

Antigen-Induced Bronchial Anaphylaxis in Actively Sensitized SD Rats

Effects of Immunization and Provocation Doses of Antigen and of Pretreatment with DSCG, Theophylline, Terbutaline and a New Anti-Allergic Xanthine Derivative, D 4026

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Bronchial anaphylactic reactions, estimated as increase in intratracheal pressure, were precipitated by intravenous injections of antigen into actively sensitized SD rats. The degree of bronchial reactivity was found to depend on both the challenge dose and the immunization dose of antigen; therefore the course of the capacity to respond was recorded as a function of these variables.

The degree of the bronchial anaphylactic response could be reduced by pretreatment with disodium cromoglycate, terbutaline or a new anti-allergic xanthine derivative, D 4026, in some groups of animals. The efficacy of each agent was found to depend on the dose of antigen used for sensitization and for provocation of the bronchial reaction rather than on the strength of the response.

Taken together, the data suggest that more than one type of homocytotropic antibody mediates bronchial anaphylactic reactivity in the SD rat.

Key words: bronchial anaphylaxis; DSCG; ovalbumin sensitized rat; terbutaline; theophylline.

Accepted for publication 2 July 1981.

CLINICAL ASPECTS

A rat model of asthma based on the principle of antigen-induced bronchial anaphylactic reactivity might more adequately be able to select agents with potential clinical usefulness in acute treatment. The present report describes the effect of varying immunization and provocation doses of antigen on the bronchial anaphylactic response capacity, measured as increased intratracheal pressure. Pretreatment of animals with DSCG, theophylline, terbutaline or D 4026, a new anti-allergic xanthine derivative, reduced the bronchial anaphylactic response in some of the immunized groups of animals.

Reagin-mediated allergic reactions are common asthma-precipitating events. The successful introduction in clinical practice of disodium cromoglycate (DSCG) stimulated interest in the development of new, orally effective “anti-allergic” agents for prophylactic use in asthma. This work primarily exploited the reagin-mediated immediate hypersensitivity reaction with

the rat skin and the release of histamine from peritoneal mast cells as discriminating tools. The disappointing clinical experience with several of the potent DSCG-like agents (for review, see 13) has clearly revealed the inadequacy of these models in their present design and has stressed the need of a reliable model of human extrinsic asthma in small laboratory ani-

* Subsidiary to AB Astra, Sweden.

Abbreviations used: DSCG, disodium cromoglycate; OA, ovalbumin; SD, Sprague-Dawley.

mals. The use of animal models of asthma based on the principle of antigen-induced bronchial anaphylactic reactivity would perhaps better select agents with potential clinical usefulness for acute treatment. Farmer et al. (18) claimed that inhibition of *passive* lung anaphylaxis in the rat is no more predictive as a screen for anti-allergic activity than passive cutaneous anaphylaxis. *Active* bronchial anaphylactic reactions have often been used as an instrument for drug selection. The guinea pig model has been discredited because IgG₁, and not IgE, is considered the major homocytotropic antibody in this species and DSCG is rarely effective in this model. However, Andersson (2) and Carney (7) have shown that bronchial anaphylactic reactivity in this species can be reduced by DSCG provided that immunization and challenge doses of antigen are carefully chosen.

Bronchial anaphylactic reactivity in rats, presumably due to the action of IgE antibodies fixed to lung tissue mast cells, has been studied by several authors. Church and coworkers sensitized animals by injection of the nematode *Nippostrongylus brasiliensis* (11, 12, 14). Church & Miller (15), like Stotland & Share (32, 33) and others (17, 26), used i.p. injection of ovalbumin (OA) in alum or *B. pertussis* (8, 9). Casey & Abboa-Offei challenged normal rats with anti-IgE (10), whereas passively sensitized animals were examined by Church (11) and by Farmer et al. (18). These studies indicate that the humoral factors involved in the mediation of human asthma and guinea pig and rat bronchoconstriction vary with the species used, but that the anaphylactic bronchoconstriction in the rat immunologically resembles that in human bronchial asthma (14).

Appropriately sensitized rats are known to be effective producers of IgE antibodies to various antigens, e.g. OA. However, it is well known that the antigen dose, the nature and dose of the adjuvant, the number of injections, the possible pretreatment with immunosuppressive drugs, the age of the rat, etc., have a profound influence on the degree and the kinetics of IgE antibody response (4, 21, 27–30). Furthermore, the antigen dose at challenge is an important

parameter determining the strength of the ensuing bronchial anaphylactic reaction and its inhibition by “anti-allergic” drugs both clinically and in bronchial anaphylaxis in guinea pigs *in vitro* (1, 2, 20). The purpose of the present study was to define, in SD rats, optimal conditions for induction of a persistent state of reagent-mediated anaphylactic reactivity and its provocation. We thought that such a model would be suitable in tests for acute and long-term effects of agents with potential anti-asthmatic effects.

MATERIAL AND METHODS

Animals and immunization

The animals used were outbred male Sprague-Dawley with a bodyweight of about 200 g obtained from Møllegaards Avlcentrum, Ejby, Skensved, Denmark. They were kept under conventional conditions. The animals were sensitized by intraperitoneal injections of ovalbumin (OA; Sigma grade III, crystallized and lyophilized) in 0.5 ml saline containing 100 mg dried and resuspended Al(OH)₃ (F 2200 Reheis Chemical Company, obtained through AB Astra, Södertälje), 10 mg amorphous Silica (Aerosil, particle diameter 25–50 nm, Degussa, Frankfurt am Main, obtained via Matak, Malmö, Sweden) or no addition of adjuvant. Selected groups of animals were given booster injections of OA alone or OA in Al(OH)₃, as specified in the text. In some groups of animals, the primary or the secondary injections of OA were preceded by i.p. injections of either 35 or 50 mg/kg cyclophosphamide (CY; Sendoxan®, Pharmacia, Sweden). Other groups of animals were given an injection of 10¹⁰ organisms of *B. pertussis* (SBL, Stockholm, Sweden) 4 days before test. Passive immunization was attempted by giving SD rats a volume of a specified serum intravenously (penis vein) at a varying interval before the test.

Respiratory measurements

At the indicated time after active or passive immunization, the animals were tested. Groups

of normal animals were also examined. The animals were anaesthetized with 50 mg/kg i.p. sodium pentobarbitone (Mebumal®, ACO, Sweden), succinylcholine chloride (Celocurin®, Vitrum, Sweden), 5 mg/kg, was administered i.p. to prevent spontaneous respiration. Respiratory measurements were made essentially as described by Stotland & Share (32).

The trachea was cannulated and the rat was ventilated with a Braun constant respiratory pump in a closed system. Tracheal pressure was recorded through a side-arm of the cannula connected to a Statham P23AC pressure transducer. The respiratory rate was set at 80 strokes/min with a tracheal pressure of 10 cm H₂O (tidal volume range 2.0–3.75 ml). The mean arterial blood pressure was recorded by a Statham P23AC pressure transducer throughout the experiment through a catheter inserted into the right carotid artery. The blood pressure pulses were transmitted to a tachogram, where the heart rate was registered. All recordings were made on a Grass polygraph model 7B. The animals were challenged with various doses of OA (5–5000 µg/animal) in 0.5 ml saline injected through a catheter inserted into the right jugular vein. The consequent increase in the tracheal pressure was used as an index of bronchoconstriction.

Selected groups of sensitized animals were treated i.v. 1 min before antigen challenge with one of the following agents: disodium cromoglycate (DSCG, a gift from Fisons, U.K.) in a dose of 1 mg/kg; theophylline (batch 11047, AB Draco, Sweden) in a dose of 0.3 mg/kg; terbutaline (AB Draco, Sweden) in a dose of 0.05 mg/kg, or 3,7-dihydro-1,8-dimethyl-3-phenyl-1H-purine-2,6-dione (D 4026, Draco, Sweden – a new anti-allergic xanthine derivative*) in a dose of 0.1 mg/kg. In these experiments, the animals were challenged with 300 µg OA followed a few minutes later by 5000 µg OA as described under Results. Separate experiments showed no difference in the strength or the characteristics of a bronchial anaphylactic response to the large challenge dose of OA whether or not it had been preceded by a chal-

lenge with a small dose of OA. Selected groups of animals, normal or sensitized, were also given serotonin (Sigma Chem. Co.) or metacholine (Sigma Chem. Co.) in a dose of 50 µg/kg, and 10 µg/kg, respectively, to induce bronchoconstriction.

All compounds, except D 4026, were dissolved and diluted in 0.9% NaCl (saline). D 4026 was dissolved in an equimolar dose of 0.5 M NaOH and diluted in 0.9% NaCl (saline).

Statistics

The figures recorded for bronchial anaphylactic response were found to be normally distributed, where a sufficient number of experiments were performed. Mean values and standard error of the mean were used for graphical illustration. Results of experiments for evaluating inhibitory capacity of drugs were statistically tested with analysis of variance.

RESULTS

Characteristics of response

At i.v. challenge with an adequate dose of OA, appropriately sensitized animals show an anaphylactic response in the form of an increase in intratracheal pressure (Fig. 1). The response is normally fully developed within 1–2 min. The arterial blood pressure is about 120 mmHg before challenge and decreases immediately after challenge to 50 mmHg. The heart rate is about 400 beats per minute before challenge, after challenge it decreases to subnormal values but soon recovers its initial ration.

Effect of varying immunization and challenge doses of OA on bronchial anaphylactic response 14 days after immunization

Groups of animals were injected with OA together with 100 mg Al(OH)₃. The dose given was either 0.1, 1, 10, 100 or 1000 µg OA. Fourteen days after immunization, the animals were used for testing. Each animal was chal-

* European patent application, publication No. 0001735.

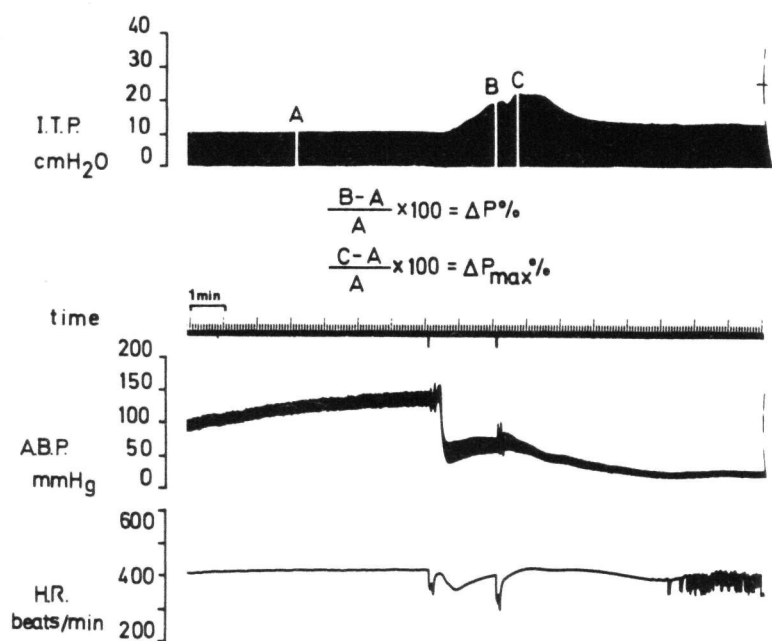


Fig. 1. Bronchial anaphylactic response to challenge with 300 µg OA (B) and 5000 µg OA (C) given i.v. to an SD rat immunized with 10 µg OA in 100 mg Al(OH)₃ 14 days before test. The way of expressing the degree of the response (Δ P %) and the maximal response (Δ P_{max} %) is indicated. Changes in arterial blood pressure (A.B.P.) and heart rate (H.R.) are also shown.

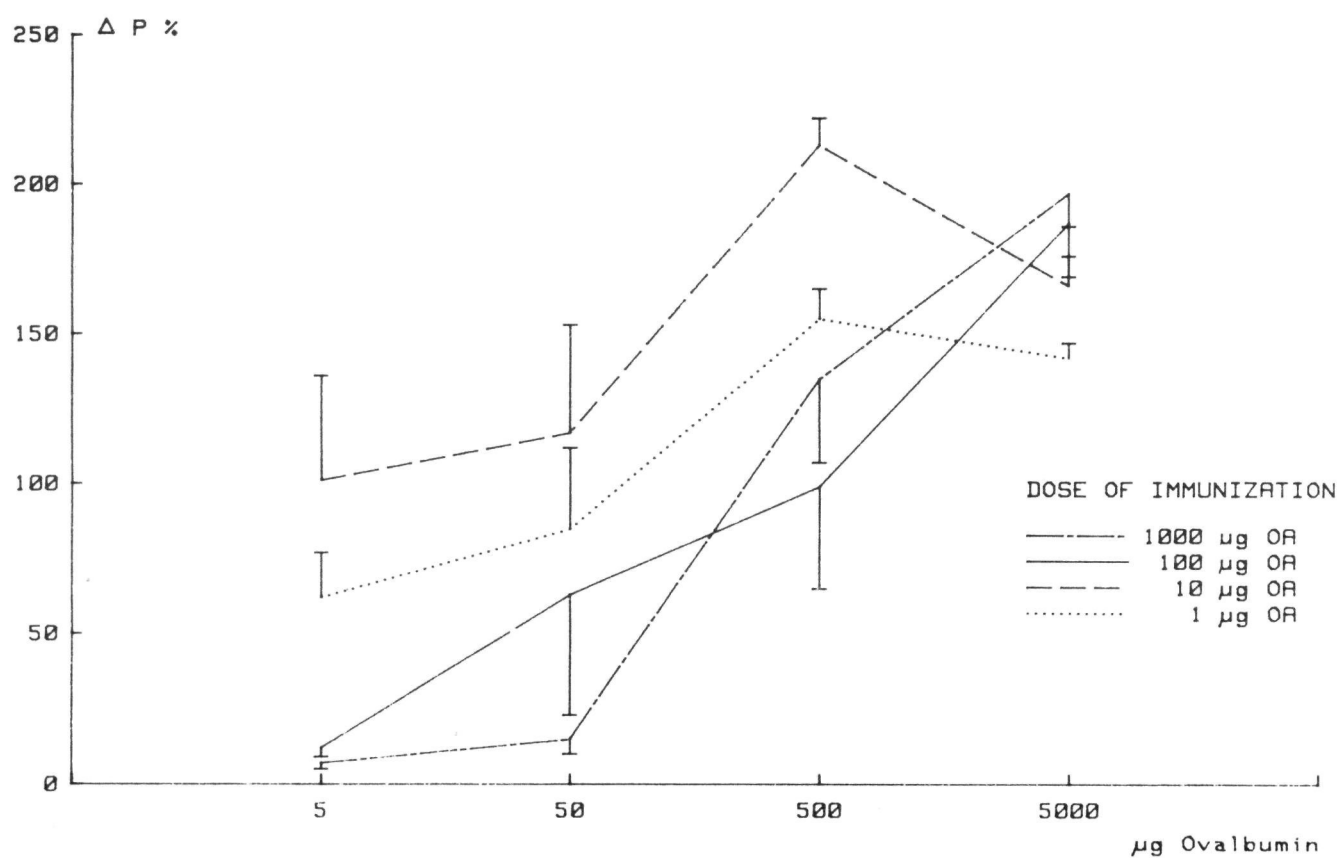


Fig. 2. Antigen-induced bronchial response (Δ P %) in animals immunized with 1, 10, 100 or 1000 µg of OA together with 100 mg Al(OH)₃ 14 days before test. Challenge dose of OA is indicated on X-axis. Each point is the mean of five experiments. Verticals denote SEM.

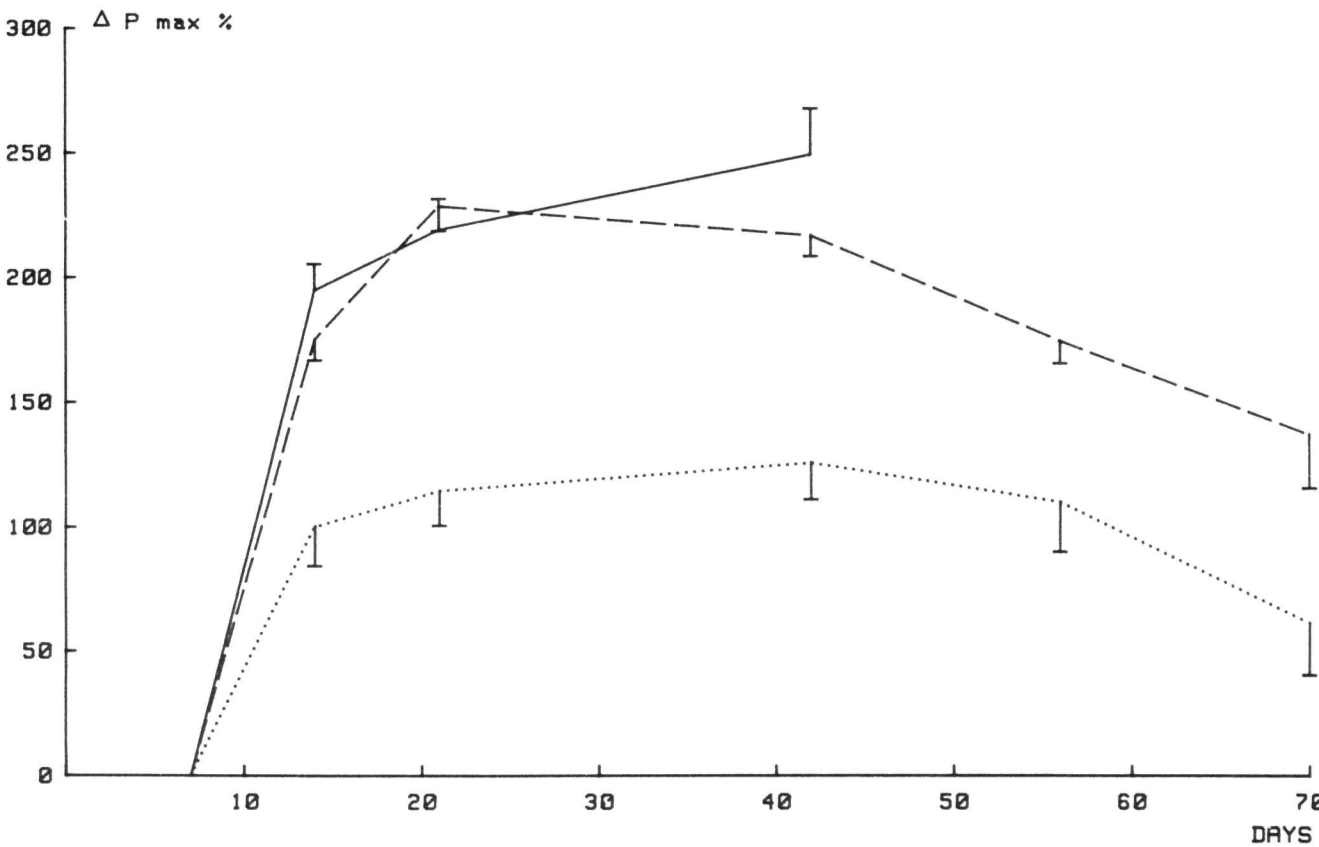
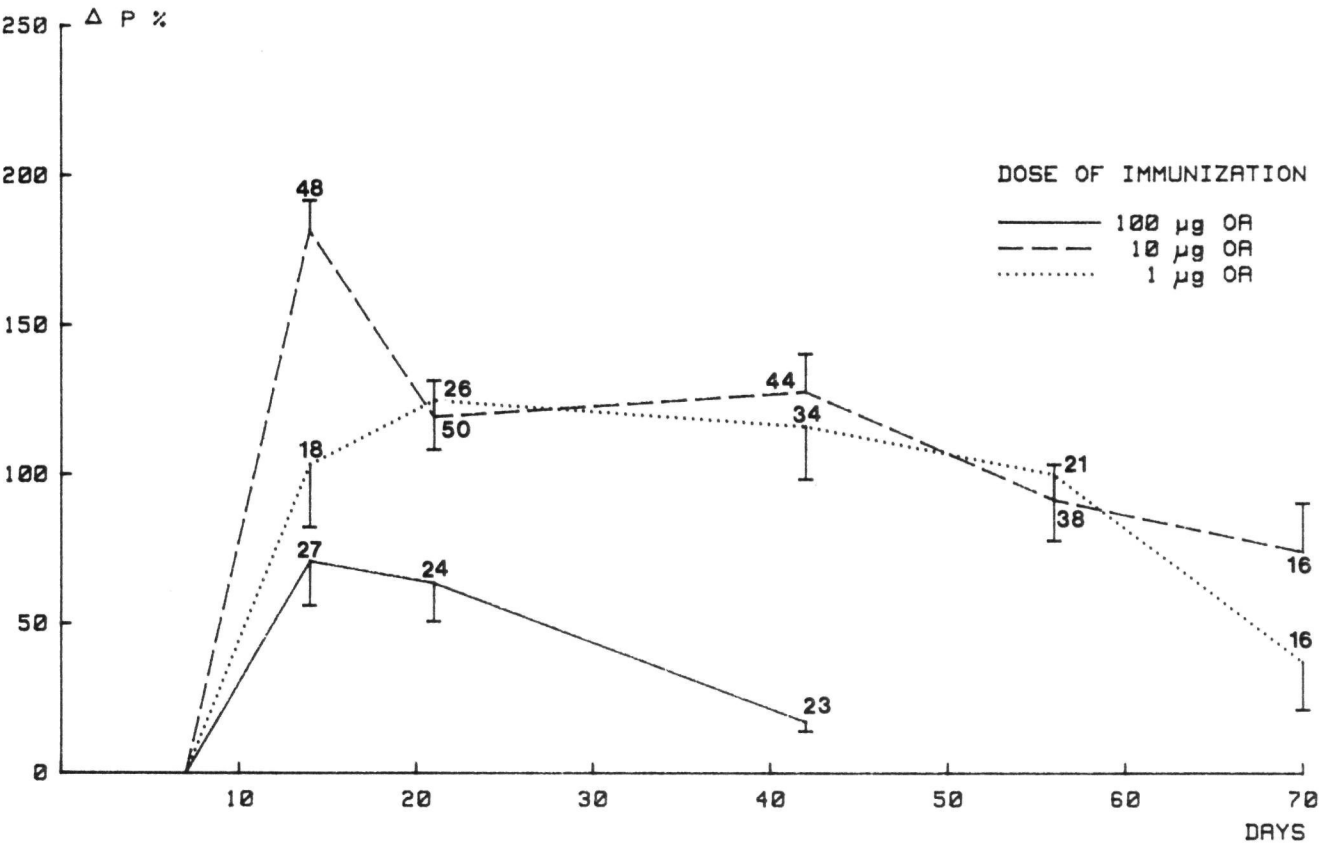


Fig. 3. Temporal pattern of development of bronchial anaphylactic response capacity in animals sensitized with 1, 10 or 100 μ g OA together with 100 mg $Al(OH)_3$ and challenged with 300 μ g OA (frame A) or 5000 μ g (frame B). Figures denote number of observations at each point.

lenged with either 5, 50, 500 or 5000 μ g OA. A single dose of antigen was given to each animal. Fig. 2 shows the results recorded. Animals

given 0.1 μ g OA did not respond to any provocation dose. However, the rats sensitized with 1 μ g OA and especially those given 10 μ g OA

and alum reacted strongly on provocation with even comparatively small doses of OA (5 or 50 μg). Such animals responded maximally to 500 μg OA; dose of 5000 μg OA did not provoke any further increase in reactivity. Rats given 100 μg OA or 1000 μg OA in alum responded only weakly to small challenge doses but maximally to large ones.

Temporal development of capacity to mount a bronchial response in rats immunized with 1, 10 or 100 μg OA in alum or Silica gel

Animals immunized with 10 μg OA 20 days before the test were further examined with respect to the effect of the challenge dose. Only two out of nine animals given 50 and 150 μg OA responded with a pressure increase of more than 50%. All animals responded to 300 μg . Based on these observations, an initial challenge dose of 300 μg OA and a cumulative dose of 5000 μg OA were chosen to monitor the temporal development of the anaphylactic reactivity in rats sensitized with 1, 10 or 100 μg OA together with alum. The capacity to respond was examined at the indicated times from 7 to 70 days after immunization. Fig. 3a shows the results obtained with the small challenge dose. Rats given 1 μg OA developed a prolonged state of bronchial anaphylactic reactivity reaching peak level at 21 days and beginning to diminish 6–8 weeks after immunization. In animals sensitized with 100 μg OA in alum the anaphylactic reactivity was not as strong on day 14 and had disappeared by 6 weeks after immunization. The development of the response capacity in rats immunized with 10 μg OA in alum apparently reflects elements from two patterns; an early short-lived peak of increased reactivity followed by a longer phase of somewhat lower reactivity. Fig. 3b shows the responses to the large challenge dose. The course of the response capacity was similar to that in the animals given the small challenge dose of 1 μg OA. However, animals sensitized with 10 μg and particularly those given 100 μg OA responded more vigorously to the large challenge dose than to the smaller one.

Table 1 shows the correlation between the response to the small (ΔP) and that to the large challenge dose (ΔP_{max} %). In the rats given 1 μg OA there was a highly significant correlation between these two parameters. However, in rats given 10 μg or 100 μg OA there was no such correlation except in the animals given 10 μg OA and tested on day 14 after immunization. We also examined the response capacity of animals pretreated with 50 mg/kg i.p. of CY 2 days before immunization with either 1 or 10 μg OA in alum. Such treatment did not clearly influence the bronchial response capacity. The effect of the use of Silica gel as an adjuvant was examined as well. Rats immunized with 1 μg OA and 10 mg Silica gel 28 days before test did not respond to the 300 μg OA provocation dose and only one out of three animals responded to 5000 μg OA. When immunized with 10 μg OA and 10 mg Silica gel 14 days before test, the animals responded to both the 300 μg OA and the 5000 μg OA challenge dose but the responses were not as strong as those observed in the animals immunized with the same amount of OA in alum (not shown).

Finally, eight SD rats were given 10 μg OA without any adjuvant. They were challenged after 14 days. None of the animals responded to the small (300 μg) or to the large (5000 μg) OA provocation dose.

Effects of secondary antigen injections with or without pretreatment with cyclophosphamide

Animals immunized with low doses of OA (0.1 or 10 μg) and alum were given repeated injections of 0.1 or 5 μg OA with or without alum and were challenged 4 or 14 days after their last injection. These animals did not differ from the controls.

The effect of treatment with CY on the secondary response in animals primarily given 10 μg OA and alum or 10 μg OA and Silica gel was then examined. The results are given in Fig. 4. In neither of these two experiments did CY-treated animals respond better to a secondary antigen injection than the animals given saline instead of CY. However, in experiment A animals given CY tended to respond better

Table 1

Correlation coefficients (r) recorded at calculation between response precipitated by a small (300 µg OA) and a large (5000 µg OA) challenge dose of antigen. The number of animals used is shown in brackets

	Time for test (day)					Immunization dose
	14	21	42	56	70	
Δ P % vs Δ P max %	0.81*** (18)	0.81*** (26)	0.90*** (34)	0.89*** (21)	0.81*** (19)	1 µg OA
Δ P % vs Δ P max %	0.76*** (48)	0.12 (50)	-0.08 (44)	0.29 (38)	0.35 (16)	10 µg OA
Δ P % vs Δ P max %	0.17 (27)	-0.10 (24)	-0.03 (23)	ND	ND	100 µg OA

*** P < 0.001.

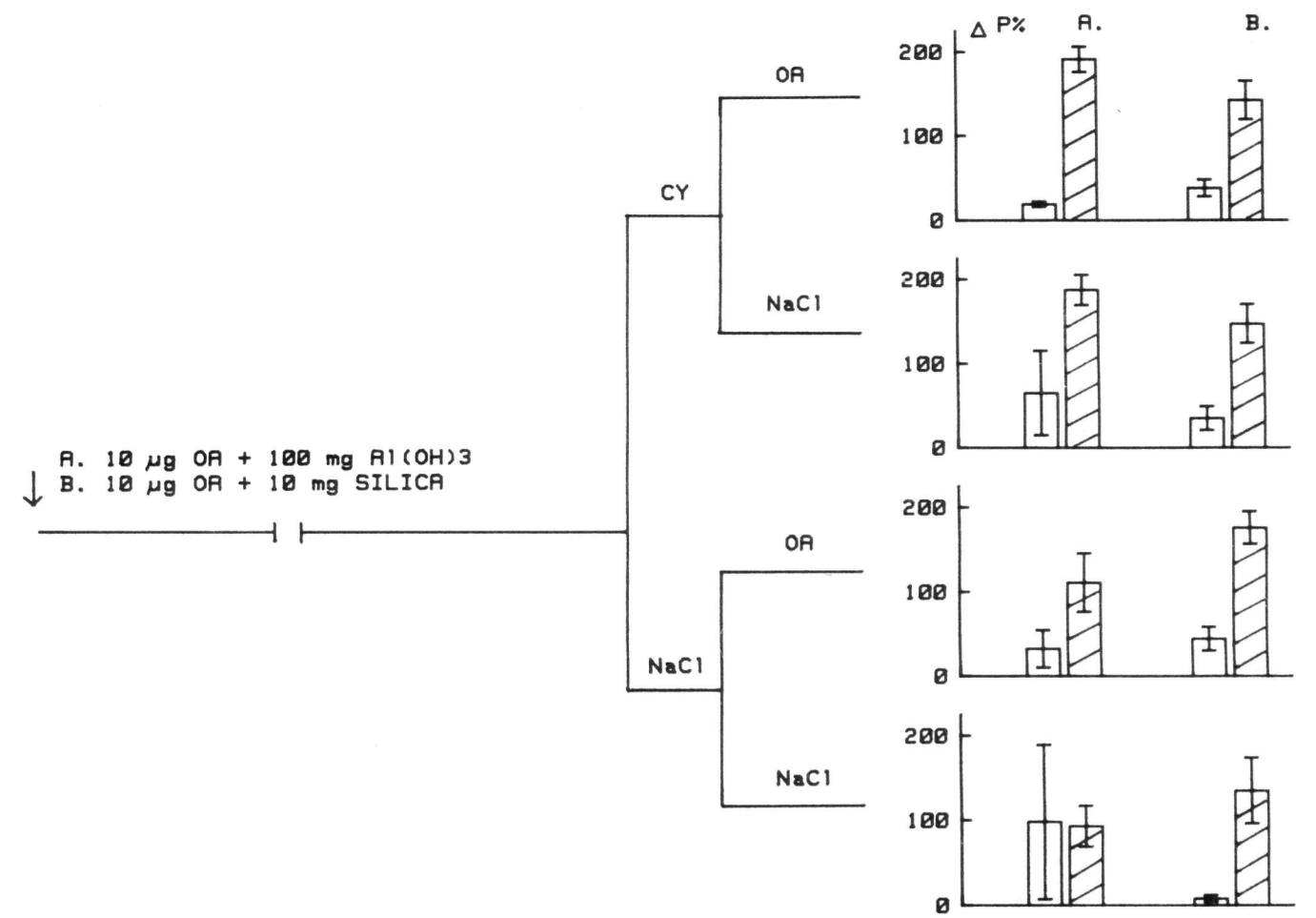


Fig. 4. Bronchial anaphylactic reactivity in rats primarily immunized on day 0 with 10 µg OA with Al(OH)₃ (experiment A) or Silica gel (experiment B) and boosted on day 61 (experiment A) or on day 30 (experiment B) with 10 µg OA with or without pretreatment 2 days before booster with 35 mg/kg i.p. of CY. Tests were performed 26 days after booster. Challenge doses of antigen: 300 µg OA (blank columns) and 5000 µg OA (hatched columns). Each value is the mean of four or five experiments. Columns denote mean of results from animals; verticals denote SEM.

to the 5000 μg OA challenge dose than animals given saline, irrespective of booster treatment.

Animals given 1 μg OA + 10 mg Silica on days 0, 28, 42, 56 and 70 were challenged 14 or 28 days after the third or the fifth antigen injection. No difference in the bronchial anaphylactic reactivity was found when the animals were given three or five injections of OA and Silica gel, but the responses persisted longer and the reactivity was higher than after a primary immunization.

A further group of SD rats were immunized by repeated injections of 1 μg OA without adjuvant 5 days a week for 2 weeks. They were challenged 10 days after the last injection. These animals responded weakly to the 300 μg OA challenge dose ($\Delta P\% = 30 \pm 13$, $n = 6$) and moderately to 5000 μg OA ($\Delta P_{\text{max}}\% = 131 \pm 27$, $n = 6$).

Effect of treatment with B. pertussis on bronchial reactivity

An i.p. injection of *B. pertussis* was given to a group of rats 26 days after immunization with 1 μg OA in $\text{Al}(\text{OH})_3$. No difference was found in bronchial anaphylactic response capacity between the animals treated with *B. pertussis* or saline 4 days after challenge (not shown).

Effects of sensitization on bronchial response to the constrictor serotonin and methacholine

Twenty-four SD rats were immunized, 12 with 1 μg OA in alum and the remaining 12 with 10 μg OA in alum. On days 25 and 56, six animals in each group and six normal animals were challenged with the bronchoconstrictors serotonin (50 $\mu\text{g}/\text{kg}$) and methacholine (10 $\mu\text{g}/\text{kg}$). In the doses used, both serotonin and methacholine induced suboptimal degree of bronchoconstriction in the normal animals. The immunized rats did not show increased bronchial reactivity compared with the controls to either of the two constrictors.

Attempted passive sensitization

SD rats with a bodyweight of 200 g were passively immunized intravenously, intraperitoneally or intramuscularly with 1 ml of a serum pool, obtained from rats actively sensitized with 10 μg OA in alum 19 days before harvest. Sera from such animals contain IgE-antibodies (3). The passively sensitized animals were challenged 72 hours after transfer, first with a small, and then with a large, antigen dose. None of them responded with changes in intratracheal pressure, but there was a clear fall in blood pressure from 120 mmHg to 50 mmHg.

Effect of anti-asthmatic agents on the bronchial anaphylactic response

Groups of animals immunized with 1 μg or 10 μg OA in $\text{Al}(\text{OH})_3$ 21 or 42 days before being tested were treated with DSCG, terbutaline, theophylline or D 4026 or with saline as a control 1 min before challenge with a small (300 μg OA + 100 mg $\text{Al}(\text{OH})_3$) the inhibitory effects of D 4026 and DSCG were less marked on day 21 than on day 42. One to two minutes later, when the response to the small antigen dose was fully developed and had begun to decline (see Fig. 1), a new antigen challenge dose of 5000 μg OA was given. The effect of pretreatment with DSCG and D 4026 was also examined in animals immunized with 10 μg OA 14 days before the test. The experimental approach is exemplified in Fig. 5, which shows that in animals given 10 μg OA + 100 mg $\text{Al}(\text{OH})_3$ the inhibitory effects of D4026 and DSCG were less marked on day 14 than on day 42 despite similar bronchial anaphylactic reactions in the control animals on days 14 and 42. The effects of the drugs are summarized in Fig. 6. Theophylline, in a sub-bronchodilatory dose, did not effectively inhibit the bronchial anaphylactic reactivity in any of the test conditions. If anything, it tended to enhance the bronchial anaphylactic response in animals immunized with 1 μg OA 42 days before the test. DSCG, terbutaline and D 4026 all showed slight, inhibitory effects in animals examined 14 or 21 days after sensitization with

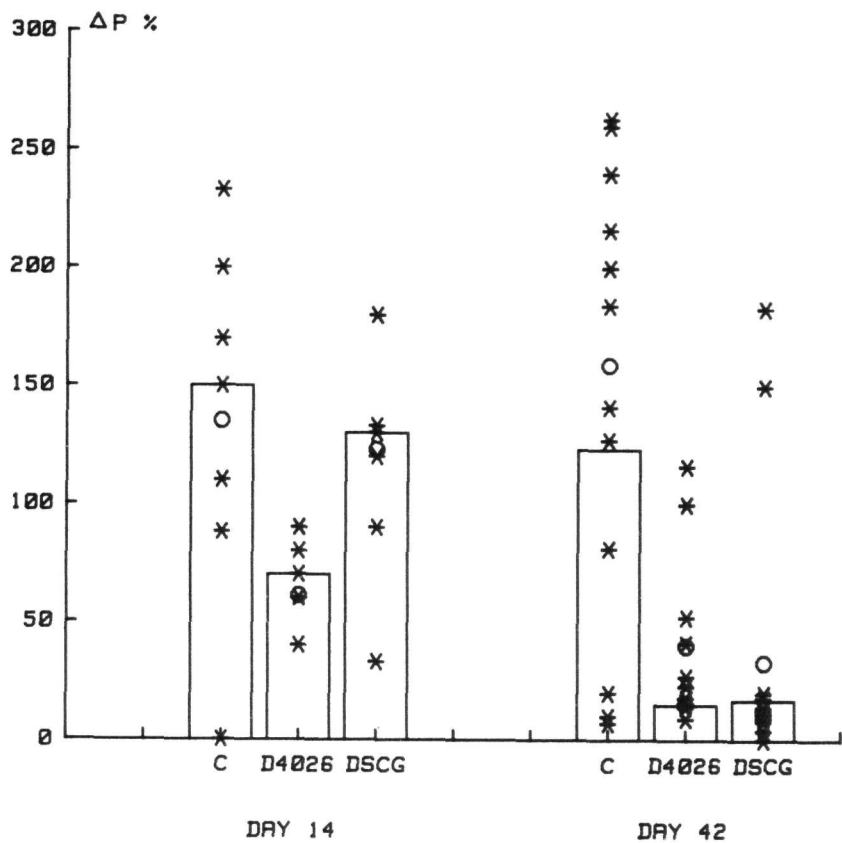


Fig. 5. Effects of D 4026, DSCG and saline (C) on the bronchial anaphylactic reactivity in animals immunized with 10 µg OA 14 or 42 days before test. The columns denote the median value; the circles, the mean value; and the asterisks the individual values.

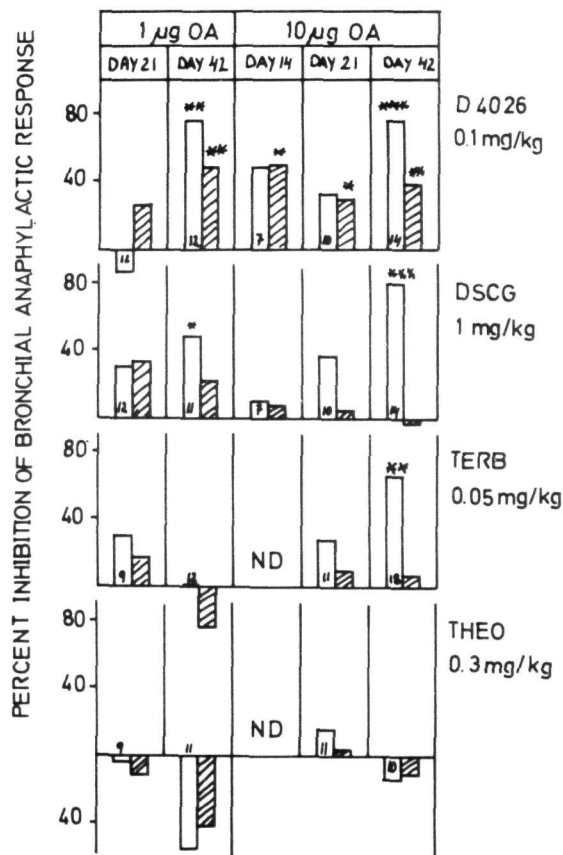


Fig. 6. Effects of DSCG, terbutaline, theophylline and D 4026 on bronchial anaphylactic reactivity in animals immunized with 1 µg OA 21 or 42 days or 10 µg OA 14, 21 or 42 days before test. Drugs were given at the indicated dose 1 min before the challenge dose of OA. Each column denotes mean percentage inhibition, i.e.

$$\left(1 - \frac{\text{mean } \Delta P \% \text{ in drug treated animals}}{\text{mean } \Delta P \% \text{ in saline treated controls}}\right) \times 100$$

Number of animals in each group is given in the Figure. Differences between groups were evaluated with analysis of variance. Blank columns denote results obtained when challenge was performed with 300 µg OA, shaded columns results obtained after a second challenge with 5000 µg OA to provoke a maximal response.
*P < 0.05, **P < 0.01, ***P < 0.001.

either 1 or 10 µg OA, but the inhibition was statistically significant only for D 4026. DSCG, terbutaline and D 4026 significantly inhibited the bronchial anaphylactic response provoked by 300 µg OA – though only D 4026 was effective in a challenge dose of 5000 µg OA – in animals tested 42 days after immunization with 10 µg OA. In animals immunized with 1 µg OA, only D 4026 showed a significant inhibitory effect at this time. It should be observed that the degree of bronchial anaphylactic reactivity *per se* was not lower on day 42 than on day 21 after immunization (see Fig. 3). The experiments with terbutaline and with DSCG thus clearly indicated that the effect of an agent varies with the immunization dose of antigen and the time after immunization; e.g. terbutaline is a very effective inhibitor in animals given 10 µg OA but not in animals given 1 µg OA 42 days before the test.

DISCUSSION

The present report describes the development course of the capacity to react with a bronchial anaphylactic response at antigen challenge of rats immunized with graded doses of OA using IgE-antibody promoting immunization regimens. The course showed several unexpected characteristics. First, animals immunized with 10 µg OA tended to show a biphasic pattern of development of their capacity to respond to a small challenge dose of antigen. We suspect that this pattern is composed of two overlapping ones. The early part of the response in such animals, a high but rapidly declining reactivity, is probably similar to the type of response observed in the animals given 100 µg OA. These animals develop an early transient reactivity, as revealed by the small challenge dose. Second, in animals given 1 µg at immunization, but clearly not in animals given 10 or 100 µg OA, there was a significant correlation between the responses to the small and the large challenge doses (Table 1). This suggests three possibilities: 1) Either the response to the large challenge dose of antigen in the latter groups is mediated by a kind of antibody other than that which

mediated the small challenge dose response. 2) blocking antibody seriously influences the outcome of the challenge; or 3) systemic anaphylactic reactions influence the response. The first possibility is strengthened by the third main finding in the present investigation. One anti-allergic agent, DSCG, and one β_2 -agonist, terbutaline, in the concentration used are somewhat effective as inhibitors 6 weeks after immunization with 10 µg OA but not after 3. Besides its interesting theoretical aspects this observation should be of value to those who use the present model for testing anti-allergic DSCG-like drugs. Previous estimates of the inhibitory efficacy of DSCG in the present system vary from low to high (14, 18, 33); apparently different conditions at the test can explain the divergence in the results. In this connection it might not be out of place to mention that a bronchial anaphylactic reaction induced by anti-IgE antibodies is very susceptible to inhibition by DSCG (10).

There is one more comment to be made in connection with the inhibition experiments. According to our experience, when bronchoconstriction was induced by 50 µg/kg serotonin, the threshold dose of theophylline for bronchodilatation was 0.5 mg/kg, while theophylline in a slightly smaller dose showed no anti-allergic properties at all. This contrasts with results obtained with a slightly different technique in guinea pigs (2).

The problem of identifying the antibodies mediating the response(s) will be dealt with in greater detail elsewhere (3). However, a few comments on experiments we performed to better define the rat bronchial anaphylactic response seem warranted.

First, Mancino & Bevilacqua (24) used Silica gel as an adjuvant in mice to induce a reaginic antibody response to OA apparently due only to IgE antibodies. This response could be boosted. We utilized Silica gel as an adjuvant and our results indicate that this agent effectively induces bronchial anaphylactic reactivity in SD rats after repeated injections. Similar results have been reported with respect to histamine release from lung tissue and mast cells (5).

Second, Taniguchi & Tada (34) made the important observation that in rats, treatment with immunosuppressive drugs or X-rays a few days before immunization with small doses of antigen delays but afterward enhances a homocytotropic antibody response. Similar results have been reported by others (for ref. see 4, 22). In the present experiments, CY given 2 days before a primary dose of 1 µg or 10 µg OA together with alum did not markedly influence the reactivity. In other words, it did not prolong the high response capacity seen on day 14 in 10 µg OA-immunized animals. On the other hand, there was a slight increase in the response to a high antigen challenge dose 4 weeks after CY-treatment of animals immunized 8 weeks before the test (Fig. 4). This effect of CY is not influenced by a booster injection of antigen 2 days after the CY treatment. These results cannot be taken as evidence for or against IgE antibodies mediating this kind of bronchial anaphylactic reactivity. It is noteworthy that a slight enhancement of IgG antibody synthesis in rats has been reported by Gagner & MacLennan (19) using a slightly different schedule for CY administration.

Third, as shown elsewhere (3), when rats are sensitized to OA together with Freund's adjuvant, their response is only weak. Taken together with serum OA-IgG_{2a} antibody determinations these results clearly indicate that IgG_{2a} antibodies are of only minor importance as mediators of the response.

Fourth, our attempts to characterize the antibodies mediating the bronchial anaphylactic reactivity by heat-treatment of serum before passive transfer have been hampered by difficulties in transferring more than a slight degree of activity with fresh non-heated serum. In this respect our data seem to corroborate those of Church (11). He transferred response capacity with 4 ml reaginic sera (PCA-titre 1:60) i.p., but the degree of reactivity transferred was low. Farmer et al. (18) and Carswell & Oliver (8) reported transfer of reactivity with high titred reaginic sera, but they used a large challenge dose of OA (25 mg/kg) or 20 mg/ml aerosolized OA to provoke the reaction. Taken together, these data indicate that systemic passive transfer

of reagin-mediated bronchial anaphylactic reactivity in rats is much more difficult to achieve than transfer of systemic anaphylactic reactivity mediated by IgG antibodies in guinea pigs (6). However, passive transfer of reactivity is not impossible. Thus, SD recipients given 4 ml each i.v. of a fresh high titred antiserum against OA produced in BN rats (OA-IgE antibody titre 238 PU/ml) responded equally well as highly reactive, actively sensitized rats (Dahlbäck, unpublished). A clearer definition of the optimal conditions for passive transfer of reactivity in SD rats must await further research.

A major characteristic of bronchial asthma is nonspecific hyperreactivity of the airways to, for example, allergic mediators. The cause of this hypersensitivity is not known but environmental factors might be a contributory cause. Cockcroft et al. (16) have reported that exposure to allergens can raise the degree of hyperreactivity in some asthmatics. It is possible that an ongoing immune response affecting bronchial tissue might increase bronchial hyperreactivity. However, our results showed no increased bronchial reactivity to i.v. serotonin or methacholine in animals immunized with 1 µg or 10 µg OA 42 days before the test. Thus, a persistent anaphylactic response capacity does not seem *per se* to lead to increased bronchial nonspecific reactivity. In these respects our results corroborate those of Church (11) in the rat and those reported by Popa et al. (31) in guinea pigs.

In certain strains of rodents, *Bordetella pertussis* enhances anaphylaxis and/or the reaction to chemical mediators, e.g. serotonin and histamine (25). This has been considered an interesting model for studying nonspecific hyperreactivity in bronchial asthma. Our efforts to enhance an antigen-induced bronchial anaphylactic reaction by treatment of sensitized animals with *B. pertussis* 4 days before the test were unsuccessful. But it is not known why; it might simply be that SD is a strain that does not respond to *B. pertussis* or that the quality of the *B. pertussis* vaccine used was not good enough to induce hypersensitivity.

Summing up, the following observations of interest were made in the investigation of the

course of development of bronchial anaphylactic reactivity in SD rats sensitized according to IgE-antibody promoting regimens: (a) In one group of animals the response to a small challenge dose tended to be biphasic, (b) the correlations between the strength of the response to a small dose and a large dose of antigen were inconsistent and (c) the efficacy of the anti-allergic agents varied with the testing conditions. These observations suggest that more than one type of homocytotropic antibody might contribute to the response recorded.

ACKNOWLEDGEMENTS

The skilful secretarial work done by Mrs. Ulla Larsson is gratefully acknowledged. I am most grateful to Fisons Ltd. for the gift of DSCG.

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