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The respiratory response in sensitized rats to aerosol challenge

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Summary. Hooded Lister rats were sensitized to DNP-ovalbumin. These rats showed a significant alteration in respiratory pattern when challenged with DNP-ovalbumin aerosol. The respiratory response was not produced by challenging sensitized rats with intragastric DNP-ovalbumin. The magnitude of the respiratory response did not correlate with the specific IgE concentration in the serum. A similar pattern of respiratory response was inducible in unsensitized rats by intravenous administration of IgE-rich sera.

INTRODUCTION

Animal models of allergic asthma have been described using the dog (Booth, Patterson & Talbot, 1970), monkey (Patterson & Talbot, 1972) and guinea-pig (Alexander, Becke & Holmes, 1926). The dog and monkey models have the disadvantage that the experimental animals are expensive and the life cycle is long. Sensitization of the guinea-pig is commonly associated with the production of both IgG_1 and IgE homocytotropic antibodies (Dobson, Rockey & Soulsby, 1971).

The rat on experimental sensitization produces IgE antibody which is analogous to human IgE antibody (Stechschulte, Orange & Austen, 1970; Kanyerezi, Jaton & Bloch, 1971) and attempts have been made to develop an appropriate rat model (Church, Collier & James, 1972: Stotland & Share, 1974). Although the anaphylactic bronchial constriction model does measure pulmonary reactivity to allergen challenge, the technique is carried out in a pithed rat. In addition, the antigen challenge is delivered intravenously rather than via the airway.

We have sensitized an inbred strain of Hooded Lister rats to DNP-ovalbumin and have demonstrated that these rats produce, on aerosol challenge, characteristic changes in their respiratory pattern as measured by body plethysmography. Similar respiratory changes may be produced by passive sensitization of rats with IgE-rich sera.

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MATERIALS AND METHODS

Animals

Hooded Lister rats, Bristol in-bred strain. Weight 200–400 g.

Antigen

DNP-ovalbumin prepared by the technique described by Williams & Chase (1967). There were 19 DNP molecules attached to each ovalbumin molecule as demonstrated by spectrophotometry.

Antisera

Antisera to DNP-ovalbumin were raised by a modification of the techniques described by Jarrett & Stewart (1974) using killed *Bordetella pertussis*

Table 1. Sensitizing regimes

Schedule	Route	Initial		Booster	
		Content	Day	Content	Day
1	Subcutaneous	10% Aluminium hydroxide 0·5 ml + 1 mg DNP-ovalbumin	16	Same	11
	Intraperitoneal	*B. pertussis* 24 × 10^9 organisms			
2	Intraperitoneal	100 μg DNP-ovalbumin *B. pertussis* 24 × 10^9 organisms	30	1 μg DNP-ovalbumin	3
3	Intraperitoneal	10 μg DNP-ovalbumin *B. pertussis* 24 × 10^9 organisms	30	1 μg DNP-ovalbumin	3

The days shown refer to days before challenge on day 0.

(Wellcome Laboratory) as adjuvant. The rats were sensitized using the illustrated regimes (Table 1). Schedule 3 was the usual method. Sera from the sensitized rats were assayed for homocytotropic antibody as shown by its ability to produce passive cutaneous anaphylaxis (P.C.A.) in recipient rats. The serum was diluted 1/5, 1/10, 1/50, 1/100, 1/250, 1/500 with phosphate-buffered saline (P.B.S.). PCA titres were carried out in duplicate in paired rats and were measured 72 h after the intracutaneous injection of 0·05 ml of the dilutions. The rats were killed 25 min after i.v. injection of DNP-ovalbumin (10 mg) and Coomassie blue (10 mg) in 1·5 ml P.B.S. The mean diameter of the blue area was measured on the visceral surface of the skin. The lowest dilution producing a blue reaction of more than 5 mm diameter was recorded as the P.C.A. titre. The individual results quoted are the mean of the two results. The PCA activity described was always reduced and commonly abolished by heating at 56° for 4 h. PCA titres at 2 h were also carried out and these were never greater than the 72 h PCA and were similarly reduced by heating at 56° for 4 h. Pooled rat serum with a high 72 h PCA titre (1/500) to ovalbumin was kindly provided by Dr Ellen Jarrett.

Aerosol challenge

Rats were placed during brief ether anaesthesia in a perspex box with the head protruding. The neck was sealed with a thick rubber collar and the animal was maintained in a vertical position. The neck seal was supplemented by a layer of KY jelly (Johnson & Johnson Ltd., Slough) placed on top of the collar. The sealed perspex box was connected to a large volume bell jar, total volume of the sealed area being 2·5 litres. The pressure changes induced in this enclosed volume by the respiratory movements of the rat were recorded by a Devices Pressure Transducer (Model UP-1) and displayed on a 6-channel Devices Recorder using a preamplifier (Devices, 352). The pressure changes so produced were calibrated against the changes resulting from the injection of measured volumes of air into the system at a similar rate to the recorded respiratory changes. Routinely two rats were set up simultaneously, one being the experimental subject and the other the control subject and the challenge procedures for both were identical. The rat was conscious and responded to stimulation throughout the experiments. To challenge the rat, 4 ml of 20 mg/ml DNP-ovalbumin or ovalbumin was added to the Bennet Twin Nebulizer which was run on compressed air. The nebulizate passed into a polythene bag chamber surrounding the head of the rat. Control periods using PBS as the nebulizate were recorded before and after the challenging of the rats. The results were recorded for 20 min prior to challenge, during the 10–20 min of aerosol challenge and for at least 30 min after the completion of challenge. Graphs of changes in respiratory rate, minute volume and expiratory flow notching were prepared. The mean changes in these parameters were recorded by sub-

traction of the mean control value from that occurring during challenge.

For intragastric challenge the rat was placed in the recording apparatus as previously without the aerosol chamber. A Portex cannula (5FG, O/D 1·65 mm) was passed into the rat's stomach after an initial control period had been recorded. Immediately after passing the tube 80 mg (4 ml) of DNP-ovalbumin was injected intragastrically and the tube withdrawn. A challenge period of 10–20 min was recorded and thereafter an appropriate control period. After a further control period with the nebulizer chamber operative, the rat previously challenged intragastrically was challenged by aerosol using 80 mg (4 ml) of DNP-ovalbumin in the nebulizer. The position of the tube in the stomach was confirmed by postmortem demonstration of large quantities of DNP-ovalbumin in the stomach and small bowel. There was no detectable DNP-ovalbumin in other sites and the oesophageal and gastro-intestinal mucosa were undamaged.

Passive sensitization

Three days before the planned aerosol challenge, the rats were injected via a tail vein with serum in volumes of less than 3 ml.

All statistics quoted refer to Students '*t*'-tests.

RESULTS

Representative recordings of the results of aerosol challenge in actively sensitized and unsensitized rats are illustrated (Fig. 1). The major changes observed in the breathing pattern of the sensitized rats are alteration of the expiratory wave-form with the appearance of a notch, a reduction in respiratory rate and a reduction in minute volume (calculated by multiplying the volume expelled in an average breath by the respiratory rate). The sudden reduction in expiratory flow (expiratory notching) suggests the

Table 2. The mean respiratory responses to aerosol challenge with DNP-ovalbumin in 20 sensitized and 10 unsensitized rats

Variable measured	Unsensitized	Sensitized	Significance of the difference (*P*)
% Increase in expiratory notching	8	30	< 0·002
% Fall in minute volume	4	13	< 0·01
% Fall in respiratory rate	4	14	< 0·002

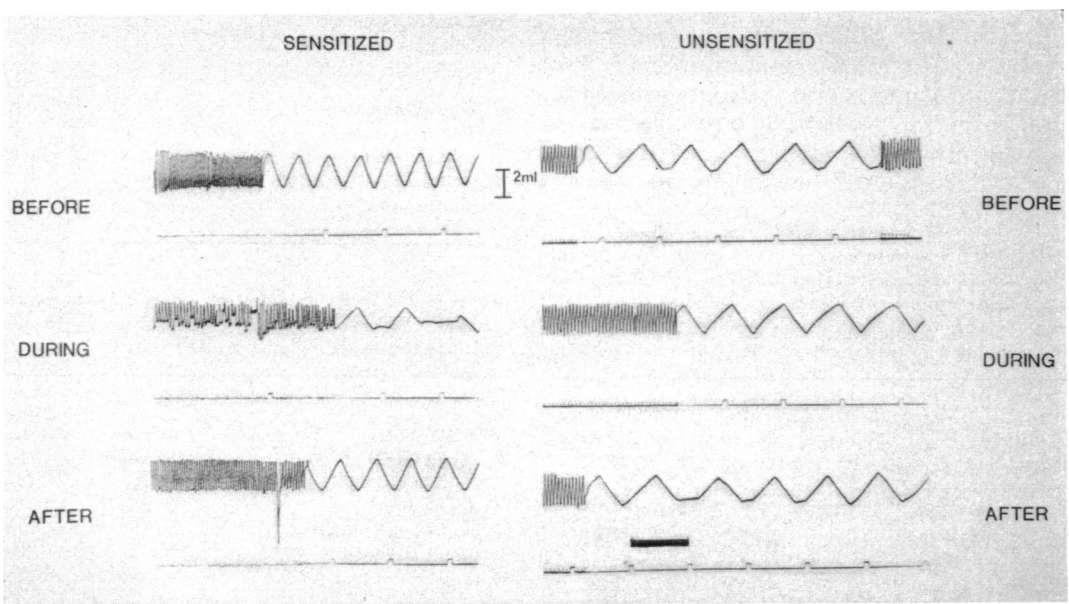

Figure 1. Photograph of parts of experimental record before, during and after DNP-ovalbumin aerosol challenge of sensitized and unsensitized rats (1/3 original size).

Recording speed is indicated by a 1 s interval; inspiration (↓); expiratory notching (↑); 2 ml calibration (I). The two recording speeds used were identical in both rats.

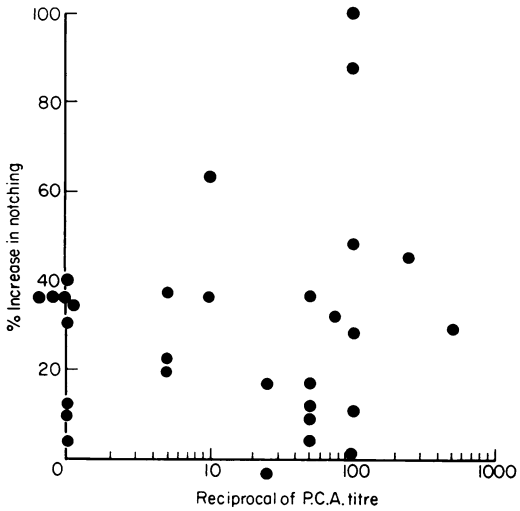

Figure 2. Comparison of P.C.A. titre and increase in expiratory notching on challenge with DNP-ovalbumin aerosol in 30 rats.

presence of airways obstruction. A statistical comparison of the mean results observed in 10 unsensitized and 20 actively sensitized rats is shown in Table 2. Significantly greater changes in expiratory notching, minute volume and respiratory rate occurred in the sensitized rats on aerosol challenge.

These rats were bled from the tail immediately before being placed in the body plethysmograph. The results of the 3-day PCA titres in the sera so obtained are shown in Fig. 2 which illustrates the lack of correlation between the extent of the respiratory change and PCA titre. Unsensitized rats did not have any specific IgE on 3-day PCA titre. There was no detectable correlation between the PCA titre and the respiratory response as measured by the increase in expiratory notching (Fig. 2), the fall in minute volume or the fall in respiratory rate. When serum from rats actively sensitized with DNP-ovalbumin was injected into unsensitized, passive recipients, we were able to demonstrate a significant respiratory response to aerosol challenge in these rats 3 days after injection. Table 3 shows the differences in the mean respiratory responses when the results of challenge are compared in the rats who received a large dose of specific IgE with those receiving less potent serum. Table 4 demonstrates that if a high potency serum containing ovalbumin-specific IgE is injected into passive recipients, it produces the characteristic respiratory response observed in the actively sensitized rats. This effect can be abolished

Table 3. The mean responses to DNP-ovalbumin aerosol challenge in 19 rats passively sensitized by i.v. injection of serum 3 days before challenge. Serum which had a low IgE content (< 0·5 ml of 1/100 titre) was designated low potency. Serum containing more than this was designated high potency

Variable measured	Low potency sera	High potency sera	Significance of difference (*P*)
% Increase in expiratory notching	1	13	< 0·04
% Fall in minute volume	−2	6	< 0·08
% Fall in respiratory rate	−3	7	< 0·02

Table 4. The mean responses to ovalbumin challenge in 16 rats passively sensitized by i.v. injection of 1 ml pooled serum 3 days before challenge. The initial P.C.A. of this serum was 1/500 but in serum heat-inactivated for 4 h at 56°, the P.C.A. was 1/3

Variable measured	Sera injected (P.C.A.) 1/3	Sera injected (P.C.A.) 1/500	Significance of difference (*P*)
% Increase in expiratory notching	1	34	< 0·002
% Fall in minute volume	4	21	< 0·03
% Fall in respiratory rate	1	34	< 0·02

Table 5. The mean responses to sequential intragastric and aerosol challenge with DNP-ovalbumin in 13 actively sensitized rats

Variable measured	Intragastric challenge	Aerosol challenge	Significance of difference (*P*)
% Increase in expiratory notching	3	30	< 0·02
% Fall in minute volume	−2	20	< 0·001
% Fall in respiratory rate	3	17	< 0·04

by prior heat inactivation of the serum. No specific IgE was found in the sera of the passively sensitized rats immediately prior to aerosol challenge.

The results of intragastric and subsequent aerosol challenge in each of 13 actively sensitized rats are shown in Table 5. A significantly greater response occurred when the rats previously challenged with intragastric DNP-ovalbumin were challenged by aerosol.

DISCUSSION

Our results demonstrate that significant respiratory reactions occur in actively sensitized rats when they are challenged by an appropriate aerosol. It is interesting that the specific IgE level in the serum of the rats immediately before challenge does not indicate the likelihood of a significant respiratory response. Jarrett & Stewart (1973) demonstrated that the concentration of IgE in the serum does not correlate well with the immediate hypersensitivity reaction in the skin. A similar lack of correlation between the specific IgE in the serum and respiratory response has been shown using the technique of anaphylactic bronchial constriction (Church, 1975). The number and avidity of mast cells for the specific IgE and its affinity presumably determines the relationship of the serum IgE to tissue IgE. We have demonstrated that sera taken from rats which have been actively sensitized were capable of producing a similar respiratory response in unsensitized rats. The magnitude of the respiratory response is related to the IgE content of the serum used (Table 3). The experiments with the ovalbumin-specific serum demonstrated that inactivation of the IgE abolishes the transmission of the respiratory response. This confirms the conclusion derived from using DNP-ovalbumin sensitive sera. The absence of any detectable specific IgE in the sera of the passively sensitized rats at the time of challenge, despite the fact that they had significant respiratory responses, supports the postulate that the tissue-bound IgE determines the respiratory response. Thus, our results suggest that the respiratory response occurring in actively sensitized rats on aerosol challenge is an IgE-mediated reaction.

It is probable that only approximately 15% of the DNP-ovalbumin taken up by the rat on aerosol challenge actually reaches the respiratory tract (Hout & Johnson, 1972). The same dose of DNP-ovalbumin was placed in the nebulizer as in the stomach on intragastric challenge. It is likely that the actual quantity reaching the respiratory tract (calculating from the quantity in the nebulizer and deducting probable losses in the circuit) was less than 5% of the intragastric challenge. This intragastric dose is also clearly greater and as rapidly present as the dose of aerosol which the rat will swallow during aerosol challenge. The experiment demonstrates that the route of challenge is of considerable importance when considering a respiratory response and would suggest that methods of challenge which do not use the respiratory airways to deliver the antigen may give erroneous results.

We have not conclusively demonstrated that there is an increase in airways resistance in the actively sensitized rat undergoing aerosol challenge as we did not record the intrapleural pressure. However, our results do demonstrate that a respiratory response does occur in these rats and the response, particularly the occurrence of expiratory notching, is suggestive of acute airways obstruction.

Our rat model offers the opportunity to examine in detail the respiratory response to aerosol challenge. It compares favourably to the monkey and dog models with regard to cost and may prove superior to the guinea-pig as the response in the rat is IgE-mediated.

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