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Pulmonary function changes in normal rats induced by antibody against rat IgE

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SUMMARY

Changes in pulmonary function, as measured by airway conductance and dynamic compliance, in normal rats have been provoked by the administration of a rabbit antisera prepared against rat IgE myeloma protein. The response is specific in that neither anti-IgG nor normal rabbit serum induce pulmonary changes. Pre-treatment with disodium cromoglycate inhibits the bronchospasm in a dose related manner. In addition, drug protection can be demonstrated with as long as 1 hr pre-dosing.

INTRODUCTION

Several animal models of pulmonary function are available for studying allergic airway diseases. Elicitation of the allergic type response is accomplished by using (a) naturally sensitive animals, such as dogs (Booth, Patterson & Talbot, 1970) or monkeys (Patterson, Talbot & Brandfonbrenner, 1971), (b) active immunization or (c) passive sensitization in other species, such as guinea-pigs (Amdur & Mead, 1958), rats (Church, Collier & James, 1972), and rabbits (Diamond *et al.*, 1975). In all three instances, the immunological reaction is provoked by challenging the animal with specific antigen either intravenously or by aerosol. The naturally sensitive animal is perhaps the most suitable model. The actively immunized animal is the least desirable and of questionable usefulness because it has a disproportionately high concentration of IgG and thus antigen challenge results in reactions more often connected with acute inflammation than with immediate hypersensitivity. The passively sensitized animal can be manipulated to select for the IgE-mediated immediate-type hypersensitivity reaction, but it suffers from inconsistency of response to antigen and the non-availability of large volumes of IgE antibody-containing sera.

Since the primary trigger mechanism for an immediate hypersensitivity reaction is the bridging of bivalent IgE antibody molecules on the mast cell surface, reactions can be provoked by at least two methods, including bridging with specific antigen or with an antibody prepared against IgE (Becker *et al.*, 1973). In this report, we describe the preparation of rabbit anti-rat IgE and the results of preliminary studies on changes in rat pulmonary function as a result of intravenous challenge of normal rats with this antiserum.

MATERIALS AND METHODS

LOU/MN rats. Breeding pairs of LOUMN rats, necessary for passage of the IgE myeloma tumour, were obtained from the National Institutes of Health, Bethesda, Maryland (NIH), and the colony was expanded in our animal facilities by strict brother–sister matings.

Rat IgE myeloma. An IgE producing myeloma (Bazin *et al.*, 1974) designated IR-162 was obtained from Dr H. Metzger, NIH, and was maintained as a solid subcutaneous tumour by passage every 14 to 21 days. To obtain ascites fluid containing

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the IgE paraprotein, rats were injected i.p. with 0·5 ml whole blood obtained from a solid tumour-bearing animal. Rats were decapitated prior to collection of the ascitic fluid to reduce the presence of erythrocytes. The fluid was centrifuged at 400 *g*, for 10 min at room temperature to remove other cells and stored frozen at −80°C.

Separation of IgE from ascitic fluid. The method for separation of IgE from ascitic fluid was modified from the procedure of Kulczycki & Metzger (1974, and personal communication). The globulins precipitated between 37% and 48% saturation by ammonium sulfate in 0·2 M borate buffer saline (BBS) pH 8·0, were dissolved in 0·1 M Tris-HCl, pH 8·0 and applied to a DEAE-Sephadex A-50 column (2·6 × 35 cm) at room temperature equilibrated in the same buffer. The column was eluted with a linear gradient to 0·05 M Tris-HCl − 0·5 M NaCl, pH 8·0. An IgE-rich fraction was eluted at a conductivity of 9–12 mhos and was further purified by molecular exclusion chromatography on a Sepharose 6B column (6 × 70 cm) at 12° in BBS. The first peak, containing IgE, was re-chromatographed on Sepharose 6B to obtain additional resolution from other proteins. The purity of the product was determined by immunoelectrophoresis developed with antisera against rat IgE and normal rat globulins. Isolated IgE was lyophilized and stored at 5°C.

Preparation of rabbit anti-rat IgE. Rabbits, weighing approximately 2 kg, were immunized with 0·5 mg rat IgE emulsified in Freund's complete adjuvant (Difco, Detroit, Michigan) and injected subcutaneously at several sites in order to stimulate several regional lymph nodes. Two months later the rabbits were bled 50 cc by cardiac puncture and subsequently every 2 weeks. Five months after the original immunization, the animals were boosted with 0·3 mg rat IgE in Freund's incomplete adjuvant and bled again every 2 weeks until they expired. Antisera with PCA inhibition titres ≥ 1/800 were pooled for use in this study.

Assay for anti-rat IgE activity. Anti-rat IgE activity was assayed in a passive cutaneous anaphylaxis (PCA) inhibition test. Various dilutions of rabbit cutaneous anaphylaxis (PCA) inhibition test. Various dilutions of rabbit anti-rat IgE were incubated for 30 min at 37°C with a standard 1/80 dilution of rat IgE anti-egg albumin prepared in our laboratory (Casey *et al.*, submitted for publication). The incubation mixtures were centrifuged at 1700 *g* for 15 min at room temperature and used to sensitize rat skin. The highest dilution of rabbit anti-rat IgE capable of completely inhibiting the PCA reaction provoked by egg albumin was considered the endpoint titre. The rabbit anti-rat IgE completely inhibited by the rat PCA reaction at dilutions of 1/800 and is therefore said to contain 800 PCA inhibition units/ml.

Pulmonary function assessment. A modification of the method of Amdur & Mead (1958) (see also Douglas *et al.*, 1972) was used to monitor changes in pulmonary function. Briefly, a rat anaesthetized with urethane (1·2 g/kg) was placed in a whole body plethysmograph with a fluid-filled catheter open into the thoracic cavity. A single air port from the plethysmograph was connected to a pneumotachograph, a transducer measuring volume and a heat sink reservoir to make a completely closed system for obtaining air flow and air volume measurements of the test animal. With the inputs of thoracic cavity pressure, air volume and air flow, an on-line computer similar to that described by Dennis *et al.* (1969) calculates a breath-by-breath analysis of airway conductance and dynamic compliance. The resulting data are expressed as the maximum percentage loss of conductance and compliance and/or the percentage inhibition of the loss due to drug treatment. Rats were challenged by an intravenous injection of whole serum or diluted rabbit anti-rat IgE.

RESULTS

In order to determine a suitable quantity of anti-IgE needed to induce a consistent bronchospasm, 0·5 ml volumes of antisera containing 25–400 PCA inhibition units were administered intravenously to normal rats. While 400 units proved fatal to some animals, 50–200 units induced bronchospasm 15–20 min after challenge (Fig. 1). Twenty-five units induced loss of airway conduction more readily than dynamic compliance and only half of the animals thus challenged exhibited changes in pulmonary function. A preliminary attempt to inhibit the responses revealed that injection of disodium cromoglycate (30 mg/kg, i.p.) 10 min prior to anti-IgE challenge inhibited the bronchospasms induced by 50 units, but not 400 units, of anti-IgE. Therefore, we decided to use 50 units of anti-IgE for the remaining studies. To date, our experience with forty-three control animals receiving 50 units anti-IgE shows an average (± s.e.m.) 82·6 ± 1·2% loss of conductance and 73·9 ± 1·6% loss in compliance. Rats challenged with either normal rabbit serum or rabbit anti-rat IgG did not experience bronchospasm.

Most animals receiving 50 units anti-IgE and experiencing bronchospasms 15–20 min later were terminated shortly after; however, if allowed to remain in the plethysmograph, they recovered fully within 1 hr. It was therefore felt to be of interest to determine whether these animals could again be induced to bronchospasm by a second challenge with anti-IgE. Three rats were challenged twice by injections of 50 units anti-IgE, separated by an interval of 40 min. Although a maximal degree of bronchospasm developed within 20–30 min, the animals were fully recovered at 40 minutes and showed no response to the second challenge (Fig. 2).

To determine whether the model was useful in evaluating anti-allergy drugs, normal rats were dosed i.p. with disodium cromoglycate (DSCG) 10 min prior to challenge with 50 units anti-IgE. Percentage

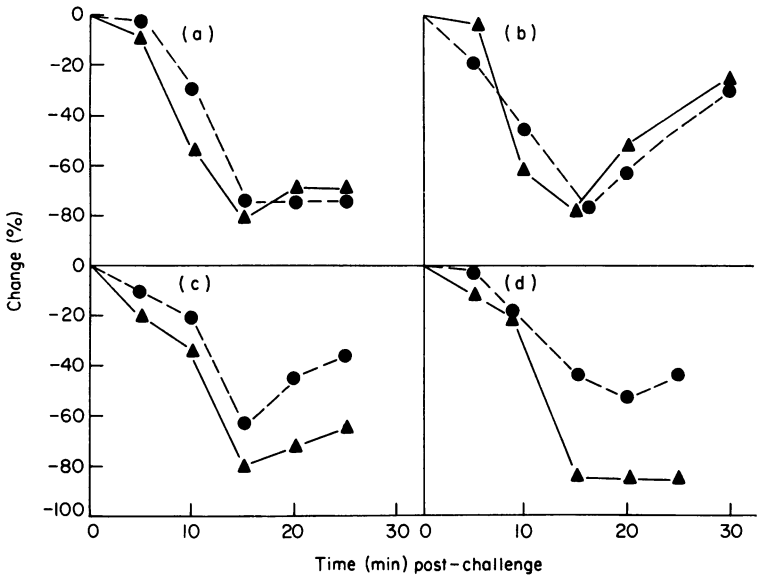

FIG. 1. Titration of rabbit anti-rat IgE induction of bronchospasm in normal rats. Anti-IgE was administered i.v. in a volume of 0·5 ml at zero time. Each plate represents the result of a single animal. (a) 200 units anti-IgE, (b) 100 units anti-I gE, (c) 50 units anti-IgE, and (d) 25 units anti-IgE. (▲———▲) Conductance, (● - - - ●) compliance.

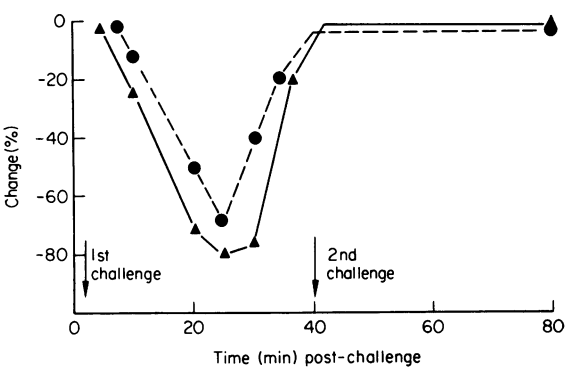

FIG. 2. Result in a typical rat double challenged with 50 units anti-IgE at zero time and 40 min later. (▲———▲) Conductance, (● - - - ●) compliance.

inhibition was calculated by comparison of the peak bronchospasm pulmonary function changes in drug-treated and non-treated rats. The results of a dose-response study are shown in Fig. 3. However, these data do not reveal that there were some animals which did not demonstrate the inhibitory effects of the drug, whereas the majority of the animals showed total inhibition at the two higher doses of 15 and 30 mg/kg, giving the appearance of an all-or-none effect. Further analysis of the dose-response data is presented in Table 1.

Another observation not apparent in the dose-response curve is the fact that in those animals not protected by drug or experiencing only slight changes in function, the peak loss of conductance and compliance occurred at a post-challenge time much later than in the control animals. In at least one animal, the peak changes occurred as late as 1 hr after challenge (Fig. 4). It was considered that the protracted response might have been due to a drug duration of activity longer than the 10 min pre-dose period. To investigate this possibility, two additional groups of rats were given DSCG (30 mg/kg, i.p.)

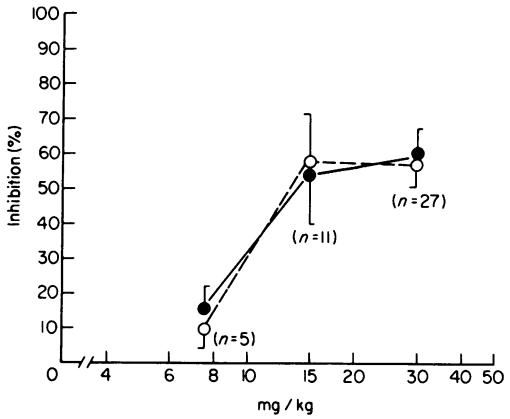

FIG. 3. Dose response inhibition of anti-IgE-induced bronchospasm by disodium cromoglycate administered i.p., 10 min prior to challenge with 50 units anti-IgE. Each point ± s.e. (●——●). Conductance, (○ - - - ○) compliance.

TABLE 1. Statistical summary of disodium cromoglycate inhibition of changes in anti-IgE induced bronchospasm

		Percentage inhibition									
		Conductance					Compliance				
Dose*	n†	Mean	(s.e.m.)	Median	Mode	Range	Mean	(s.e.m.)	Median	Mode	Range
30 mg/kg	27	60·5	(±8·3)	90	100	0–100	58·9	(±7·9)	73	100	0–100
15 mg/kg	11	55·1	(±14·8)	100	100	0–100	58·0	(±14·0)	100	100	0–100
7·5 mg/kg	5	15·6	(±6·7)	9	None	0–39	10·2	(±5·8)	0	0	0–32

* i.p., 10 min prior to anti-IgE challenge.
† Number of animals.

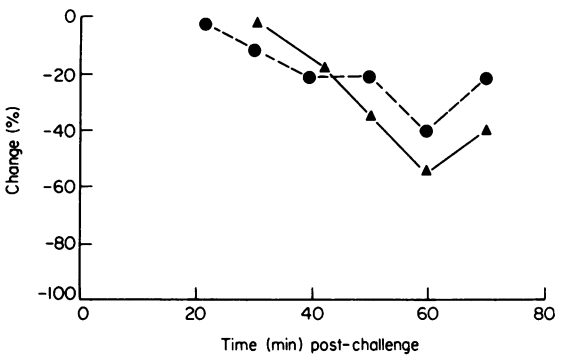

FIG. 4. Protracted response of an animal only partially protected by disodium cromoglycate, 30 mg/kg, i.p., 10 min prior to challenge with 50 units anti-IgE. (▲——▲) Conductance, (● - - -●) compliance.

either 1 hr or 2 hr prior to anti-IgE. As can be seen in Fig. 5, substantial activity was demonstrated after 1 hr, but not 2 hr pre-dosing. Again, those animals pre-dosed 10 min or 1 hr prior to challenge and not fully protected, had a peak loss of pulmonary changes at a time point much later than contol animals.

As mentioned previously, animals were generally refractory to a second administration of anti-IgE following successful and complete recovery from a primary bronchospasm. It was of interest to see if

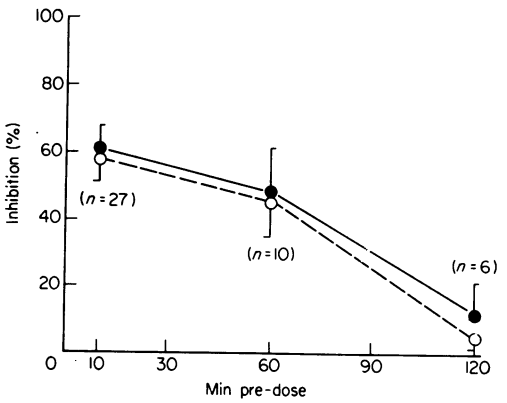

FIG. 5. Relationship of pre-dose time to inhibition of anti-IgE (50 units) induced bronchospasm in normal rats treated with disodium cromoglycate, 30 mg/kg, i.p. Each point ± s.e. (●———●) Conductance, (○ - - - ○) compliance.

animals that were totally protected by DSCG from the primary bronchospasm would also be refractory to a subsequent challenge. Animals were dosed with DSCG 30 mg/kg, i.p., 10 min prior to primary anti-IgE challenge and those totally protected were re-challenged 1 hr after the first challenge. Of the six animals tested, only one experienced bronchospasm following the second challenge; all others were refractory.

DISCUSSION

The results of this study reveal a new small animal model system with clear advantages over previous models used in this laboratory and by other investigators. It is possible to use normal animals rather than actively immunized animals of mixed Ig response, or passively sensitized animals in which it is not feasible to pre-determine the degree of sensitization prior to testing. The animals challenged with the anti-IgE gave a very reproducible response in terms of percentage change in conductance and compliance, as well as the peak time of these changes. The response was specific in that normal rabbit serum and anti-IgG failed to induce any changes.

Disodium cromoglycate was an effective inhibitor of the anti-IgE-induced bronchospasm in contrast to a previously described active lung anaphylaxis model where only marginal inhibition was demonstrated (Stotland & Share, 1974). However, it was quite clear that certain animals were non-responders with respect to drug efficacy while most others were fully protected at the two higher doses of 15 and 30 mg/kg. This wide range of inhibition is reflected in the rather large standard errors obtained in the dose-response and pre-dose time studies.

Another unique feature of the model is the time delay in the occurrence of maximum broncho-constriction of animals only partially protected by drug. Farmer *et al.* (1975) reported a passive lung anaphylaxis model in which rats are sensitized with high titre IgE-containing rat serum 24 hr prior to antigen challenge. Disodium cromoglycate, as well as FPL 55712 and methysergide, were efficacious in their system; however, the time course of bronchospasm was identical in drug-treated animals to that in control animals.

The observation that DSCG could demonstrate efficacy 1 hr after administration was unexpected in the light of its limited duration of action in the rat PCA (Thomson & Evans, 1977). The results of this study appear to be related more to the clinical situation in which DSCG has been demonstrated to have a dose-related duration of activity relative to changes in one second forced expiratory volume (FEV_1) in allergen-challenged asthmatic patients (Kolotkin, Lee & Townley, 1974; Orr, 1977). It is clear that DSCG at 30 mg/kg was capable of inhibiting bronchospasm after 1 hr, but not 2 hr pre-dosing. The pattern of bronchospasm and recovery in the animals in this experiment is similar to that shown by the afore-

mentioned patients (Orr, 1977) undergoing changes in FEV_1. The results reported here suggest that the anti-IgE model may be more useful in evaluating the duration of drug activity.

It was interesting that the animals were unresponsive to challenge following recovery from a bronchospasm. This lack of response is similar to desensitization typified by monkeys spontaneously sensitive to parasitic antigens (Patterson, Irons & Harris, 1975) or by actively sensitized guinea-pigs (Popa, Douglas & Bouhuys, 1974). The exact mechanism of the refractory state observed in this study is not known although it may be due to insufficient nascent IgE to re-sensitize the mast cell receptors, or a change in the IgE-receptor complex to a non-active state. The fact that such refractoriness was observed following successful protection by DSCG would tend to favour the latter possibility, assuming that the mechanism for refractoriness is the same in both instances.

The failure of the animals to respond to a second challenge after being successfully protected from the first challenge by DSCG may also reflect a mode of action of the drug. It is possible that part of the prophylactic nature of DSCG is to allow the individual to become refractory to his allergen environment while DSCG blocks the mediator release induced by primary allergen encounter. Thus, not only does DSCG block mediator release, but it also allows desensitization to occur. A similar hypothesis based on patient studies has been suggested previously (Kolotkin, Lee & Townley, 1974).

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