

Anaphylactic bronchoconstriction in immunized guinea pigs provoked by inhalation and intravenous administration of hexahydrophthalic anhydride and methyltetrahydrophthalic anhydride

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We established a guinea-pig model of anaphylactic bronchoconstriction provoked in immunized animals by inhalation and intravenous administration of 4,4-methyltetrahydrophthalic anhydride (MTHPA) and hexahydrophthalic anhydride (HHPA). Guinea pigs were immunized intradermally with either MTHPA ($n=8$) or HHPA ($n=8$) suspended in olive oil. Control animals ($n=8$) were injected with olive oil alone. After 4 weeks, the animals were challenged during mechanical ventilation by inhalation or intravenous administration of MTHPA or HHPA conjugated with guinea-pig serum albumin (GPSA). Airway flow, and airway and esophageal pressures were measured. Resistance (R) and static compliance (C_{st}) of the respiratory system (rs), lung (l), and chest wall were studied with the flow-interruption technique. After challenge with MTHPA-GPSA or HHPA-GPSA, R , rs and R , l increased dramatically while C_{st} , rs and C_{st} , l decreased, and severe arterial hypoxia developed. The reaction occurred at a well-defined dose of anhydride and lasted about 30 min. When the same dose was repeated after 30 min, the response was much attenuated. MTHPA and HHPA can induce asthma in guinea pigs. The dose-response curve at antigen challenge is steep. Once a threshold dose is reached, a severe reaction occurs. The reactivity is then exhausted. This model may be suitable for assessing occupational asthma caused by acid anhydrides and possibly by other low-molecular-weight chemicals.

Key words: asthma; gas exchange; guinea pigs; hexahydrophthalic anhydride; lung mechanics; methyltetrahydrophthalic anhydride.

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Exposure to organic acid anhydrides has been reported to cause a wide range of symptoms including symptoms from the eyes; rhinitis; and asthma of immediate, late, or dual type. Various pathophysiologic mechanisms may be involved in the asthmatic reaction: reflex bronchoconstriction, inflammatory bronchoconstriction, hypersensitivity bronchoconstriction, and "pharmacologic bronchoconstriction" (1–3).

Organic acid anhydrides, like other reactive low-molecular-weight chemicals, react with macromolecules in the body (haptenization) and induce

an antibody response. Thus, the induction of IgE antibodies may have a possible role in the induction of organic acid-anhydride-associated immediate asthma. Specific IgE antibodies to acid-anhydride conjugates with serum albumin have been found in workers exposed to several acid anhydrides (1). A remarkably high prevalence has been found in workers exposed to methyltetrahydrophthalic anhydride (MTHPA) (4) and hexahydrophthalic anhydride (HHPA) (5, 6). The specific IgE to MTHPA and HHPA have been associated with respiratory tract disease (3, 7, 8).

Guinea-pig models have been frequently employed in the investigation of occupational asthma, including organic acid-anhydride-related asthma. These models may be important tools in the investigation of the pathophysiologic mechanism of occupational asthma and dose-response relationships (9). However, many different methods have been used in guinea-pig models of occupational asthma in regard to sensitization regimen, mode of challenge, and protocol for the monitoring of airway reactivity. In studies on reactive organic chemicals such as acid anhydrides and isocyanates, challenges have been performed by intravenous injection (10), inhalation of hapten (11, 12), inhalation of aerosol (13, 14), or intratracheal instillation (15, 16). Airway reactivity has been monitored by the registration of respiratory rate and tidal volume (11, 13, 14), pulmonary inflation pressure (17), lung resistance (16, 18, 19), or dynamic compliance (18). Studies have been performed on trimellitic anhydride (TMA) and, to some extent, phthalic anhydride (PA). To our knowledge, MTHPA and HHPA have not been studied in experimental models.

A precise method for the study of mechanics of the respiratory system in man has been developed by our group (20). A new method for the delivery of aerosols makes possible a more controlled deposition in the periphery of the lung even in small animals (21, 22). The aim of the present study was to use these techniques to establish a sensitive and reproducible guinea-pig model of anaphylactic bronchoconstriction induced by MTHPA and HHPA. Methods for studies of respiratory mechanics under the influence of well-defined doses of inhaled and intravenously injected MTHPA-GPSA or HHPA-GPSA were developed. The response to challenge with these antigens was characterized with respect to dose response and to a repeated dose equal to that which induced an initial and severe reaction.

Material and methods

Animals and immunization

Male, inbred, Hartley strain guinea pigs (GP) weighing 300–450 g (Sahlins AB, Malmö, Sweden), were maintained in the laboratory 1 week before immunization. We intradermally injected 5% MTHPA or HHPA (Merck, Darmstadt, Germany) in olive oil at two sites (0.05 ml/site). Control animals received pure olive oil. Groups of animals with a narrow range of antibody titers were chosen for the provocation studies. The animals weighed 500–700 g when they were challenged by antigens 4 weeks after immunization.

Conjugates

MTHPA and HHPA were conjugated with guinea-pig serum albumin, GPSA, according to a method described by Welinder & Nielsen (23). Briefly, 462 mg of MTHPA or 154 mg of HHPA was added to a cooled solution of 300 mg GPSA in 100 ml of 0.1 molar NaHCO_3 . The reaction mixture was stirred overnight and filtered. The conjugate was purified from low-molecular-weight compounds (< 30 kDa) by ultrafiltration and stored at -20°C after lyophilization. The MTHPA/GPSA or HHPA/GPSA molar ratio was found to be 24 mol of anhydride per mol of GPSA (24).

Antibody determinations

Specific IgG antibodies were analyzed by ELISA according to Welinder et al. (25). The procedure was as follows. 1) Wells of polystyrene microtiter plates (Nunc-Immuno Plate, Nunc, Kamstrup, Denmark) were coated by adding a 100- μl solution of the anhydride-GPSA conjugate, and 2) post-coated with 4% bovine serum albumin (BSA) to block uncoated protein-binding sites. 3) GP serum (1:50) was added to the wells and incubated for 1 h at room temperature. 4) A volume of 100 μl of alkaline phosphatase-conjugated goat anti-GP IgG antisera (Fc fragment, Jackson Immunoresearch Laboratories, West Grove, PA, USA) was then added and incubated for 1 h at room temperature. Washing between the different steps was performed with a Titertek Microplate Washer (Flow Laboratories, Rickmansworth, UK). 5) A volume of 100 μl (1 mg/ml) substrate solution (*p*-nitrophenyl phosphate [Sigma Chemical Company] in diethanolamine buffer 1 mg/ml) was added. The wells were read after 120 min at room temperature by a filter photometer (Titertek Multiskan, Eflab Oy, Helsinki, Finland) at 405 nm. All samples were analyzed in triplicate and read against a GPSA antigen, as a control for nonspecific binding. The results were expressed as the net absorbance (*A*), corrected for the absorbance of positive controls on each microtiter plate.

Protocol

Initially, 15 experiments served to define the intravenous dose that could cause a reaction in sensitized animals. The final study comprised 24 animals divided into six groups as shown in Table 1.

Animal preparation

Anesthesia was induced with a 1.0 ml/kg body weight intramuscular injection of ketamine (30 mg/ml) and

Table 1. Groups of animals in final study. Optical density (OD, median and range) of specific IgG to conjugates of methyltetrahydrophthalic anhydride (MTHPA) or hexahydrophthalic anhydride (HHPA) and guinea-pig serum albumin (GPSA) analyzed by ELISA (serum dilution 1:50) in immunized guinea pigs and controls. Sera were collected 4 weeks after immunization

	Controls		Immunized guinea pigs	
	(n=4)	(n=4)	(n=4+4)	(n=4+4)
Immunization with	Olive oil	Olive oil	MTHPA in olive oil	HHPA in olive oil
Challenged by	MTHPA-GPSA	HHPA-GPSA	MTHPA-GPSA	HHPA-GPSA
Mode of challenge				
Inhalation	0.02 (0.01–0.03)	0.01 (0.01–0.02)	1.07 (1.05–1.13)	1.23 (1.18–1.28)
Intravenous			1.15 (1.01–1.22)	1.15 (1.13–1.28)

xyzazine (9 mg/ml). Subsequently, a half dose of the mixture was delivered 1 h later if necessary. The right carotid artery was cannulated to sample blood and monitor blood pressure. The right external jugular vein was cannulated for injections. Animals were placed in the supine position with an intra-tracheal cannula (1.85 mm ID) connected to a ventilator (Servo Ventilator 900C, Siemens-Elema AB, Sweden) in volume-control mode, delivering 30% O₂ at a tidal volume of 10 ml/kg, a frequency of 60 breaths/min, an I : E ratio of 1 : 2, and 2 cm H₂O of positive end-expiratory pressure. The added dead space including the tracheal cannula was 2.7 ml. Spontaneous ventilation was stopped by intravenous injection of 50 mg/kg of suxamethonium chloride (Kabi Vitrum AB, Stockholm, Sweden). A blood sample was taken for determination of antibodies.

Measurements and calculations

Airway flow ($\dot{V}$) was measured with a pneumotachograph (Fleisch no: 000) at the proximal end of the endotracheal cannula connected to a differential pressure transducer (SX01DN, Sontec GmbH, Germany). Tidal volume (V_T) was calculated by numeric integration of flow. Another Sensym pressure transducer for determination of airway opening pressure (P_{ao}) was connected to the port of the pneumotachograph where the internal diameter was 6 mm, which is large enough to avoid significant Bernoulli effects. Esophageal pressure (P_e) was measured with a water-filled catheter (0.6 mm ID) with side holes at the tip, connected to a pressure transducer (PDCR75/1, Druck Ltd, UK). Its proper position was assessed before the animal was paralyzed. The airway was then occluded for a period during which inspiratory efforts caused variations in P_e and P_{ao} , which should be equal to ensure optimal placement of the

esophageal catheter. The circuit of the ventilator and tracheal connection was carefully checked for leaks.

The pressure transducers were calibrated against a water manometer. The pneumotachograph was calibrated with a 40-ml syringe. The frequency response of the systems for flow and pressure determination was flat up to 20 Hz. The time shift between signals did not exceed 3 ms. The flow resistance of the added equipment (tracheal cannula included) was constant up to flow rates of 30 ml/s and amounted to 0.03 cm H₂O/(ml/s). After passing through antialiasing filters, $\dot{V}$, P_{ao} , and P_e signals were fed into a 12-bit, AD converter (PC-30, Boston Technology), sampled at 200 Hz for 12-s periods, and stored in an IBM-compatible personal computer. Signals were displayed for visual quality control.

Respiratory mechanics was measured by a flow-interruption technique (20). Flow ($\dot{V}$) was interrupted at end-inspiration and end-expiration of ordinary breaths by using the end-inspiratory and end-expiratory pause-hold functions of the ventilator. The static pressure at end-inspiration and end-expiration (P_{ei} and P_{ee}) was measured after a 2-s pause. Tidal volume (V_T) was obtained from integrated flow. Static compliance (C_{st}) was calculated by the following formula:

$$C_{st} = V_T / (P_{ei} - P_{ee})$$

Inspiratory resistance (R) was calculated by the following formula:

$$R = (P_{t0} - P_{t1} + \Delta V / C_{st}) / \dot{V}_{t0}$$

P_{t0} was measured just before the onset of flow decline (t_0). P_{t1} was measured from the moment of zero flow (t_1). The volume change between t_0 and t_1 was measured as ΔV . $\Delta V / C_{st}$ is a compensation for change of elastic recoil during the period of flow cessation.

Aerosol and intravenous challenge

A volume of 3 ml of MTHPA-GPSA or HHPA-GPSA solution (10 mg/ml) was placed in a nebulizer (Acorn, Vix, USA). The pneumotachograph was disconnected. During a period in late expiration, an airflow of 8 l/min was fed to the nebulizer. During that period, the inspiratory line was filled with aerosol. The aerosol was carried into the lungs by the following undisturbed inspiration (21, 22). The aerosol particles had a mass median diameter of $4.9\ \mu\text{m}$ with a geometric standard deviation of 1.9, as measured with a light-scattering technique. MTHPA-GPSA or HHPA-GPSA aerosol was generated and administered to the airway by five or more breaths. In five animals, we measured the volume of aerosol deposited in the lungs and trachea. In the same way as in the other animals, we nebulized a solution of human serum albumin labeled with technetium ($^{99\text{m}}\text{Tc}$ -labeled HSA), but gave 80 breaths of aerosol. The deposition was studied with a gamma camera with a pinhole collimator (Portacamera, General Electric, USA) (Fig. 1). We then performed perfusion lung scintigraphy after giving a well-defined dose of macroaggregates of $^{99\text{m}}\text{Tc}$ -labeled albumin intravenously. These particles were quantitatively trapped in the pulmonary circulation. The radioactivity of the latter dose was many times higher than the dose given as aerosol. The volume of aerosol deposited was calculated from the count rate over the lung fields and the trachea before and after the injection of macroaggregates, the dose injected in MBq, and the concentration of the liquid in the nebulizer (MBq/ μl).

In an initial series of provocation experiments, immunized animals were given various doses of

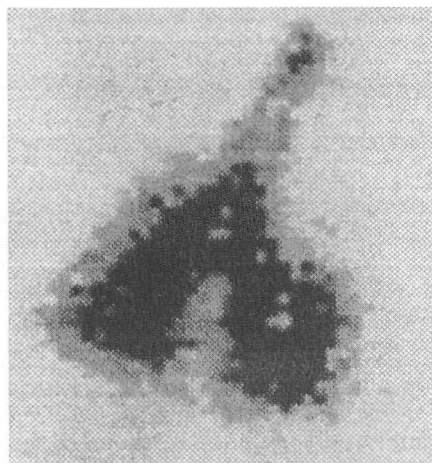


Fig. 1. Gamma-camera image after inhalation of $^{99\text{m}}\text{Tc}$ -labeled HSA, showing dominant intrapulmonary distribution of aerosol.

antigen to establish suitable doses for the final study. In the latter, MTHPA-GPSA or HHPA-GPSA was injected into the jugular vein in a dose of $20\ \mu\text{g/kg}$ body weight. In the aerosol challenge group, five breaths were given. If the initial dose did not cause a reaction, the dose was doubled stepwise after 10 min until 20 breaths or $160\ \mu\text{g/kg}$ i.v. had been given. The dose that caused an obvious reaction with respect to mechanics or PaO_2 was denoted the critical dose. When the reaction was observed or when the maximum dose had been given, measurements were performed after 5, 10, 15, and 30 min. The last given dose was then repeated, as was the sequence of measurements.

Statistical analysis

All values represent the mean $\pm$ SE. ANOVA and paired *t*-test were used to compare differences among and within groups, respectively, by the SPSS program. $P < 0.05$ was considered to be significant.

Results*Antibody titers*

Each of the control animals in the final study had an optical density value in the ELISA for specific IgG antibodies of <0.03 . Each of the animals immunized with MTHPA or HHPA in both the initial and the final study had a value >1.0 (Table 1).

Aerosol deposition

Gamma-camera images showed an even distribution of aerosol over the lung fields (Fig. 1). There was an accumulation of activity at the tip of the tracheal cannula. The activity was low in the mediastinal field below that point. The volume of aerosol deposited in the lung fields was $0.03 \pm 0.008\ \mu\text{l/breath}$ and $0.004 \pm 0.003\ \mu\text{l/breath}$ in the mediastinal field.

Initial provocation experiments

The three first animals immediately died after injection of 1–10 mg/kg of antigen. In the following five animals, no response to MTHPA-GPSA at concentrations of 0.01, 0.1, 1.0, and $10\ \mu\text{g/kg}$ body weight was detected, after which the animals died or went into an irreversible shock from intravenous injection of $100\ \mu\text{g/kg}$ of MTHPA-GPSA (Table 2). We then reduced the difference between successive doses to a factor of two, starting at 20 or $40\ \mu\text{g/kg}$ of MTHPA-GPSA or HHPA-GPSA. An important fall in PaO_2 and rise in peak airway pressure

occurred at injection of 40–80 µg/kg of MTHPA-GPSA and HHPA-GPSA in all seven animals. After repetition of the critical or a higher dose, an attenuated response was observed in PaO_2 and other parameters. A critical dose was accordingly established to be approximately 40 µg/kg.

Final provocation experiment

Mechanics determined on the basis of esophageal pressure which reflects properties of the chest wall showed trivial and, from most aspects, statistically insignificant changes in conjunction with provocation in all groups. Accordingly, observed reactions to challenges referred to the lungs rather than to the chest wall. The findings representing changes within the total respiratory system, based upon Pao , and those representing the lungs, which were based on the difference between Pao and Pe , were nearly identical. As studies of the respiratory

system are easier to perform and are independent of the use of an esophageal catheter, we report the results of the respiratory system only.

Arterial blood gases and mechanics of the respiratory system were similar at baseline observations in all groups (Table 3). In the nonimmunized control animals, challenged by inhalation of five plus 10 breaths of either MTHPA-GPSA or HHPA-GPSA, no response was obtained with respect to either compliance, resistance, or PaO_2 (Fig. 2).

The response to the first challenge

After intravenous challenge, 6/8 immunized animals reacted after 40 µg/kg of either MTHPA-GPSA or HHPA-GPSA. One animal reacted after 80 µg of HHPA-GPSA, and one after 160 µg/kg MTHPA-GPSA. After inhalation of the antigen complex, 5/8 animals responded after five breaths, one

Table 2. Response of sensitized animals to various amounts (µg/kg) of antigen (MTHPA and HHPA)

Animal no.	0.01	0.1	1.0	10	20	40; 1st	40; 2nd	80; 1st	80; 2nd	100	160	1000	5000	1000
1														Died
2													Dying	
3												Dying		
4	(-)	(-)	(-)	(-)						Died				
5	(-)	(-)	(-)	(-)						Died				
6	(-)	(-)	(-)	(-)						Died				
7	(-)	(-)	(-)	(-)						Dying				
8	(-)	(-)	(-)	(-)						Dying				
9					(-)	(+ +)								
10					(-)	(+ +)		(-)						
11						(-)		(+ +)			(+)			
12						(+ +)		(+ +)	(+)					
13					(-)	(+ +)	(+)							
14					(-)	(+ +)	(+)							
15*					(-)	(+ +)	(+)							

* Animal received HHPA; (-): negative response; (+): slight response; (+ +): obvious response.

Table 3. Lung-function parameters at baseline conditions and at time of individual maximum response to challenge. Controls were not immunized. Other groups were challenged either by aerosol or intravenously

	MTHPA-GPSA-challenged animals					HHPA-GPSA-challenged animals				
	Controls <i>n</i> =4	Aerosol challenge in immunized group <i>n</i> =4		Intravenous challenge in immunized group <i>n</i> =4		Controls <i>n</i> =4	Aerosol challenge in immunized group <i>n</i> =4		Intravenous challenge in immunized group <i>n</i> =4	
		Baseline value	Maximum response	Baseline value	Maximum response		Baseline value	Maximum response	Baseline value	Maximum response
Cst,rs (ml/cm H_2O)	0.49 ± 0.04	0.56 ± 0.03	0.21 ± 0.04	0.47 ± 0.04	0.15 ± 0.04	0.41 ± 0.04	0.38 ± 0.02	0.23 ± 0.04	0.39 ± 0.03	0.12 ± 0.01
R,rs (cm $\text{H}_2\text{O}/\text{l/s}$)	0.10 ± 0.01	0.10 ± 0.004	0.67 ± 0.23	0.08 ± 0.02	0.65 ± 0.29	0.09 ± 0.003	0.11 ± 0.01	0.29 ± 0.12	0.11 ± 0.006	0.70 ± 0.26
PaO_2 (kPa)	14.3 ± 0.9	13.7 ± 1.6	8.1 ± 0.6	14.9 ± 1.4	5.8 ± 0.4	14.3 ± 0.7	16.2 ± 0.7	5.4 ± 0.6	14.5 ± 0.4	4.4 ± 0.3

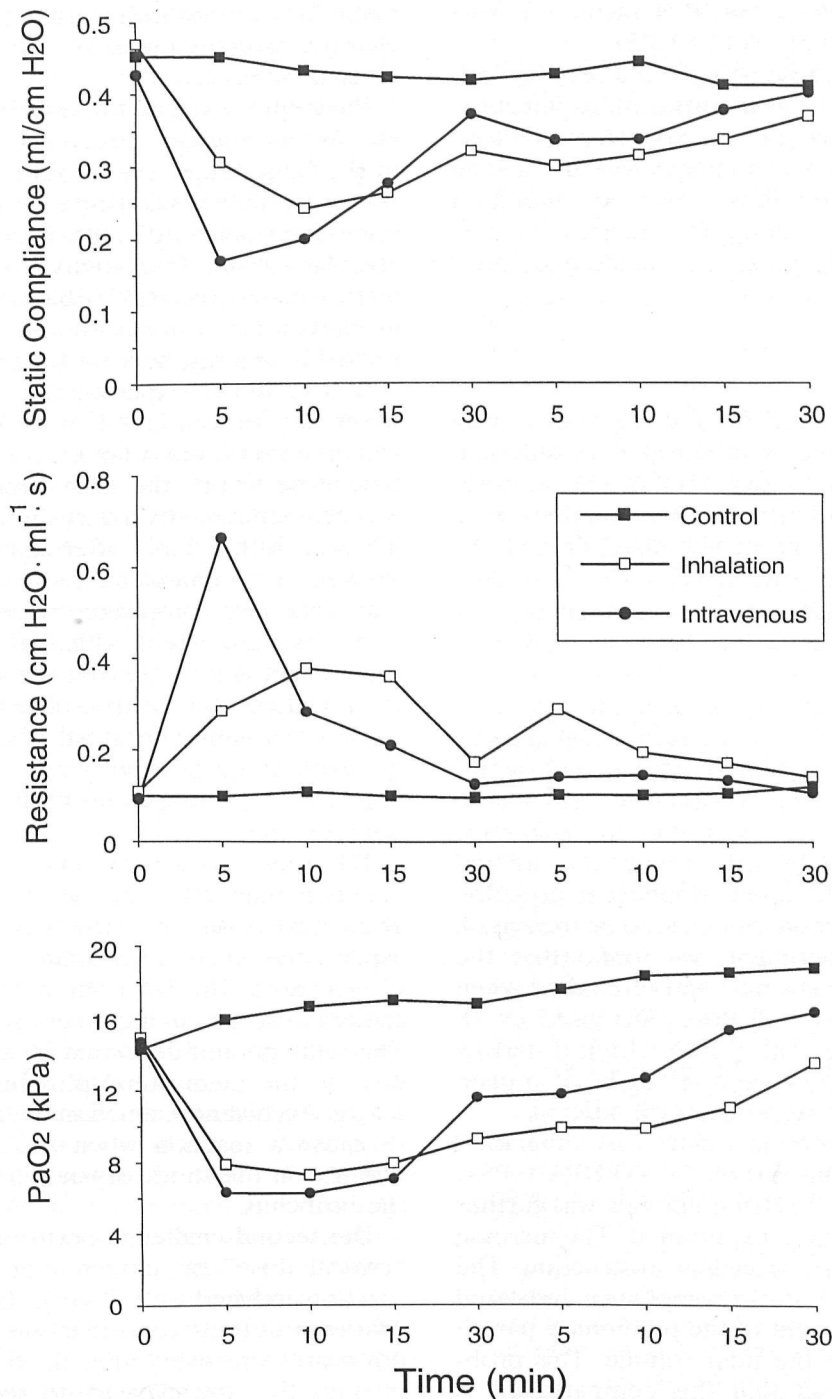


Fig. 2. Compliance, resistance, and PaO₂ during final provocation experiments in control animals and immunized animals.

needed 10 breaths of MTHPA-GPSA, and two needed 20 breaths of HHPA-GPSA. The reaction to the two substances was similar (Table 3). The results from the groups sensitized and challenged with MTHPA and HHPA were therefore merged (Fig. 2). Cst,rs and PaO₂ fell and R,rs increased significantly after both inhalation and intravenous challenge by MTHPA-GPSA and HHPA-GPSA compared to control groups ($P < 0.001$). For each

parameter, the reaction was maximal within 10 min after aerosol challenge and 5 min after intravenous injection. In the aerosol group, the mean value of Cst,rs fell from 0.47 to 0.24 ml/cm H₂O and for PaO₂ from 15.0 to 7.3 kPa ($P < 0.01$), while R,rs rose significantly from 0.11 to 0.38 cm H₂O ml⁻¹·s ($P < 0.05$). In the intravenous injection group, Cst,rs and PaO₂ also significantly decreased from 0.43 to 0.17 ml/cm H₂O and from 14.7 to 6.3 kPa,

respectively ($P < 0.001$), but R_{rs} increased from 0.09 to 0.66 cm H₂O ml⁻¹·s ($P < 0.05$).

When the critical dose was given a second time, either as an aerosol or as an intravenous injection, the reaction was weak (Fig. 2). No statistical difference in the lung-function values before the second challenge (30 min) and those observed 5 min later was observed in either group. The changes of $C_{st,rs}$, PaO_2 , and R_{rs} were significantly smaller than after the first critical dose ($P < 0.01$).

Discussion

A model was developed for the study of airway reactivity in guinea pigs immunized by intradermal injection of MTHPA and HHPA. Very dose-dependent, clear, and reproducible reactions were obtained after challenge of sensitized animals by both intravenous and airway routes. The reproducibility of the reactions even after challenge by the airway route may reflect the even and well-standardized distribution of aerosol in the lung which is possible with the aerosol-delivery technique (22). After challenge, the guinea pigs showed a significant increase of lung resistance, and significant decreases of lung compliance and PaO_2 . The mechanical properties of the thoracic cage remained unaffected by the provocation, as was shown by data based upon esophageal pressure. Accordingly, the latter did not need to be measured.

In the initial experiment, we found that the animals reacted very strongly and often died when they were challenged with doses increased in 10-fold steps. We found that a dose within a narrow range, such as between 40 and 160 µg/kg of antigen i.v., produced a clear response in all animals.

The bronchoconstriction induced by inhalation or intravenous administration of MTHPA-GPSA or HHPA-GPSA in sensitized animals was further characterized in the final experiment. The increase of resistance reflected bronchial obstruction. The dramatic decrease in static compliance indicated that a large compartment of the pulmonary parenchyma did not share the tidal volume. This probably reflected the fact that this compartment is deprived of ventilation because of airway closure. The pronounced hypoxia that was observed in spite of an increased fraction of inspired oxygen indicates that a large fraction of pulmonary perfusion was shunted through the closed compartment. All physiologic observations can accordingly be explained by bronchial obstruction related to spasm, secretion, and/or edema. Studies on the effect of bronchial smooth-muscle contraction induced by methacholine infusion in sheep have shown that a substantial decrease in lung compliance may reflect contraction of conducting airways larger than ter-

minal bronchioles rather than the more peripheral airways, alveolar ducts, or other elements in the alveolar wall (26).

We cannot exclude the possibility that the allergic reaction engages structures other than bronchi in the lung. Organic acid anhydrides, e.g., inhalation of trimellitic anhydride, have been reported to cause lung injury (27, 28). It is also possible that alveolar surface forces may be affected and may modify compliance (29). The negative provocation in the control groups shows that the reaction was caused by allergy, and not toxicity.

In the initial experiments, the animals could often not be ventilated immediately after intravenous injection of a large dose of antigen. In the final experiment, the time course of obstruction was characterized by a very fast onset that became maximal within 5 min after challenge. All animals showed a spontaneous recovery. After 30 min, resistance and compliance were still affected but only to a slight extent, while PaO_2 showed a slower recovery. It is possible that the slower recovery of PaO_2 reflects the collapse of peripheral lung units secondary to more proximal airway closure. We did not explore this possibility by trying to recruit such units by the prolonged insufflation of an increased tidal volume.

The time course was faster after intravenous injection than after inhalation, and the response after intravenous injection was stronger. In other aspects, the reaction was similar after the two types of challenge. The total amount of allergen in the critical dose given intravenously averaged 36 µg. The total amount deposited by aerosol as a critical dose in the mediastinal plus lung field was only 2.3 µg. Accordingly, much smaller doses are needed to cause a reaction when the dose is deposited directly on the shock organ, which, presumably, is the bronchus.

The second challenge performed with the same "critical dose" of antigen that induced the first reaction induced only a very slight reaction. The character of the second reaction was like a slowing down of the recovery after the first reaction. It was obvious that the capacity to react soon after an initial reaction was exhausted. In two animals in the initial experiment (nos. 2 and 11, Table 2), we doubled the second dose after a first positive reaction and did not observe a clear response to the increased dose. The attenuation of the response appears not to be a matter of dose. The mechanism behind the attenuation of responsiveness soon after a first response is unclear. Possible mechanisms are cell depletion of mediators or desensitization (30).

The guinea pigs used in this model showed a negative or very low IgE response after a single-dose immunization by HHPA or MTHPA as measured

by the passive cutaneous anaphylaxis test (25). Provocation tests after passive sensitization with both untreated and heat-treated sera (to destroy IgE) showed similar effects on lung resistance and plasma exudation (Zhang et al., unpublished observation). Thus, IgG1 seems to be the major anaphylactic antibody in the present model.

Although the other mechanisms behind manifestations of MTHPA- and HHPA-induced asthma remain unclear, it is known that several mediators may be engaged in the acute allergic airway response in guinea pigs, such as histamine, leukotrienes, thromboxane, platelet-activating factor, and cytokines (10, 31–33). The release of these mediators is involved in the development of bronchial constriction and edema and leads to airflow obstruction and plasma exudation, resulting in the pathophysiologic picture. It is possible that stores of these mediators may be emptied after the first challenge. Another possibility is that the first response may have led to activation of protective systems. For example, asthma is associated with endogenous production of nitric oxide, as shown by increased amounts of nitric oxide in exhaled air (34).

Sensitization of the airways was achieved by intradermal immunization with MTHPA and HHPA. Sensitization to trimellitic anhydride (12) and phthalic anhydride (15) has earlier been reported. The reactions at provocation were identical for the MTHPA- and HHPA-sensitized groups with respect to both critical dose and reaction. Both MTHPA and HHPA are known human sensitizers (3–6). The similarity in response was interesting with regard to the difference in chemical structure between the two anhydrides.

We conclude that intradermal immunization with MTHPA and HHPA leads to asthma in guinea pigs. The dose-response relationship when sensitized animals are exposed to the antigen is extremely steep. The reactivity appears to be exhausted after an initial positive provocation. This model, which represents a development of previous methods (16), may be suitable to assess the induction of asthma by other acid anhydrides and, possibly, other low-molecular-weight chemicals.

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