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A new *in vivo* method for the measurement of repetitive anaphylactic responses in the guinea-pig

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1 The established Konzett-Rossler bronchorespiratory model has been combined with a unique ovalbumin sensitization procedure to give a novel method to measure anaphylaxis in the anaesthetized guinea-pig.

2 Following antigen challenge, up to eight equal bronchoconstrictor responses to the same dose can be generated from a single animal over a 120 min period.

3 Total inhibition of the anaphylactic response can be elicited by four different classes of compound, namely salbutamol, mepyramine, theophylline and dimaprit. Cromoglycate failed to cause any inhibition.

4 The method is discussed with particular reference to the antigen sensitization procedure, which differs substantially from other regimens previously employed and gives rise to heat labile antibody.

Introduction

Today, one of the major therapeutic classes of compound employed in the treatment of asthma is the β-adrenoceptor agonist, but the precise mechanism of action is still not clear. The property of these agents in inhibiting mediator release has long been established (Assem & Schild, 1969) and more recently salbutamol was reported to inhibit histamine release from human chopped lung (Church & Young, 1981). However, whether this mast cell stabilizing activity manifests itself at therapeutic doses *in vivo* has been questioned (Jack, Harris & Middleton, 1978). Histamine itself, by acting on H_2-receptors, is known to inhibit its own release from human basophils (Bourne, Melmon & Lichtenstein, 1971; Lichtenstein & Gillespie, 1973). In addition, Chand (1979) and, more recently work from our laboratory using the H_2-agonist dimaprit (Rising & Lewis, 1982), has indicated that activation of the H_2-receptor inhibits antigen-induced mediator release from the guinea-pig isolated chopped lung.

As the first step in the demonstration of similar effects *in vivo*, an appropriate whole animal model for anaphylaxis would be required. Although numerous methods are available for use, both the sensitization procedures and the specific methods of measuring bronchorespiratory activity vary considerably. To our knowledge no method has been reported for the guinea-pig which allows repeated anaphylactic responses to be recorded in one animal. For the purpose of drug screening this experimental approach would enable each animal to act as its own control, and the duration of action to be estimated.

Initial data on such an animal model have previously been presented (Rising, Steward, Bottomley & Lewis, 1982) and are now given in more detail. Theoretical immunological considerations have been given to the sensitization procedure, and the actions of a β-adrenoceptor agonist (salbutamol), an H_2-agonist (dimaprit) and compounds normally associated with bronchorespiratory activity have been determined.

Methods

Sensitization procedure

Male Dunkin Harley guinea pigs weighing 250–300 g were divided into groups of 8–12 animals. Each group was placed in a perspex box measuring 400 × 250 × 250 mm and sprayed with an aqueous solution of ovalbumin delivered from a thin layer chromatography spray gun (Shandon). The particle size of the droplets delivered was on average 25–50 and each guinea-pig received approximately 5 ml over a 30 min period. The percentage ovalbumin concentrations (w/w) given were 1.0, 0.1 and 0.001 on days 0, 5 and 19 respectively.

Measurement of anaphylactic responses

Experiments to measure anaphylactic responses were carried out 12 to 17 days after completion of the sensitization programme. The guinea-pigs were premedicated with diazepam (2.5 mg kg^{-1} i.p.) and anaesthetized with Hypnorm (fentanyl 0.2 mg kg^{-1} plus fluanisone 10 mg kg^{-1}), intramuscularly. The jugular vein and trachea were cannulated and bronchorespiratory function was measured essentially by the method of Konzett & Rossler (1940). Briefly, the animals were automatically ventilated by means of a piston-pump with inspiratory pressure set at 100 mm H_2O, a tidal volume between 6 and 10 ml and a frequency of 48 strokes min^{-1}. Changes in bronchial reactivity were determined by measuring air overflow by means of a bronchospasm transducer.

Ovalbumin was adminstered intravenously at 15 min intervals until two anaphylactic responses of similar intensity were obtained.

When drug effects were being studied, the test compound was given intravenously, followed 2 min later by the antigen at a dose that had previously caused two similar bronchospasms. When a reduced anaphylactic response was observed, further ovalbumin was administered at 15 min intervals to test for duration of action.

Determination of antibody levels

Sera were obtained from two different batches of guinea-pigs undergoing the ovalbumin sensitization, at various times during the treatment period. Each sample was divided into two, one half being heated at 56°C for 4 h to inactivate any labile antibody present. Four skin sites were injected intradermally with 0.1 ml dilutions of the antisera on each lateral shaved side of a guinea-pig (female, Dunkin Hartley, 450–500 g). After 4 h the guinea-pigs were challenged by intravenous administration of a physiological saline solution containing ovalbumin and Evans blue, each at 10 mg kg^{-1}. The antisera titres were determined by observing the maximum dilution of sera which gave a blue skin lesion 20 min after challenge. Results were calculated as mean values from 4–8 animals bled on each occasion, with all serum samples assayed in duplicate.

Materials

Salbutamol, disodium cromoglycate and mepyramine were obtained from Glaxo Laboratories Ltd, Fisons Ltd and May & Baker Ltd respectively and dimaprit was synthesized in our own laboratories.

Results

Control anaphylactic responses

A typical series of anaphylactic responses produced in the sensitized guinea-pig is shown in Figure 1a. In general two low priming doses were required prior to the antigen level which subsequently gave equivalent responses, and this latter ovalbumin concentration

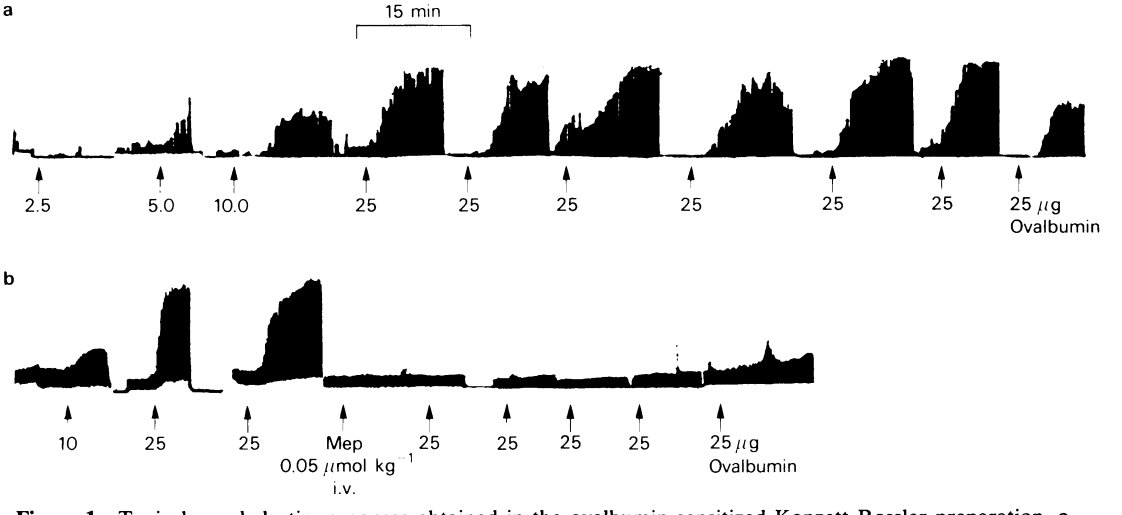

Figure 1 Typical anaphylactic responses obtained in the ovalbumin sensitized Konzett-Rossler preparation. **a** Control responses produced from the concentration of ovalbumin indicated, administered intravenously at 15 min intervals; (b) The effect of a single intravenous dose of mepyramine (Mep) (0.05 μmol kg^{-1}) on the immediate and subsequent anaphylactic responses produced by 25 μg ovalbumin.

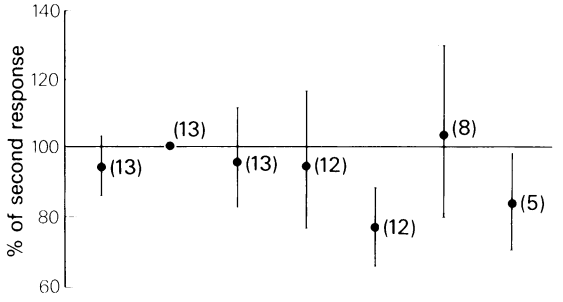

Figure 2 Variations in the anaphylactic responses elicited by ovalbumin. Of the 2 responses of similar intensity, following the initial priming doses of ovalbumin, the 2nd has been designated a level of 100% and the relative sizes of the previous and subsequent responses have been calculated as percentages. The mean with s.e.mean (vertical lines) of these percentage values is presented, for 7 succesive responses with the number of determinations on each response given in parentheses (e.g. the relative size of the 6th response was determined on 8 occasions).

was commonly 25 or 50 μg per guinea-pig. For all guinea-pigs sensitized by the prescribed protocol, approximately 75% were found to give a similar pattern to that depicted in Figure 1a, although the actual size of the response varied. Departures from the given sensitization procedure (e.g. 0.1% ovalbumin on day 19 or the second dose on day 7) appeared to result in atypical and unusable responders.

The reproducibility of the anaphylactic response is indicated in Figure 2. For several control experiments, all responses following those produced by the initial priming doses are presented, having been expressed as a percentage of the second full response. Although there is a degree of variation, there were no statistically significant differences ($P > 0.05$) between the calculated values. Thus for testing the bronchorespiratory effects of compounds in this model, the consistency of the response can be assumed.

Antibody titres

Serum antibody titres were determined on days 0, 5 and 19 directly preceding ovalbumin sensitization, and on day 31, when the guinea-pigs were normally used experimentally. Zero titres were observed on days 0 and 5. On day 19 the antibody titre was 170, of which approximately 20% was heat labile, whereas on day 31 following the third antigen sensization, the titre had increased dramatically to 1920, of which about 42% was heat labile. Analysis of the total and heat stable titres of each sample by a paired *t* test, gave a statistically significant difference ($P < 0.05$) between the values for the day 31 sera.

Table 1 Inhibition of the anaphylactic response in the sensitized Konzett-Rossler model

Drug	ED_{100} (μmol kg^{-1})	*No. of experiments*
Salbutamol	0.35	4
Mepyramine	0.05	8
Theophylline	38.8	3
Dimaprit	100.0	4
Sodium cromoglycate	Inactive at 20	6

ED_{100} values were the minimum intravenous doses which gave 95–100% inhibition of the maximum anaphylactic response.

Drug effects on the anaphylactic response

The β-agonist salbutamol, the H_2-agonist dimaprit and the H_1-antagonist mepyramine totally inhibited the antigen-induced bronchoconstriction at widely differing doses (Table 1). A typical experimental result is shown in Figure 1b for mepyramine, where following ovalbumin challenge at 15 min intervals, the anaphylactic responses gradually returned to the control value, normally after approximately 90 min. Similar results were obtained with all 3 compounds, the duration of inhibition depending on the level of dose administered.

Theophylline also inhibited the anaphylactic response, thus allowing an ED_{100} value to be calculated (Table 1). However, disodium cromoglycate at doses

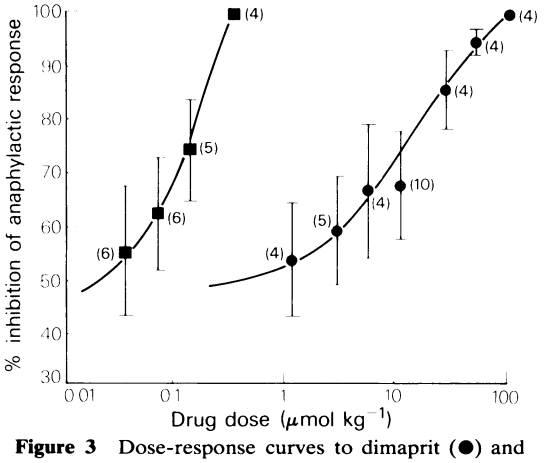

Figure 3 Dose-response curves to dimaprit (●) and salbutamol (■) in the ovalbumin-sensitized Konzett-Rossler preparation. Each value represents the mean for the number of experiments given in parentheses, vertical lines indicate s.e.mean.

up to 20 μmol kg^{-1} (i.v.) repeatedly failed to diminish the induced bronchospasm.

The potential of the model for estimation of ED_{50} values was assessed using salbutamol and dimaprit. With both compounds dose-response curves were constructed (Figure 3), and ED_{50} values were calculated to be 0.02 and 0.50 μmol kg^{-1} for the β-agonist and H_2-agonist respectively.

Discussion

The present animal model for studying drug effects on the anaphylactic response combines the well-established Konzett-Rossler anaesthetized guinea-pig method with a novel procedure for antigen sensitization. Although the former has been used for similar studies (Collier & James, 1967), the latter is of prime importance in obtaining reproducible bronchospasm responses and it is of value to discuss it in relation to other sensitization regimens often employed before *in vivo* experimentation.

Although ovalbumin is the normal antigen administered, sera have also been employed (Takashima, Ono, Ohtsuka, More & Kumada, 1979; Martin & Romer, 1978a). Using ovalbumin, both intravenous and intramuscular routes have been reported and adjuvant in the form of *Bordetella pertussis* (Holroyde, Smith & Holme, 1980), *Haemophilus pertussis* (Martin & Romer, 1978b) or Freund's adjuvant (Advenier, Mallard, Santais & Ruff, 1979) have been co-administered. More recently, Andersson (1981) sensitized guinea-pigs with differing concentrations of ovalbumin plus various levels of $Al(OH)_3$ with and without cyclophosphamide. These variations produced marked changes in the observed anaphylactic response, which were also influenced by the timing of booster antigen doses. Such booster injections are often a feature of the sensitization programme, but normally only one is given and this is administered less than 10 days after the initial antigen dose. The final major variable is the dose level, which with ovalbumin can vary from 5 mg (Advenier *et al.*, 1979) to 0.5 μg (Andersson, 1981) per guinea-pig.

In the present sensitization regimen an ovalbumin aerosol inhalation procedure is employed, which although in itself is not entirely novel, when combined with the timing and the relative concentrations of the doses, results in unique anaphylactic responses. The rationale behind such timings is that lower antigen booster injections at specific intervals might induce the synthesis of high affinity homocytotropic antibodies. It was found that guinea-pigs undergoing the full sensitization procedure had heat-labile reaginic antibodies in significant amounts. Using adjuvants, Perini & Mota (1973) and more recently Andersson (1981, 1982) have obtained similar results and have characterized the antibody as IgE. In the present study the identity of the heat labile antibodies was not determined.

The various drugs examined, with the exception of cromoglycate, gave positive results in the model. The lack of effect with cromoglycate was somewhat disappointing in a procedure designed in part to investigate mast cell stabilizers. However, this anti-asthmatic drug has frequently been reported to be either inactive or inconsistent in its action, particularly in the guinea-pig, as discussed by Cairns (1979). Total inhibition of the antigen-induced bronchoconstriction was observed with salbutamol, mepyramine, theophylline and dimaprit, and with the β_2- and H_2-agonists ED_{50} values were also determined. Similar *in vivo* data have not been reported for dimaprit. However, in the Konzett-Rossler preparation with acetylcholine used as spasmogen, salbutamol had ED_{50} and ED_{100} values of 0.07 and 0.35 μmol kg^{-1} respectively (Brittain, 1971). These can be compared with corresponding values of 0.02 and 0.35 μmol kg^{-1} determined in the present study. The observation that the H_1-antagonist mepyramine acts as a potent inhibitor, is evidence for the direct involvement of histamine in the observed ovalbumin-induced bronchospasm.

The nature of the antibodies involved requires further study. It is possible that both IgG and IgE are responsible for the observed effects, as suggested by Andersson (1981) who also used similarly low antigen challenge doses, but this remains to be determined.

Overall, the present experimental procedure provides a model where, in a single guinea-pig, control anaphylactic responses, acute drug effects and duration of drug action can be monitored. The relevance to similar allergic responses in man will have to await further evaluation.

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