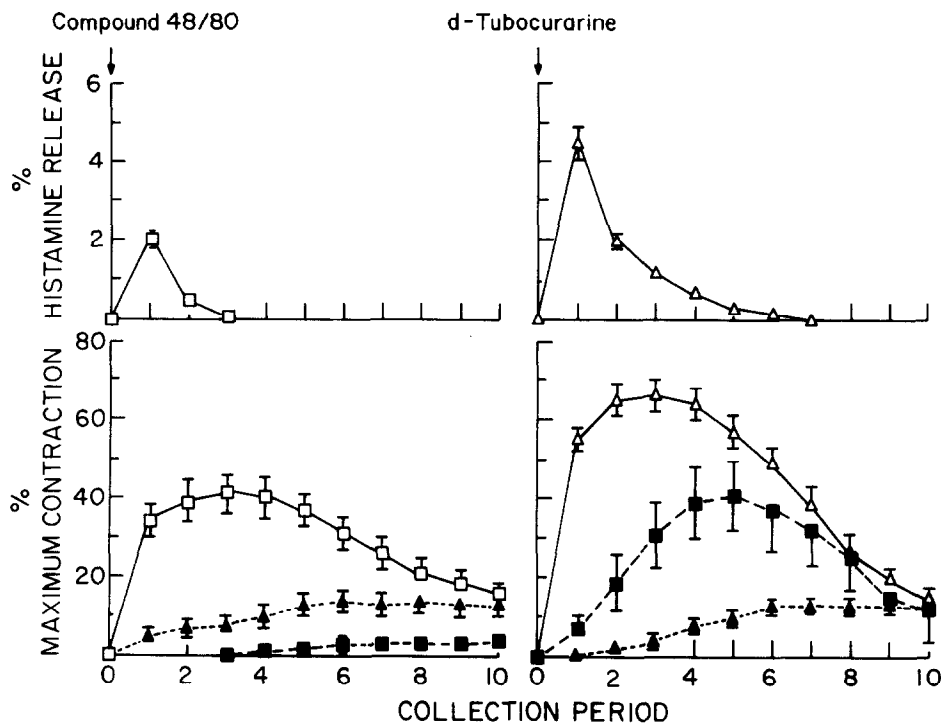


FIG. 1. Time course for contraction (**lower panels**) and HR (**upper panels**) evoked by compound 48/80 (**left panels**) or D-tubocurarine (**right panels**) in superfused guinea pig trachea without prechallenge (control: □, compound 48/80; △, D-tubocurarine) and after homologous or heterologous exposure to secretagogue (compound 48/80, 10^{-1} mg/ml, ■; D-tubocurarine, 3×10^{-3} mol/L, ▲). Results represented in the figure were obtained with the same concentrations of each secretagogue. Peak contractions (percent carbachol) determined independent of collection period were 43 ± 5 (□; $n = 14$), 5 ± 2 (■; $n = 8$; $p < 0.05$), and 15 ± 3 (▲; $n = 8$; $p < 0.05$) for compound 48/80 and 68 ± 4 (△; $n = 18$), 43 ± 9 (■; $n = 6$; $p < 0.05$), and 14 ± 2 (▲; $n = 10$; $p < 0.05$) for D-tubocurarine. Total HR values (percent of total) during challenge in control experiments were 2.6 ± 0.4 and 8.7 ± 0.9 for compound 48/80 and D-tubocurarine, respectively. Histamine was not found in any of the superfusate samples obtained with challenge after homologous or heterologous desensitization, and those zero values are not illustrated in the figure. Vertical lines represent SEM and are not illustrated when they lie within the limits of the symbol or overlap another. Where SEM did not overlap, there were statistically significant ($p < 0.05$) differences from control contraction values.

analyzed for residual tissue histamine content. The histamine content of each superfusate sample was analyzed, and the total superfusate and residual tissue contents were combined to determine total histamine content. The amount of histamine in each superfusate sample was expressed as a percentage of the total histamine content remaining after the preceding collection period. Histamine was not found in any of the spontaneous-release samples collected before beginning challenge.

Histamine assay

Histamine was measured by an automated continuous-flow extraction (with dialyzer) and fluorometric analyzer as described by Siraganian.²² At concentrations used in these studies, the antigens and secretagogues did not elicit non-

specific fluorescence or quenching of histamine standard solutions.

Analyses

Means and SEMs were calculated for values obtained in each experimental protocol. Differences between means were determined with analysis of variance and Student's *t* test for unpaired samples. A *p* value of <0.05 was considered statistically significant.

Drugs and solutions

The following substances were used: histamine free base, D-tubocurarine chloride, compound 48/80 (Sigma Chemical Co., St. Louis, Mo.), and carbamylcholine chloride (carbachol, Aldrich Chemical Co., Inc., Milwaukee, Wis.). All

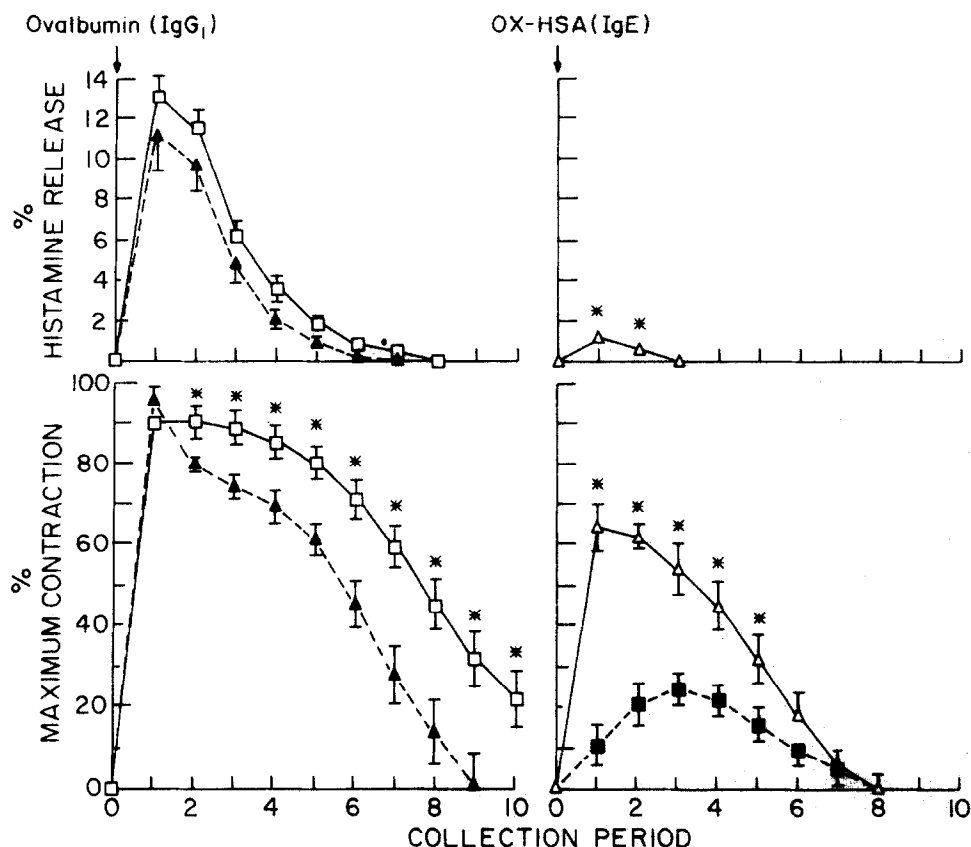


FIG. 2. Time course for contraction (lower panels) and HR (upper panels) evoked by OA (left panels) or OX-HSA (right panels) in superfused trachea taken from guinea pigs passively sensitized with both IgG1 (anti-OA) and IgE (anti-OX) antibodies. Experiments were conducted without prechallenge (control: □, OA; △, OX-HSA) and after heterologous antigen exposure (OX-HSA, 10^{-1} mg/ml, ▲; ovalbumin, 10^{-1} mg/ml, ■). Results represented in the figure were obtained with the same concentrations of each antigen. Peak contractions (percent carbachol) determined independent of collection period were 93 ± 3 (control, □; $n = 8$) and 95 ± 4 (▲, after OX-HSA prechallenge; $n = 8$; $p > 0.05$) for OA and 67 ± 6 (control, △; $n = 8$) and 28 ± 4 (■, after OA prechallenge; $n = 8$; $p < 0.05$) for OX-HSA. Total HR values (percent of total) were 32.2 ± 2.6 (control, □) and 25.6 ± 3.6 (▲, after OX-HSA prechallenge; $p > 0.05$) during challenge with OA and 1.7 ± 0.3 (△) during control challenge with OX-HSA. Histamine was not found in any of the superfusate samples obtained with OX-HSA challenge after prechallenge with OA, and those zero values are not illustrated in the figure. Vertical lines represent SEM and are not illustrated when they lie within the limits of the symbol or overlap another. An asterisk denotes a point at which there was a statistically significant ($p < 0.05$) difference between a value obtained with and without heterologous antigen prechallenge.

drug solutions were prepared on the day of each experiment. Drugs were dissolved in 0.9% sodium chloride and diluted or dissolved directly in the superfusion solution.

RESULTS

Homologous antigen or secretagogue desensitization

There was a statistically significant reduction in second challenge contraction and histamine release

that developed as a result of a single exposure of tracheal strips to either compound 48/80, 10^{-1} mg/ml, or D-tubocurarine, 3×10^{-3} mol/L (Fig. 1). In the case of these two secretagogues, a small contractile response was still evident, but no histamine could be detected in superfusate samples during the second consecutive homologous challenge.

One challenge of tracheal strips with 10^{-1} mg/ml of either OA ($n = 2$) or OX-HSA ($n = 2$) after sen-

TABLE I. Summary of effects of compound 48/80 and D-tubocurarine on responses of guinea pig trachea to immunologic challenges

Challenge (mg/ml)	Peak contraction* with SEM (n)			Total HR† with SEM		
	Control‡	After D-tubocurarine§	After compound 48/80	Control‡	After D-tubocurarine§	After compound 48/80
OA¶ (10 ⁻⁴)	34 ± 4(9)	38 ± 5(8)	41 ± 3(8)	2.4 ± 0.3	2.4 ± 1.0	5.3 ± 2.8
OA¶ (10 ⁻¹)	70 ± 3(8)	70 ± 4(4)	72 ± 3(4)	19.3 ± 2.8	29.2 ± 3.0	27.1 ± 2.0
OX-HSA(IgG1)# (10 ⁻¹)	77 ± 2(9)	68 ± 4(12)	ND	14.3 ± 2.1	12.0 ± 2.1	ND
OX-HSA(IgE)# (10 ⁻¹)	71 ± 4(10)	59 ± 7(8)	ND	4.2 ± 0.8	6.2 ± 1.2	ND

n, Number of experiments; ND, not determined.

*Peak contraction determined independent of collection period and expressed as a percentage of the contraction produced by carbachol, 10⁻³ mol/L, added at the end of each experiment.

†Total HR during the period of antigen challenge and superfusate collection expressed as a percentage of total histamine content.

‡Responses to antigen without secretagogue prechallenge.

§Tissues were prechallenged with D-tubocurarine, 3 × 10⁻³ mol/L.

||Tissues were prechallenged with compound 48/80, 10⁻¹ mg/ml.

¶Trachea were obtained from actively sensitized guinea pigs.

#Trachea were obtained from passively sensitized guinea pigs.

sitization of guinea pigs with both IgG1 (anti-OA) and IgE (anti-OX) was adequate to prevent contractile responses and histamine release evoked by a second challenge with the same concentration of each antigen (homologous desensitization, data not presented). Since these tissues responded in a normal manner to subsequent superfusion with carbachol, the absence of a contractile response to the second antigen challenge cannot be attributed to a nonspecific smooth muscle depression (see below).

Heterologous secretagogue desensitization

Cross-desensitization between compound 48/80 and D-tubocurarine was also evident (Fig. 1). Although small contractile responses remained to one substance after prechallenge with the other substance, histamine release was not observed during the second challenge with either substance.

Heterologous antigen desensitization

The effects of a single challenge with 10⁻¹ mg/ml of either OA or OX-HSA on subsequent tracheal responses to the other antigen after sensitization of the guinea pigs with both IgG1 (anti-OA) and IgE (anti-OX) are illustrated in Fig. 2.

Prechallenge with OX-HSA had minimal effect on subsequent responses to OA. There were no changes

in either the pattern of HR or total HR by OA. Even though the peak contraction in response to OA was similar to response obtained without antigen prechallenge, the duration of the contraction was slightly shorter after OX-HSA (Fig. 2).

In contrast, prechallenge of the trachea with OA resulted in a statistically significant ($p < 0.05$) decrease in tracheal contraction and abolition of HR in response to subsequent superfusion with OX-HSA (Fig. 2).

Secretagogue-antigen challenges

Data in Table I demonstrate that prechallenge with desensitizing concentrations of either compound 48/80 or D-tubocurarine did not alter contractile responses or HR produced by small and large concentrations of OA in trachea from actively sensitized guinea pigs. Furthermore, D-tubocurarine did not significantly alter contractions or HR evoked by OX-HSA after passive sensitization of the guinea pigs with either IgG1 (anti-OX) or IgE (anti-OX).

Antigen-secretagogue challenges

In trachea from guinea pigs passively sensitized with IgG1 (anti-OA) and IgE (anti-OX), sequential challenge with both antigens (OA and OX-HSA) resulted in small, but statistically significant, decreases

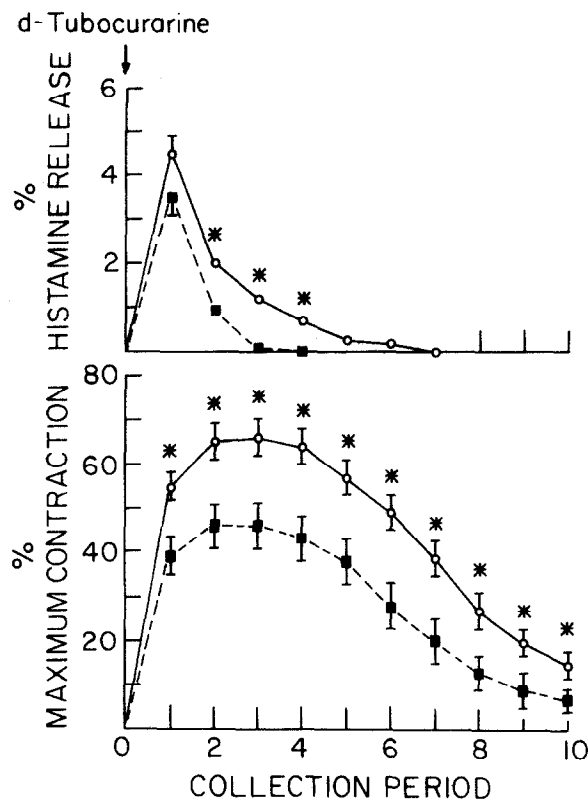


FIG. 3. Time course for contraction (**lower panel**) and HR (**upper panel**) evoked by D-tubocurarine, 3×10^{-3} mol/L, in superfused trachea taken from guinea pigs passively sensitized with both IgG1 (anti-OA) and IgE (anti-OX) antibodies. Experiments were conducted without prechallenge (control: ○, D-tubocurarine; $n = 18$) and after two consecutive challenges (antigens, ■; $n = 16$), one with OA, 10^{-1} mg/ml, and the other challenge with OX-HSA, 10^{-1} mg/ml, as described in METHODS. Half the tissues received OA and half received OX-HSA as the first prechallenge. In both sequences, the results were similar, and the values for antigen challenge were pooled. Peak contractions (percent carbachol) induced by D-tubocurarine determined independent of collection period were 68 ± 4 (control, ○) and 51 ± 4 (■, after OA and OX antigens prechallenge; $p < 0.05$). Total HR values (percent of total) were 8.7 ± 0.9 (control, ○) and 4.6 ± 0.6 (■, after OA and OX antigens prechallenge; $p < 0.05$) during superfusion with D-tubocurarine. Vertical lines represent SEM, and an asterisk denotes a point at which there was a statistically significant ($p < 0.05$) difference between a value obtained with and without antigen prechallenge.

in subsequent contractions and HR induced by D-tubocurarine (Fig. 3).

DISCUSSION

The results of this study do not support the postulate that the guinea pig trachea contains a population of mast cells having unique characteristics of desensitization or cross-desensitization. Rather, the desensi-

tization patterns observed in this study were similar, for the most part, to patterns found in earlier studies³⁻⁷ with isolated mast cells or basophils. Specifically, desensitization and cross-desensitization could be demonstrated for nonimmunologic stimuli (compound 48/80 and D-tubocurarine in this study), but this desensitization did not extend to immunologic activation, regardless of the class of sensitizing antibody activated. The small decrease in response to D-tubocurarine after establishment of immunologic desensitization for both IgG1 and IgE antibodies suggests that the process for antigen desensitization also does not extend to any major degree to involve a nonimmunologic stimulus.

Although support for the original hypothesis was not found, the results of this study provide the first demonstration of cross-desensitization between compound 48/80 and D-tubocurarine for provoking contraction and HR in the guinea pig trachea. This observation is consistent with suggestions that these secretagogues act at a similar site on mast cells²³ and strengthens the case for the existence, in guinea pig trachea, of a population of mast cells containing this site.¹⁷

Whether the absence of cross-desensitization between immunologic and nonimmunologic stimuli is related to release by each class from different populations of mast cells cannot be ascertained at the present time. Since activation of IgG1 resulted in desensitization to activation of IgE, it appears that these two antibodies can sensitize a similar population of tracheal mast cells. The inability of activation of IgE to provide cross-desensitization to activation of IgG1 raises the possibility that an additional mast cell population sensitized by IgG1, but not IgE antibody, exists. Alternatively, this difference in cross-desensitization could be related to the type of desensitization produced by each antigen. Clearly, the magnitude of HR evoked by maximum activation of IgG1 in this study was larger than that by maximum activation of IgE. This distinction is consistent and typical between the two types of antibody²⁰ and raises the possibility that the type of desensitization produced by antigen directed against IgG1 is "nonspecific" and that produced by antigen directed against IgE is "specific".^{4, 24} The occurrence of these two different types of desensitization could therefore account for the different degrees of cross-desensitization, depending on the sequence of antigen challenge. It is also possible that there is a similar differential effect of the non-immunologic stimuli used in this study. Additional experiments will be necessary to clarify these issues. However, since we have previously demonstrated that IgG1 and IgE sensitize different Fc receptors in guinea pig pulmonary tissues,¹⁸ it appears likely that acti-

vation of IgG1 in the present studies resulted in the "nonspecific" type of desensitization.

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