

A comparison of immediate-type respiratory reactions to immunologic and pharmacologic agents in rhesus monkeys

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Threshold cutaneous and respiratory reactivity to Ascaris antigen, histamine, compound 48-80, and bradykinin were compared in a group of 6 rhesus monkeys. Three of these monkeys had reproducible immediate-type respiratory reactivity of long duration following respiratory challenge with Ascaris antigen. The other 3 animals had cutaneous but no respiratory reactivity to Ascaris antigen. As a group, the animals with respiratory reactivity to Ascaris antigen did not demonstrate increased cutaneous or respiratory responsiveness to the pharmacologic agents. The patterns of respiratory response in animals reacting to aerosolized Ascaris antigen, aerosolized histamine, intravenous compound 48-80, and rabbit anti-IgE (reverse passive respiratory response) were similar in time of onset, time of peak abnormality, and time of recovery. All of these responses met the criteria established for an acute immediate-type airway reaction in rhesus monkeys. Although immediate-type cutaneous reactions to bradykinin and compound 48-80 were demonstrated, no response to these agents occurred after aerosolization under the conditions of the experiments.

Immediate-type cutaneous and respiratory reactivity in rhesus monkeys can be produced by challenge with pollen antigens after passive systemic sensitization with human reaginic sera.¹ Active cutaneous and respiratory responses occur following challenge of sufficiently sensitive monkeys with *Ascaris* antigen.² A third system is the acute respiratory response of rhesus monkeys to aerosol challenge with anti-IgE.³ The latter reaction was termed the reverse passive respiratory reaction and may be compared with the reverse passive cutaneous reaction with anti-IgE in the same animal. These passive, active, and reverse passive respiratory responses in rhesus monkeys are considered to be IgE mediated.¹⁻³ Respiratory mast cells obtained from these monkeys may be observed in short-term tissue culture preparations and morphologic changes observed after active immunologic challenge with antigen or reverse passive immunologic challenge with anti-IgE.⁴

The objectives of the current study were to determine whether or not three pharmacologic agents—histamine, compound 48-80, and bradykinin—would

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result in acute airway responses in rhesus monkeys similar to the IgE-mediated reactions. Second, we wished to determine if such pharmacologic responses would be similar in time of onset and duration to the immunologic responses. Third, an attempt was made to determine if there was a correlation between a threshold cutaneous dose and a threshold respiratory dose of antigen or the reactive pharmacologic agent in the same animal. Finally, the group of animals used as test subjects is of additional interest because it included 3 monkeys which had been studied for over 2 years. These 3 animals had persistent cutaneous and respiratory reactivity to *Ascaris* antigen³ associated with respiratory eosinophilia⁵ in the apparent absence of parasitic infestation. Three other monkeys in the group had no respiratory reactivity to *Ascaris* antigen. The fourth objective of these studies was to determine whether or not there was a difference in cutaneous or respiratory reactivity of these two groups of animals to challenge with histamine, compound 48-80, or bradykinin.

METHODS AND MATERIALS

Animals

Young adult female rhesus monkeys were used in this study. They were in apparent good health at the onset of the experiments. The reactivity of these animals has been described in detail elsewhere,^{2, 3} and certain characteristics which were established in previous studies are summarized in Table I. All of these animals were selected initially on the basis of the degree of their cutaneous reactivity to *Ascaris* antigen.

Cutaneous testing

Animals were anesthetized with intravenous sodium pentobarbital. One milliliter of 0.5 per cent Evans blue dye was injected intravenously. Skin tests were done by intracutaneous injections of 0.1 ml. of the test solution as previously described.⁶ Cutaneous end-point titrations were done in duplicate with the use of serial tenfold dilutions of reagents and were reproducible within that range.

Respiratory testing

Animals were anesthetized by intravenous injection of sodium pentobarbital. A cuffed endotracheal tube was inserted. A control period of respiratory function was recorded before and after aerosol administration of 0.15M NaCl. Aerosol challenge with immunologic or pharmacologic agents was done by nebulization through the endotracheal tube with a Bird Mark 7 positive pressure respirator with an in line nebulizer. The same positive pressure and controlled respirations were used in all experiments by maintaining constant dial settings for inspiratory pressure and inspiratory flow. The respiratory rate was controlled at approximately 20 breaths per minute. No respiratory challenges were done at intervals of less than one week.

Lung function recordings

These were obtained before and after aerosol delivery of 0.15M NaCl and at periodic intervals after aerosol challenge with immunologic or pharmacologic agents. The lung function recordings were made with a pneumotachograph screen cone (25 L. per minute flow range, Sanborn, Waltham, Mass.) and by recording with a direct-writing oscillograph (Sanborn). Changes in respiratory frequency (f), peak expiratory flow rate (PEFR), and the ratio between the duration of expiration and inspiration (E/I) were determined by comparison of the post-saline aerosol control tracings with postchallenge tracings. The results were expressed as the per cent of change which occurred following aerosol challenge with each agent. Criteria for a positive respiratory response are shown in Table II.

TABLE I

	1	2
Response to challenge with aerosolized <i>Ascaris</i> antigen*	Persistently positive for 31 months	Persistently positive for 32 months, subsequently negative
Respiratory eosinophilia after aerosol challenge with <i>Ascaris</i> antigen†	Positive	Positive
Response to challenge with aerosolized anti-IgE*	Positive	Positive

*See Reference 3.

†See Reference 5.

TABLE II. Criteria for determination of positive immediate-type respiratory reactions to aerosol challenge with *Ascaris* antigen in rhesus monkeys

Variables	Criteria
Onset of response	Within 5 min. of antigen challenge
Duration of response	At least 20 min.
Required changes in respiratory function at first measurement	Increase of at least 100% in frequency Decrease of at least 25% in PEFr Increase of at least 20% in ratio of expiratory time/inspiratory time

Immunologic and pharmacologic cutaneous and aerosol test materials

Ascaris antigen. An extract of *Ascaris suum* purified by Sephadex G-50 and Sephadex G-75 and standardized as previously described⁶ was used. The standard *Ascaris* antigen (SAA) contained 0.66 mg. of protein per milliliter.

Anti-IgE. Antihuman IgE was prepared by immunization of rabbits with a purified preparation of an IgE myeloma protein. The anti-IgE used was the same pool as that previously described.³ Evidence for cross-reactivity of this rabbit antihuman IgE with monkey IgE is its activity in the production of reverse passive cutaneous anaphylaxis in high dilution in monkey skin,³ reverse passive respiratory reactions in monkey respiratory tracts,³ and degranulation of monkey respiratory mast cells.⁷

Compound 48-80. This was obtained from Burroughs-Wellcome & Co., Inc., Tuckahoe, New York, and dissolved in sterile, 0.15M NaCl, pH 7.35. Dilutions were made in the same solution.

Histamine. Histamine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.) was prepared fresh for each experiment. It was dissolved in sterile, 0.15M NaCl, pH 7.35, and dilutions were made in the same diluent.

Bradykinin. Bradykinin triacetate was obtained from Sigma Chemical Co., St. Louis, Mo. The bradykinin was stored at -20° C. until just prior to use and was then dissolved in 0.15M NaCl, pH 7.35, and further diluted for cutaneous and aerosol testing.

RESULTS

Threshold respiratory and cutaneous response to challenge with *Ascaris* antigen

The characteristics of the 6 monkeys used in these studies are listed in Table I. These animals were skin tested to serial tenfold dilutions of *Ascaris* antigen and increasing concentrations of the same material by aerosol challenge. The latter was done at 20 minute intervals until a definite respiratory response was

Animal No.			
3	4	5	6
Persistently positive for 33 months	Positive on initial challenge, subsequently persistently negative for 7 months	Persistently negative for 9 months	Persistently negative for 24 months
Positive	Negative	Negative	Negative
Positive	Positive	Positive	Negative

TABLE III. Comparison of cutaneous skin titration and threshold respiratory response of Ascaris-sensitive monkeys to standard Ascaris antigen (SAA)

Reactivity	Animal No.					
	1	2*	3	4	5	6
Cutaneous†	10 ⁻⁶	10 ⁻⁴	10 ⁻⁵	10 ⁻³	10 ⁻²	10 ⁻⁴
Respiratory‡	10 ⁻¹	10 ⁻¹	10 ⁻¹	Neg.§	Neg.	Neg.

*Respiratory reactivity no longer present. Response determined prior to loss of reactivity. Cutaneous reactivity remained unchanged.

†Recorded as the highest dilution of SAA giving positive skin reaction.

‡Recorded as the highest serial tenfold dilution of SAA giving positive respiratory reaction.

§Undiluted SAA used as highest concentration tested.

obtained as determined by criteria previously established (Table II). Results of these studies are shown in Table III. The threshold respiratory reactivity of Monkey 2 was determined during the long persistent respiratory responsiveness which subsequently disappeared (Table I). All 6 animals had marked cutaneous reactivity since they were selected on the basis of their skin reactivity.

The 3 animals with respiratory reactivity appeared to have a somewhat greater degree of cutaneous reactivity, but no consistent difference in the degree of cutaneous reactivity of animals with positive respiratory reactivity to SAA and those without respiratory reactivity was demonstrated. The minimal concentration of SAA required to produce a definitive respiratory response in the respiratory reactors was 1,000 to 100,000 times the threshold cutaneous dose. The characteristic pattern of a respiratory response to SAA is shown in Fig. 1, A. This is the response of Animal 1, Table III.

Threshold respiratory and cutaneous response to histamine

The results of cutaneous and aerosol challenge of rhesus monkeys to histamine are shown in Table IV. A relative consistency of response to cutaneous testing with histamine is shown with 3 animals reacting to a dilution of 10⁻⁴ and 3 reacting to 10⁻³. The animals with respiratory responsiveness to SAA (Table I) were the most reactive to cutaneous testing. In contrast, there was no significant correlation between the respiratory response of these animals to histamine and the respiratory response to SAA. There were 2 animals in each group not react-

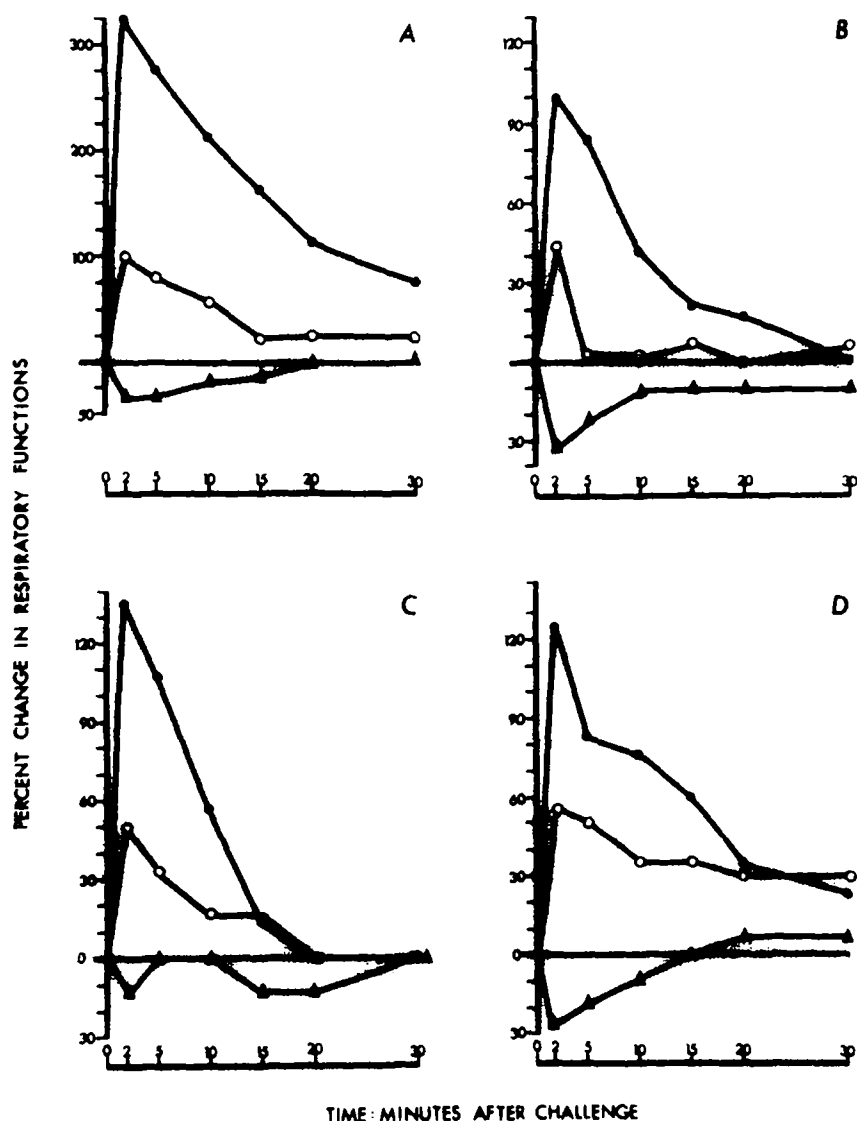


FIG. 1. Respiratory responses of rhesus monkeys to pharmacologic or immunologic challenge. Breathing frequency, ●; peak expiratory flow rate, ▲; ratio of expiratory to inspiratory time, ○. **A**, Response to aerosolized *Ascaris* antigen (0.066 mg. per milliliter); **B**, response to aerosolized histamine (2.5 μ g per milliliter); **C**, response to intravenous compound 48-80 (1,000 μ g); **D**, response to aerosolized anti-IgE (1:2 dilution).

ing to the highest concentration of histamine tested. The animal reacting to the lowest concentration of histamine (Table IV) was not reactive to aerosol challenge with SAA at the highest concentration of SAA used (Table III). The aerosol challenges were done with concentrations of 1, 2.5, 5, and 10 μ g per milliliter instead of serial tenfold dilutions, because preliminary experiments had shown that responsiveness of different animals could be detected with the use of these concentrations. The results of the studies with histamine appeared

TABLE IV. Threshold cutaneous and respiratory response of monkeys to histamine

Reactivity	Animal No.					
	1	2	3	4	5	6
Cutaneous*	10 ⁻⁴	10 ⁻⁴	10 ⁻⁴	10 ⁻³	10 ⁻³	10 ⁻³
Respiratory†	2.5	Neg.	5	1.0	2.5	Neg.

*Highest serial tenfold dilution of 10 μ g histamine per milliliter producing a positive cutaneous reaction.

†Lowest concentration of histamine expressed as micrograms per milliliter producing a positive respiratory response; highest concentration used was 10 μ g per milliliter.

TABLE V. Cutaneous and respiratory response to compound 48-80 after cutaneous, aerosol, and intravenous administration

Reactivity	Animal No.					
	1	2	3	4	5	6
Cutaneous*	1.0	0.1	0.1	0.1	1.0	0.1
Respiratory†	Negative	Negative	Negative	Negative	Negative	Negative
Respiratory‡	ND§	Negative	ND	Positive	Positive	Negative

*Expressed as the lowest concentration of compound 48-80 (micrograms per milliliter) producing a positive cutaneous reaction.

†Response after aerosol challenge with a solution of 10,000 μ g per milliliter.

‡Response after intravenous injection of 1,000 μ g.

§ND = not done; see text.

to demonstrate no significant difference in response to histamine challenge in those animals with a respiratory response of long duration to SAA. The animals which responded to aerosolized histamine required a concentration 1,000 to 10,000 more than the minimal reactive cutaneous dose. The respiratory response of Animal 5 (Table IV) to histamine is shown in Fig. 1, B.

Cutaneous and respiratory responses to cutaneous, aerosol, and intravenous administration of compound 48-80

The cutaneous reactivity of the 6 monkeys to intracutaneous testing with compound 48-80 is shown in Table V. All animals reacted either to 1.0 or 0.1 μ g of compound 48-80 per milliliter. There was no correlation between reactivity of animals to compound 48-80 (Table V) and reactivity of animals to SAA (Tables I and III). The animals were challenged by aerosol with compound 48-80 and none reacted, even with the highest concentration (10 mg. per milliliter) used. This concentration was 10,000 or 100,000 times the minimal concentration resulting in a positive cutaneous reaction. Because of the negative respiratory responses to the highest concentration of compound 48-80 used by aerosol, the respiratory response to increasing intravenous doses of compound 48-80 was evaluated. Two monkeys had respiratory responses after the intravenous injection of 1,000 μ g of compound 48-80 (Table V). The respiratory response of Animal 5 to intravenous injection of compound 48-80 is shown in Fig. 1, C. The response of Animal 4 was similar in pattern. Although both Animal 4 and Animal 5 appeared to recover from the acute response to com-

TABLE VI. Cutaneous and respiratory responses to cutaneous and aerosol challenge with bradykinin

Reactivity	Animal No.				
	1	2	3	5	6
Cutaneous*	10 ⁻²	10 ⁻⁵	10 ⁻²	10 ⁻²	10 ⁻⁴
Respiratory†	Neg.	Neg.	Neg.	Neg.	Neg.

*Expressed as the highest serial tenfold dilution of 1 mg. of bradykinin per milliliter producing a positive test.

†Using 1 mg. per milliliter as highest aerosolized concentration.

pound 48-80 by clinical observation and by the improvement in pulmonary function as shown in Fig. 1. Animal 4 died within 24 hours. Because of the possible relation of this death to the administration of intravenous compound 48-80 and because of the value of Animals 1 and 3 as persistent respiratory reactors to aerosol challenge with SAA, this agent was not administered intravenously to those animals.

Cutaneous and respiratory responses to cutaneous and aerosol challenge with bradykinin

Marked variability to cutaneous testing with bradykinin was observed in the 5 monkeys remaining for this study (Table VI). No correlation between cutaneous responsiveness to bradykinin and any other cutaneous reactivity was observed. Aerosol challenge of the animals with a concentration of 1 mg. per milliliter of bradykinin did not result in a respiratory response in any animal, although this was 100 to 100,000 times the minimal dose necessary to produce a positive cutaneous reaction.

Respiratory response to anti-IgE

Animal 1 was challenged by aerosolized anti-IgE with a 1:2 dilution. This concentration was known from previous studies to produce a positive respiratory response. The resulting respiratory reaction is shown in Fig. 1, *D*. The similarity of respiratory responses in time of onset and duration to aerosolized *Ascaris* antigen, histamine, and anti-IgE and to intravenous compound 48-80 are shown in Fig. 1.

DISCUSSION

It was considered possible that the 3 monkeys with long duration of marked respiratory reactivity² and respiratory eosinophilia⁵ following aerosol challenge to *Ascaris* antigen might constitute a group of animals more responsive to pharmacologic agents demonstrable by immediate-type cutaneous or respiratory reactions. This was not demonstrated, since no definite pattern of increased responsiveness to histamine, compound 48-80, or bradykinin was found in the 3 animals with a history of immediate-type respiratory reactivity to SAA. The different degree of respiratory reactivity to *Ascaris* in the two groups of monkeys is presumed to be the result of different degrees of sensitivity.

The patterns of respiratory response in time of onset and duration appeared similar (Fig. 1) whether these were induced by *Ascaris* antigen, anti-IgE, histamine, or intravenous compound 48-80. Thus, an acute respiratory reaction of similar onset, severity, and duration can be produced in primates with an antigen, rabbit anti-IgE, histamine, and a mast cell degranulating agent—compound 48-80.

Other studies of compound 48-80 with the use of live respiratory mast cells from these animals have demonstrated degranulation of these mast cells *in vitro*.⁴ The number of cells degranulating increased as the concentrations of compound 48-80 increased from 5 to 50 μg per milliliter, and at the latter concentration over 90 per cent of respiratory mast cells degranulated.⁷ In the current studies, it was expected that aerosolized compound 48-80 would result in an acute respiratory reaction, yet this did not occur even at a concentration of 10,000 μg per milliliter. Whether the absence of a respiratory response to aerosolized compound 48-80 is due to inability of the compound to reach reactive cells because of poor absorption through respiratory mucosa or due to rapid local metabolism of the agent is unknown. That compound 48-80 can produce an acute respiratory reaction in monkeys was demonstrated by the responses in 2 of the 4 monkeys tested by intravenous challenge. Using intracardiac injection of compound 48-80, Colebatch and associates⁸ showed that acute respiratory responses, including increased resistance, occurred in cats. Their experiments used doses of 0.25 to 3 mg. of compound 48-80 per kilogram body weight, which is higher than those used in the current study. The monkeys weighed approximately 4 kilograms, and the intravenous dose of 1,000 μg would result in a concentration of compound 48-80 in the plasma space similar to the concentration which results in degranulation and release of histamine from rat peritoneal mast cells⁹ and the degranulation of primate respiratory mast cells.⁷ A significantly higher concentration of compound 48-80 appeared necessary to release the majority of histamine from human peripheral leukocytes.¹⁰

Marked variability in cutaneous end-point titrations with bradykinin was observed in different monkeys. No respiratory responses to aerosolized concentrations of bradykinin 100 to 100,000 times those giving the minimal cutaneous reaction were observed. Bradykinin contracts guinea pig trachea *in vitro*.¹¹ Although it has been reported to reduce the vital capacity of human patients with asthma,¹² it does not result in contraction of the isolated human bronchus.¹³

The criteria for a positive immediate-type respiratory response in rhesus monkeys are the result of multiple experiments.¹⁻³ Less marked respiratory responses occur as manifestations of immediate-type reactions in these animals, and it is likely that minor acute changes in the airways occur which are not apparent by the recording measurements used. The criteria for positive responses (Table II) shown in Fig. 1 have been adopted because they indicate unequivocally positive reactions which may be applied to a variety of test systems. For certain applications of the monkey respiratory response system, less marked respiratory reactions may be adequate.

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