Strain Difference in an Allergic Asthma Model in Rats

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Abstract—A new rat asthma model was devised, and with the model, allergic bronchoconstrictor responses and effects of disodium cromoglycate (DSCG) were compared among Wistar, Lewis and Fischer 344 rats. Rats were actively sensitized with DNP-Ascaris antigen (DNP-As) and killed Bordetella pertussis vaccine. After eight days, asthmatic response was provoked by inhalation of DNP-As. The bronchomotor response was measured with a modified Konzett-Rössler method in diaphragm-sectioned rats. The inhalation of DNP-As using a newly devised apparatus caused a marked asthmatic response with negligible effects on systemic blood pressure and heart rate. The extent of the bronchoconstriction provoked was of the following order: Wistar > Lewis > Fischer 344. There was no relationship between the individual 48 hr PCA titer and the bronchoconstriction that occurred in any strain of rats. The bronchoconstrictions were inhibited by DSCG (10 mg/kg, i.v.) and the inhibition ratios were 28%, 36% and 33% in Wistar, Lewis and Fischer 344 rats, respectively. The inhibitions were statistically significant in the latter two strains. Fischer 344 rats were more susceptible to the damage resulting from the operative procedures. The above findings suggest that Lewis rats are the most suitable among the above strains as a model for studying the effects of antiallergic agents.

Although bronchial asthma models have been developed in several animal species, there is an obvious need for useful and reproducible models resembling human allergic asthma using small animals. Such models would facilitate the basic pathophysiological research relevant to this disorder and the development of novel drugs.

Rats mainly produce IgE in some conditions, so that they have been recently used for screening antiasthma drugs. However, since provocation by antigen is usually done by intravenous administration, systemic anaphylaxis accompanied by severe hypotension and sometimes death inevitably occurs (1). Thus, there is a limitation for accurate and quantitative investigation on the allergic airway responses by using such routes of antigen administration. Moreover, outbred rats such as Wistar rats and Sprague-Dawley rats are usually used. Recently, inbred rats have been increasingly used for pharmacological studies because of the reproducible responses obtainable in such animals. Asthmatic responses in inbred rats and their strain difference have been poorly reported, although the selection of a suitable strain is important for developing models of bronchial asthma.

In the present study, we devised a new method for quantitative measurement of the allergy-induced bronchoconstriction in rats by inducing local bronchial anaphylaxis with a new technique for inhalation of antigen in actively sensitized rats. With this model, strain differences of both allergic asthma responses and effectiveness of DSCG were compared among Wistar rats (outbred) and Lewis and Fischer 344 rats (inbred).

Materials and Methods

Experimental animals: Male Wistar (230–
280 g), Lewis (220–270 g) and Fischer 344 (240–290 g) rats purchased from Charles River Japan, Inc. were used. Animals were immunized with 2,4-dinitrophenylated Ascaris extract (DNP-As, 2 mg protein) together with killed Bordetella pertussis (2×10^{10}) as an adjuvant and were boosted by DNP-As (0.5 mg protein) 5 days later according to the method of Tada and Okumura (2). Eight days after the first immunization, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and placed in the supine position and ventilated artificially through a tracheal cannula at a frequency of 70 breaths/min. Respiratory volume was adjusted at the beginning of the experiment for ventilation overflow to be 1.0 ml in each rat. After the midline incision of the abdomen, the diaphragm was partially incised to cease the spontaneous respiratory movement.

**Determination of airway constriction:** The scheme of the preparation devised in the present study is shown in Fig. 1. In anesthetized rats, the bronchomotor tone was measured by a modification of the Konzett-Rössler method (3). The lung was inflated at a fixed volume of air at room temperature and humidity under a constant pressure (6 cmH₂O), and ventilation overflow was continuously recorded with a combination of a pneumotachograph (MFP-1T, Nihon Kohden) and an integrator (RFJ-5, Nihon Kohden) as an index of change in airway resistance. Systemic arterial blood pressure was monitored with a pressure transducer (MPU-0.5, Nihon Kohden) from a cannula inserted into the right carotid artery, and heart rate was measured with a tachometer (RT-5, Nihon Kohden) using systolic blood pressure as the trigger. All the above parameters were recorded using a polygraph (Nihon Kohden, RM-85).

**Induction of allergic asthma attacks:** DNP-As (3.0 mg protein/ml, total of about 3 mg protein) was inhaled for 5 min into the animals by aerosolizing antigen solution in a

![Fig. 1. Scheme of the experimental method. (A) Diagram of rat asthma model. DNP-Ascaris antigen was inhaled with an ultrasonic nebulizer to induce asthmatic responses in anesthetized rats. (B) The specially devised plastic cylindrical chamber for inhalation of antigen set into the ultrasonic nebulizer.](image-url)
specially devised plastic cylindrical chamber (diameter: 4.0 cm and height: 8.0 cm) (Fig. 1B) which was introduced in an ultrasonic nebulizer (Tur-3000, Nihon Kohden). The plastic chamber was placed in the respiratory circuit so that the aerosolized mist was inhaled into the airways each time of ventilation. When DNP-As solution was inhaled, the humidity of the air insufflating the lungs approximated 100%.

**Serum reagin titers:** Antisera were collected before the inhalation of DNP-As to determine reagin-like immunoglobulin titers by 48 hr passive cutaneous anaphylaxis (PCA) using the corresponding strain of rat as a recipient.

**Drugs:** Disodium cromoglycate (DSCG; Fujisawa) was dissolved in saline at the time of usage, and administered 30 sec before the inhalation of DNP-As. The dose of DSCG was expressed in terms of its salt.

**Statistical analysis:** All values were expressed as the mean with S.E. Statistical significance of difference was determined by Student's t-test.

**Results**

**Antibody titer in the serum:** Titers of the antisera against DNP-As, determined by 48 hr homologous PCA, in Wistar, Lewis and Fischer 344 rats are shown in Fig. 2. Titer levels of \( \leq 32 \leq 1024 \leq \) (the average of 380: \( \leq 32 \) and 1024: \( \leq 1024 \) were taken as 32 and 1024 in the calculations, respectively). \( 64 \leq 1024 \leq \) (the average of 512) and \( \leq 32 \leq 256 \) (the average of 96) were obtained in the three strains, respectively.

**Asthmatic bronchoconstriction by inhalation of DNP-As:** Immunized Wistar, Lewis and Fischer 344 rats were challenged inhalationally by DNP-As for 5 min at a total dose of about 3 mg protein. DNP-As caused a remarkable increase in ventilation overflow with only negligible effects on systemic blood pressure and heart rate in any strain of rats. A typical recording of the asthmatic response in the Wistar rat is shown in Fig. 3. Ventilation overflow began to increase in 1–2 min after the start of DNP-As inhalation. Maximal increases in ventilation overflow of \( 1.14 \pm 0.18 \text{ ml} \ (N=8) \), \( 0.71 \pm 0.09 \text{ ml} \ (N=13) \) and \( 0.57 \pm 0.04 \text{ ml} \ (N=9) \) were obtained in Wistar, Lewis and Fischer 344 rats, respectively, about 6 min after the start of DNP-As inhalation (Fig. 4). The bronchoconstrictor response tended to recover gradually toward the pre-level in Wistar rats, but did not in Lewis and Fischer 344 rats within 10 min after the end of antigen inhalation. On the other hand, inhalation of DNP-As had no substantial effect on ventilation overflow in nonsensitized Wistar rats.

**Relationship between antiserum titer and asthmatic bronchoconstriction:** Relationships
Fig. 4. Time course of the bronchoconstriction induced by inhalation of DNP-Ascaris antigen (DNP-As) to three strains of rats sensitized with DNP-As/ killed Bordetella pertussis (adjuvant). Each point represents the mean with S.E. from 8 (Wistar), 13 (Lewis), 9 (Fischer 344) and 5 (Wistar (Naive)) experiments. “Naive” means the animals which inhaled DNP-As without presensitization.

Fig. 5. Relationship between titer of antiserum and increase in ventilation overflow induced by inhalation of DNP-Ascaris antigen to Wistar (○), Lewis (▲) and Fischer 344 rats (■).

between 48 hr homologous PCA titer of the antiserum and increase in ventilation overflow induced by inhalation of DNP-As in Wistar, Lewis and Fischer 344 rats are shown in Fig. 5. The coefficients of correlation (r) were -0.5427, -0.1400 and 0.3300, respectively, which were non-significant statistically.

Effect of DSCG on the asthmatic bronchoconstriction (Fig. 6): Effect of DSCG was estimated by the maximal increase value of in ventilation overflow in each animal. Maximal increases in ventilation overflow of 1.23±0.19 ml (N=8), 0.77±0.07 ml (N=13) and 0.58±0.04 ml (N=9) were obtained in Wistar, Lewis and Fischer 344 rats, respectively. The increases in ventilation overflow were inhibited by DSCG at a dose of 10 mg/kg, i.v., to the values of 0.89±0.03 ml (N=8), 0.49±0.09 ml (N=5) and 0.39±0.04 ml (N=6), respectively. The inhibition ratios were 28%, 36% and 33%, respectively, and the inhibitions were statistically significant in Lewis and Fischer 344 rats.

Discussion

Suitable animal models of allergic asthma are required for studying both the mechanisms involved in asthma and the evaluation of potential new antiasthma drugs. As for the antibodies responsible for systemic anaphylaxis in rats, as well as in humans, they are reaginic or of the IgE family (2). Therefore, rats seem to be appropriate for models of anaphylaxis, including anaphylactic bronchoconstriction, from the immunological aspect.

In the present study, remarkable bronchoconstrictions were provoked by inhalation of DNP-As in rats with negligible effects on systemic blood pressure and heart rates.
Systemic anaphylaxis caused by i.v. treatment with antigen has been usually used to induce experimental asthma in rats (4-7). However, systemic anaphylaxis is inevitably accompanied by circulatory collapse or shock (1), which provokes extreme influences on various systemic signals through nervous, hormonal, respiratory and biochemical phenomena. So, it is inadequate to cause allergic bronchoconstriction by systemic i.v. administration of allergen. Carswell and Oliver (8) and Piechuta et al. (9) reported an asthmatic response obtained by challenge of antigen aerosols in conscious rats which were actively sensitized by DNP-ovalbumin or egg albumin. In their papers, rats were placed in a plastic box, and the pressure change in the box or air flow between the box and its surroundings was evaluated as the parameter of the airway response. The responses they measured were expiratory and inspiratory patterns, but not the responses of airway smooth muscles.

The allergic bronchomotor response provoked by inhalation of antigen in the present study was restricted in the airways, without substantial changes in systemic blood pressure and heart rate. Moreover, quantitative and continuous measurement of airway resistance was possible. Incision of the diaphragm of anesthetized rats was made in order to cease spontaneous respiration. For measuring the response of airway musculature accurately, spontaneous breathing should be ceased. Rats were found to be relatively insensitive to muscle relaxants such as decamethonium and d-tubocurarine in our preliminary experiments. After incision of the diaphragm, the spontaneous respiratory movement completely disappeared, with no effect on airway resistance. Furthermore, the bronchomotor tone, systemic blood pressure and heart rate were stable at least for an hour after the operation. Antigen inhalation was done with an ultrasonic nebulizer in which a specially elaborated small plastic chamber was introduced. The small chamber allowed the determination of relatively minute changes in bronchomotor response in small animals. The nebulizer was placed into the respiratory circuit; and thus, this technique made it possible to inhale drugs into the airways in a certain manner and enabled the recording of the airway response continuously even during the antigen inhalation phase.

Relatively invariable titers of antisera were produced in Lewis and Fischer 344 rats as compared with those in Wistar rats. The mean titers of antisera were in the following order: Lewis > Wistar > Fischer 344. The reason for this strain difference is now unexplainable, but genetic factors may probably be involved.

The extent of the bronchoconstriction that occurred did not correlate with the serum reagin titer level in any strain of rats tested. Church (10) reported that no correlations were observed among titer of antiserum, active cutaneous anaphylaxis and active systemic anaphylaxis in Wistar rats sensitized with Nippostrongylus brasiliensis. Carswell and Oliver (8) and Piechuta et al. (9) reported that no correlation was observed between titer of antiserum and bronchial anaphylaxis in Sprague-Dawley and Hooded Lister rats sensitized with DNP-ovalbumin and egg albumin, respectively. Titer of antiserum represents the activity of free IgE in the serum. However, IgE has high affinity with mast cells in the tissues, so that serum titer level would not reflect the extent of sensitization of mast cells in the airways. As a possible reason for the lack of a relationship between titer of antiserum and bronchial response, differences of the extent of chemical mediators released from mast cells and sensitivity of airways to mediators among individual animals may also be involved.

As to models for screening new drugs, it is desirable that reproducible responses can be obtained. Recently, for this reason, inbred rats have been increasingly used in elaborating models of diseases. However, asthmatic responses in inbred rats have been poorly documented. In our preliminary study, Wistar rats produced higher titers of antisera than Sprague-Dawley rats (outbred), and both the titers of antisera and the passive cutaneous anaphylaxis varied from animal to animal in much the same degree in the two strains of rats. In the present study, we
therefore investigated the strain difference among Wistar rats (outbred), Lewis and Fischer 344 rats (inbred), to determine their usefulness as asthma models. Furthermore, the three strains of rats with approximately the same range of body weights were used to compare airway constrictions invoked among them.

The extent of bronchoconstriction provoked in the present model was of the following order: Wistar>Lewis>Fischer 344. However, in Wistar rats, the bronchomotor response varied greatly from animal to animal. On the other hand, the basal tidal volume in Fischer 344 rats (average 4.1 ml) was much less than those in Wistar and Lewis rats (averages of 6.7 and 6.1 ml, respectively). Fischer 344 rats were, furthermore, more susceptible to the damage resulting from the operative procedures. Namely, in about one third of the Fischer 344 rats used, the airway resistance spontaneously kept increasing with hypotension after the operation, so that they could not be used for the experiments. The strain difference of the asthmatic response would be derived from genetic and constitutional factors, especially from differences in the ability to produce specific IgE antibody and differences of airway responsiveness.

The antiasthmatic effects of disodium cromoglycate (DSCG) were compared among Wistar, Lewis and Fischer 344 rats using the present asthma model. DSCG at a dose of 10 mg/kg, i.v., almost inhibited the passive cutaneous anaphylaxis in rats in our preliminary study. The inhibitory effects of DSCG were comparable to each other, and the inhibitory ratios were 28–36% in the three strains of rats, although the effect was not significant only in Wistar rats.

The above findings indicate that 1) marked asthmatic responses are provoked in Wistar, Lewis and Fischer 344 rats by inhalation of DNP-As antigen; 2) Wistar rats are less adequate for asthma models because the response varies so much from animal to animal, and moreover, the effect of DSCG is hard to detect in this strain; and 3) Lewis rats seem to be more suitable than Fischer 344 rats for technical reasons.

The present asthma model may be useful for screening antiasthmatic drugs and studying the mechanisms of asthma.

References
10 Church, M.K.: Correlation of anaphylactic bronchoconstriction with circulating reaginic antibody level and active cutaneous anaphylaxis in the rat. Immunology 29, 527–534 (1975)