Increase in Respiratory Resistance after Exercise in Conscious Guinea Pigs. 
As a Model for Exercise-Induced Asthma

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We have developed an experimental model for exercise-induced asthma (EIA) using conscious guinea pigs. Respiratory resistance (Rrs) was measured before and after exercise (running). When a 0.05% lipopolysaccharide (LPS) was inhaled by guinea pigs which had been pretreated with a corticosteroid biosynthesis inhibitor metyrapone (50 mg/kg, i.v.), Rrs significantly increased 24 h after exercise. Metyrapone had no effect, however, on the LPS-induced increase in the numbers of macrophages, eosinophils, neutrophils and lymphocytes in bronchoalveolar lavage fluid (BALF). In order to examine the role of airway inflammation, the effects of murine recombinant interleukin-5 (mrIL-5) and platelet activating factor (PAF) were investigated in guinea pigs. The exercise-elicited increase in Rrs, was observed 24 h later than the treatment with mrIL-5 in metyrapone-treated animals. The number of macrophages, eosinophils and neutrophils increased in the BALF of mrIL-5-treated animals. In contrast, a 0.05% PAF aerosol caused an increased number of eosinophils in BALF, but did not affect Rrs after exercise in either metyrapone-treated or non-treated animals. Moreover, to evaluate the value of this model as a pharmacological tool, the effect of ketotifen and prednisolone on the exercise-induced increase in Rrs was investigated. Prior administration of ketotifen and prednisolone showed a tendency to prevent, or clearly inhibited, the exercise-induced increase in Rrs in animals treated with LPS and metyrapone.

Keywords exercise-induced asthma; metyrapone; interleukin 5; asthma; lipopolysaccharide

Exercise-induced asthma (EIA) is caused by bronchoconstriction and hyperinflation after strenuous exercise. 1–3 There have been numerous clinical investigations of EIA. As for the pathophysiology of EIA, recent studies have demonstrated that the exercise-related airway response in asthmatic individuals is proportional to heat loss from the respiratory epithelium. 4–6 In addition, extensive research has shown the participation of airway inflammation in the onset of bronchial asthma. 7–9 The relationship between airway inflammation and EIA, however, has not yet been investigated. Therefore, the present study was conducted to develop an animal model for EIA and to examine the participation of airway inflammation in the onset of exercise-induced airway obstruction in conscious guinea pigs. In the present study, exercise in on exercise function was examined to compare the changes in airway resistance (Rrs) before and after exercise.

MATERIALS AND METHODS

Animals Male Hartley guinea pigs weighing 300–400 g (Nihon SLC, Japan) were used.

Materials Murine recombinant interleukin 5 (mrIL-5) was provided by Suntory Co., Ltd. (Osaka, Japan) and Dr. K. Takatsu (University of Tokyo, Japan). Ketotifen was provided by Sandoz Ltd. (Basel, Switzerland). Prednisolone acetate (Takeda Chemical Ind., Ltd., Osaka, Japan), platelet activating factor (PAF, 1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphorylcholine) (Bachem Feinchemikalien AG, Bubendorf, Switzerland), bovine serum albumin (BSA) (fraction V, Miles Inc., Illinois, U.S.A.), lipopolysaccharide (LPS; from Escherichia coli, Serotype; Sigma Chemical Co., St. Louis, U.S.A.), 2-methyl-1,2-di-3-pyridyl-1-propanone (metyrapone; Aldrich Chem. Co., Milw, WI, U.S.A.), May–Gruenwald solution, Giemsa solution (Mercer Co. Inc., N.J., U.S.A.), Turk solution (Wako Pure Chemicals, Japan) and sodium pentobarbital (Abbott Lab. Co., Chicago, U.S.A.) were commercially purchased. BSA, LPS and metyrapone were dissolved in 0.9% NaCl (saline). PAF was dissolved in saline containing 0.1% BSA. Prednisolone acetate and ketotifen were suspended in saline containing 0.5% carbamoyl methyl cellulose-sodium (CMC-Na).

Schedule for Experiments Guinea pigs were trained by running 1–20 m/min on a treadmill which was partitioned into 6 equal sections (one section: length 47 cm, width 7 cm) (Takei Kiki Kogyo Co., Ltd.) 1–5 times at one-day intervals. After sufficient training, the animals were used for the experiments. Exercise was performed by running on the treadmill at 1 m/min for 1 min, 5 m/min for 1 min, 10 m/min for 1 min, 15 m/min for 15 min and 20 m/min for 5 min, successively, 2 and/or 24 h after the treatment with LPS, PAF or mrIL-5. Rrs was measured 30 min before and after exercise. Bronchoalveolar lavage fluid (BALF) was recovered 30 min after exercise at 24 h.

Treatment with LPS, PAF and mrIL-5 Guinea pigs were exposed to LPS aerosol and PAF aerosol by the following methods. Animals were positioned inside a plastic body apparatus which was partitioned into 10 equal sections, with the head and body compartments separated by a neck restrainer. The snout of each animal was placed in a nose-space connected to an ultrasonic nebulizer Tur-3200 (Nihon Koden, Japan). A 0.05% LPS aerosol or a 0.05% PAF aerosol spray (spray volume 3 ml/min, mass particle diameter distribution 2.0–6.0 μm) was
generated for 30 min (LPS) or 10 min (PAF) using the ultrasonic nebulizer Tur-3200. For mrlL-5 exposure, the previously described method was used. In brief, the trachea was surgically exposed, and 0.25 ml of sterile mrlL-5 solution was injected into the trachea using a 27G syringe under sodium pentobarbitone anaesthesia. The wound was closed with sterile stitches.

Metyrapone, propranolol, cimetidine and indomethacin were injected i.v. immediately before exposure to LPS, PAF or mrlL-5. Ketotifen was administered i.p. at 30 min before, and prednisolone was given i.p. 2 h before the inhalation of LPS.

**Measurement of \( R_n \)** \( R_n \) was measured using the oscillation technique described by Iwama et al. according to the modified method of Mead. In brief, each guinea pig was positioned inside a body plethysmograph with the head outside the chamber. The respiratory airflow from the face mask at the snout and the oscillating pressure in the body chamber at 10 cm H2O with a 30 Hz sine wave pressure were recorded with a differential pressure transducer, and these signals were displayed simultaneously on an X-Y oscilloscope and recorded on a polygraph. \( R_n \) was calculated using the following formula: \( R_n = \frac{\text{pressure/d flow}}{\text{cm H}_2\text{O/ml/s}} \).

**BALF Study** Guinea pigs were killed by an intraperitoneal injection of sodium pentobarbitone (150 mg/kg). The trachea was cannulated and the airway lumen was washed with two aliquots (5 ml) of saline containing 0.1% bovine serum albumin (BSA) at 37°C. BALF from each animal was pooled in a plastic tube cooled in ice and then centrifuged (150 × g) at 4°C for 10 min. The cell pellets were resuspended in physiological saline (2 ml). The total number of leukocytes was counted after Turk staining, and the differentiated cell type count was made on a smear prepared with a cytocentrifuge followed by May–Grunwald and Giemsa dye staining under a microscope (×500). Results are expressed as the total number of cells recovered in the BALF.

**Statistical Analysis** All data are represented as the mean ± S.E.M. and were analyzed using a paired t-test and Dunnett’s multiple range test.

**RESULTS**

**Effect of Exercise on \( R_n \) of Guinea Pigs Treated with LPS and/or Metyrapone** The exercise challenge was carried out 2 and 24 h after treatment with LPS, metyrapone or the combination of LPS and metyrapone. As indicated in Fig. 1, a significant increase in \( R_n \) was observed in animals which had been running 24 h after treatment with both 0.05% aerosol LPS and metyrapone (50 mg/kg i.v.) (D), but not with LPS (B) or metyrapone (C) only.

**Changes in Bronchial Leukocyte Number in Guinea Pigs Treated with LPS and Metyrapone** BALF was recovered after the measurement of \( R_n \) following exercise. Inhaled LPS caused a significant increase in the numbers of macrophages, eosinophils and neutrophils, but not in the number of lymphocytes, in BALF at 24 h. Metyrapone (50 mg/kg) had no effect on these LPS-induced bronchial

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Fig. 1. Change in \( R_n \) after Exercise in Conscious Guinea Pigs Treated with LPS and/or Metyrapone

\( R_n \) was measured before and after exercise challenge 2 and 24 h after A) no treatment, B) inhalation of 0.05% LPS, C) intravenous injection of metyrapone (50 mg/kg) and D) the inhalation of 0.05% LPS following the intravenous injection of metyrapone (50 mg/kg). base = baseline of \( R_n \) in animals before \( R_n \) before exercise challenge. after = \( R_n \) after exercise challenge. Each value represents mean ± S.E. of 6–12 animals. a) p < 0.05, significantly different from \( R_n \) before exercise challenge.
Fig. 2. Effect of Metyrapone on Changes in the BALF Leukocytes after Exercise in LPS-Inhaled Guinea Pigs

Metyrapone at a dose of 50 mg/kg was given intravenously, followed by LPS inhalation. BALF was obtained after the measurement of $R_n$ following exercise 24 h after LPS inhalation. Macro = macrophages, Eosi = eosinophils, Neut = neutrophils, Lymph = lymphocytes. Each value represents mean ± S.E. of 4—10 animals. a) $p<0.05$, significantly different from the normal groups by Dunnett’s multiple range test. □, normal; ◆, LPS; ■, LPS + metyrapone.

Fig. 3. Effect of Exercise on A) $R_n$ and B) BALF Leukocyte Numbers in Conscious Guinea Pigs Treated with an Intratracheal Injection of mrlIL-5

A) $R_n$ was measured before and after exercise challenge 24 h prior to the inhalation of 0.05% PAF pretreated with and without metyrapone (50 mg/kg, i.v.). B) BALF was obtained immediately after the measurement of $R_n$ following exercise challenge at 24 h. Base = baseline of $R_n$ in animals. before = $R_n$ before exercise challenge. after = $R_n$ after exercise challenge. Macro = macrophages, Eosi = eosinophils, Neut = neutrophils, Lymph = lymphocytes. Each value represents mean ± S.E. of 6 animals. a) $p<0.05$, significantly different from A) $R_n$ before exercise by paired t-test and B) the BSA response by Dunnett’s multiple range test. A) □, baseline; ◆, before exercise; ■, after exercise. B) □, vehicle; ◆, PAF; ■, PAF + metyrapone.

leukocyte increases (Fig. 2).

Effect of Exercise in Guinea Pigs Treated with mrlIL-5 or PAF

The effects of mrlIL-5 and PAF on $R_n$ after exercise were investigated in guinea pigs. A significant increase in $R_n$ after exercise was observed in guinea pigs 24 h after treatment with a combination of mrlIL-5 (15 μg/animal, intratracheally) and metyrapone (50 mg/kg, i.v.), but not in animals treated with mrlIL-5 alone. (Fig. 3). The number of macrophages, eosinophils and neutrophils in BALF were increased by IL-5 treatment, but the number of these leukocytes did not increase after metyrapone treatment (Fig. 3B). When 0.05% PAF was inhaled, however, neither metyrapone (50 mg/kg, i.v.)-treated nor untreated animals showed an exercise-induced increase in $R_n$ 24 h after treatment (Fig. 4A). However, PAF increased the number of eosinophils in BALF in untreated animals, and increased the number of eosinophils and macrophages in BALF in metyrapone-treated animals (Fig. 4B).

Effect of Ketotifen and Prednisolone on the Exercise-
Fig. 5. Effect of Ketotifen and Prednisolone on Exercise-Induced Increases in $R_n$ in Conscious Guinea Pigs Treated with LPS and Metyrapone

$R_n$ was measured before and after exercise challenge 24 h after the inhalation of LPS following the intravenous injection of metyrapone (50 mg/kg). B) Ketotifen (2 mg/kg, i.p.) was given 30 min before $R_n$ measurement. C) prednisolone (20 mg/kg, i.p.) 2 h before and D) prednisolone (30 mg/kg, i.p.) 1 h before the LPS and metyrapone treatment. base = baseline of $R_n$ in animals before $R_n$ measurement. after = $R_n$ after exercise challenge. Each value represents mean ± S.E. of 6 animals. *p < 0.05, significantly different from $R_n$ before exercise challenge.

Induced Increase of $R_n$ in Guinea Pigs Treated with LPS and Metyrapone

To evaluate the above EIA-like model as a pharmacological tool, the effects of ketotifen and prednisolone on exercise-induced increases in $R_n$ were investigated. Experiments were carried out 24 h after LPS inhalation in combination with metyrapone. As indicated in Fig. 5, ketotifen (2 mg/kg) and prednisolone (20 mg/kg) showed a tendency to inhibit the exercise-induced increase in $R_n$. However, prednisolone at a dose of 30 mg/kg i.p. inhibited an exercise-induced increase in $R_n$.

DISCUSSION

The present study was conducted in order to produce an experimental model for EIA in guinea pigs and to investigate the role of airway inflammation in the onset of experimental EIA. A significant increase in $R_n$ after exercise was observed after treatment with LPS and metyrapone combined. No increase in $R_n$ was observed when either of these agents was used alone. These results suggest that the combined use of LPS and metyrapone is important for the induction of EIA. Our previous report demonstrates a decrease in serum cortisol levels after treatment with metyrapone in guinea pigs. Lowered cortisol levels, therefore, may play a role in this EIA-like phenomenon.

LPS is known to cause airway inflammation and hyperresponsiveness in guinea pigs. In the present study, LPS aerosol induced an increase in the numbers of macrophages, eosinophils, neutrophils, and lymphocytes in BALF 24 h after the treatment. $R_n$ after exercise was increased by pretreatment with metyrapone 24 h after treatment with LPS, but the accumulation of bronchial leukocytes was not affected. These results suggest that metyrapone may be important in inducing the increase in $R_n$ after exercise, but that it has no direct relation to the airway inflammation caused by LPS. In addition, our previous results demonstrated that the combined use of LPS and metyrapone provokes airway hyperresponsiveness to acetylcholine without modifying airway inflammation. These results suggest that metyrapone may also be involved in both the increase in airway responsiveness to stimulants such as exercise and acetylcholine, and in the decrease in the glucocorticoid level. The precise mechanism of metyrapone-induced airway hyperresponsiveness is still obscure. Further experiments, including measurement of the responsibility of isolated tracheal muscle against electric stimulation after the treatment of metyrapone, will be necessary.

Although glucocorticoids have little effect on EIA in humans, high doses of prednisolone inhibited the onset of exercise-induced broncho-constriction in guinea pigs in our study. The efficacy of prednisolone may be due to a recovery from the lowered cortisone levels in metyrapone-treated animals. Prednisolone also reduced the accumulation of macrophages, but not the accumulation of other inflammatory leukocytes in BALF. In order to determine the relationship between airway inflammation and exercise-induced increases in $R_n$, the effect of two other phlogistic agents, PAF and IL-5, were tested. The present results indicate that the exercise-induced increase in $R_n$ was induced in animals treated with metyrapone in combination with IL-5. This treatment increased the numbers of macrophages, eosinophils and neutrophils in BALF, but PAF had no effect. The activation of macrophages may partially participate in the induction of the present EIA-like airway obstruction. However, further detailed experiments are necessary.

Many investigators have reported on the mechanisms of EIA pathophysiology. Lee et al. suggested that mast cell degranulation and some mediators such as histamine and neutrophil chemotactic factor are released during EIA. Other investigators, however, have reported that the onset of EIA is not associated with mast cell activation and mediator release. Furthermore, a recent work demonstrates that heat loss from the respiratory epithelium may play the most important role in the onset of EIA. Several investigators believe that heat loss is important for the onset of EIA. The present results demonstrate that the combined effect of hypo-glucocorticoid and the accumulation of certain types of airway leukocytes may also accelerate the onset of EIA. There is, however, little clinical evidence to support the above experimental results.

In conclusion, the present results indicate that the exercise-induced increase in $R_n$ occurs in LPS- or IL-5-treated guinea pigs which had been pretreated with metyrapone. These results suggest that the participation
of certain kinds of inflammatory cells in BALF and a
decrease in serum glucocorticoid levels are necessary for
the onset of EIA. More detailed clinical investigations are
necessary to clarify the relationship between airway
inflammation and EIA.

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