Strain Differences in Guinea Pigs' Bronchial Sensitivity to Acetylcholine

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The bronchial sensitivity to acetylcholine (ACh) of guinea pigs of various strains was investigated to clarify strain differences. Inbred Strain 2, Strain 13 and JY-1 and non-inbred Hartley strain (two colonies) were used in this experiment. (1) Guinea pigs were exposed to 0.08% ACh aerosol and the time needed to produce falling down (TNPFD) was determined. Mean ± standard error of TNPFD (n = 14 per group) of animals was 182 ± 28 sec, 148 ± 22 sec, 210 ± 30 sec, 342 ± 24 sec and 406 ± 36 sec in Strain 2, Strain 13, JY-1, Hartley (Japan SLC) and Hartley (Hitachi), respectively. There was a significant difference in TNPFD between inbred strains and non-inbred strains (P <0.05 or P <0.01), indicating that inbred strains had higher sensitivity. (2) Guinea pigs were exposed to 20-5000 µg/ml ACh for 2 min. The mean dose threshold as determined by transcutaneous oxygen pressure was 524 µg/ml, 424 µg/ml, 614 µg/ml, 1317 µg/ml and 1651 µg/ml (n =14 per group) in Strain 2, Strain 13, JY-1, Hartley (Japan SLC) and Hartley (Hitachi), respectively. Inbred strains showed lower dose thresholds than non-inbred strains. (3) Isolated trachea-lungs of 5 guinea pigs were perfused with 10⁻⁹-10⁻⁵ g/ml ACh to determine strain differences. Dose response curves of animals of inbred strains shifted to the left (lower concentrations), unlike those of non-inbred strains, suggesting that inbred strains had higher sensitivity to ACh than non-inbred strains. These results suggested that bronchial sensitivity was markedly higher in inbred strains than in non-inbred strains, whereas the differences between the three inbred strains and the two non-inbred colonies were not significant.

Guinea pigs have been widely used as bronchial asthmatic and allergic animal models because of their high bronchial sensitivity to stimulants and allergens [1, 2].

Investigations on bronchial sensitivity using mice, rats, hamsters, guinea pigs and rabbits have been previously reported, and it has been shown that guinea pigs have congenitally higher bronchial sensitivity than other experimental animals [3, 4]. We have reported that a wide range of individual differences is observed in the bronchial sensitivity of Hartley guinea pigs [4]. Despite their widespread use in experiments, strain differences in the bronchial sensitivity of guinea pigs have not yet been reported. We thought it would be useful to clarify such strain differences. Therefore, this became the object of the present study.

Materials and Methods

Experimental animals : Eight-week-old male guinea pigs of specific pathogen free (SPF) Strain 2 (Japan SLC, weighing from 467 to 552 g), SPF Strain 13 (Japan SLC, weighing from 476 to 543 g), conventional JY-1 (KIWA Laboratory Animals, weighing from 412 to 461 g), and SPF Hartley strain (Japan SLC, weighing from 497 to 543 g, and Hitachi Animal Medical Research Institute, weighing from 480 to 538 g) were used. Eight-week-old guinea pigs were used because after eight weeks...
Hartley guinea pigs show constant bronchial sensitivity as determined by the time required to produce falling down after exposure to acetylcholine [5]. Seventy animals (5 groups) were used for measurement of the time needed to produce falling down (TNPFD) or dose threshold, and 5 (one group) and 35 (5 groups) for perfusion experiments with isolated trachea-lung preparations.

Throughout the experiments, these animals were kept in polycarbonate cages (740 × 450 × 230 mm, 3 animals/cage) with wood shavings and hay on the floor. The animals were maintained in a clean, air-conditioned animal room (22±1°C in room temperature and 55±10% in relative humidity) with artificial lighting for 12 hours from 7 : 00 a.m. to 19 : 00 p.m., and a continuous flow of fresh air filtered through microfilters was supplied horizontally from the ceiling. They were given a commercial diet (RC-4: Oriental Yeast Co., Tokyo) and tap water ad libitum throughout the experimental period.  

Drugs: Acetylcholine chloride (ACh, Ovisot, Daiichi Seiyaku Co., Tokyo) was dissolved in physiological saline solution just before use in the experiment. Concentrations of ACh used for this experiment were 0.08% for measurement of TNPFD, 20, 39, 78, 156, 312, 625, 1250, 2500 and 5000 µg/ml for measurement of dose threshold, and 10⁻⁸, 10⁻⁷, 10⁻⁶ and 10⁻⁵ g/ml for the perfusion experiment in isolated trachea-lung preparations.

Inhalation: Our usual method [4, 5] was followed. The guinea pig was placed in a glass receptacle (volume, 10 l) and exposed to ACh aerosol using a glass nebulizer (particle size 6.2-21.8 µm, mean diameter 14.7 µm, nebulizing volume 0.1 ml/min, Nippon Shoji Co., Osaka) connected to a compressor (air delivery 61/min, Nippon Shoji Co., Osaka). Aerosol leaked out constantly from the bottom of the glass receptacle [5]. Inhalation was performed in a room where the condition was the same as in the animal room (in order to avoid environmental changes).

Assessment of inhalation test:
1) Time needed to produce falling down (TNPFD): The sensitivity was expressed by the time interval (sec) from the start of inhalation to falling down caused by ACh [5].
2) Dose threshold (method of transcutaneous oxygen pressure): The ear of the guinea pig was placed on a transcutaneous electrode connected with a transcutaneous oxygen pressure system (Oxykapnomonitor, Hellige Co., West Germany). We recorded transcutaneous oxygen pressure (tcPO₂) while animals were exposed to ACh (20-5000 µg/ml) at 2 min intervals. Dose threshold was calculated as the ACh concentration (µg/ml) causing about a 20% decrease in tcPO₂ curve [6-9].

Assessment of perfusion in isolated trachea-lung preparation: A guinea pig was killed by exsanguination. Both the trachea and lung were taken out and placed in Locke’s solution (37°C). The surface of lung was washed with Locke’s solution and a cannula was inserted in the trachea. Furthermore, it was perfused with Locke’s solution by using Mariott’s bottle, and the height of fluid (Locke’s solution) in Mariott’s bottle (cm H₂O) was changed to allow 20-30 drops of solution/min. Several doses of ACh (10⁻⁸-10⁻⁵ g/ml), from lower to higher doses, were applied to the isolated trachea-lung preparation for 3 min each [10, 11]. Assessment of response was done by the maximum inhibitory rate (%) of drops of ACh solution compared with that of Locke’s solution alone.

Statistical analysis: Significant values for TNPFD were calculated by Scheffé’s multiple comparison and Bartlett’s test for variance, and for dose threshold by cumulative x² test.

Results

Fig. 1 shows differences in bronchial sensitivity in all strains by means of the time needed to produce falling down (TNPFD) when 0.08% ACh was inhaled. The mean ± standard error of TNPFD was 182±28 sec, 148±22 sec, 210±30 sec, 342±24 sec and 406±36 sec in Strain 2, Strain 13, JY-1, Hartley (Japan SLC) and Hartley (Hitachi), respectively. Significant differences were observed between inbred strains and non-inbred strains (P <0.05 or P <0.01), indicating that inbred strains had higher bronchial sensitivity than non-inbred strains. The TNPFD (bronchial sensitivity) of guinea pigs had large strain-to-strain variations. The TNPFD of Hartley guinea pigs (Hitachi colony, with the lowest bronchial sensitivity) was approximately 2.7 times longer than that of Strain 13 (with the highest bronchial sensitivity).
Fig. 1. Strain differences in the time needed to produce falling down (TNPFD) of guinea pigs of various strains following exposure to 0.08% acetylcholine. Each column with vertical bar represents mean ± S. E. of 14 animals. NS means not significant. A : Strain 2 B : Strain 13 C : JY-1 D : Hartley (Japan SLC) E : Hartley (Hitachi) * : P < 0.05, ** : P < 0.01, using Bartlett's test and Scheffe's multiple comparison.

Fig. 2. A record of continuous transcutaneous measurement of PO2 (tcPO2) during exposure to acetylcholine

Fig. 2 shows an example of continuous measurement of tcPO2. In this example, at 312 µg/ml of ACh, tcPO2 decreased by about 20%. Thus, the dose threshold was determined to be 312 µg/ml.

The dose threshold (µg/ml) in all guinea pigs is shown in Fig. 3. The mean dose threshold of animals was 524 µg/ml, 424 µg/ml, 614 µg/ml, 1317 µg/ml, and 1651 µg/ml in Strain 2, Strain 13, JY-1, Hartley (Japan SLC), and Hartley (Hitachi), respectively. Significant differences between of Strain 2, Strain 13, JY-1 and the Hartley strains were noticed (P < 0.05 or P < 0.01, respectively), whereas the dose thresholds of the three inbred strains were not significantly different.

Fig. 4 shows an example of dose response curves in isolated trachea-lung preparations perfused with different concentrations of ACh [Hartley guinea pigs (Japan SLC) were used]. ACh dose dependency was observed. The peaks of dose response curves were observed at 5-20 min, and these responses became weaker with time.

The inhibitory rates calculated by these ACh dose response curves in all strains are...
Fig. 4. Dose response curves (inhibitory rate in %) of isolated tracheal lungs of Hartley guinea pigs (Japan SLC) perfused with acetylcholine (ACh). Each point with vertical bar represents mean ± S. E. of 5 animals. Perfusion with Locke's solution (37°C) containing each dose of ACh was carried out for 3 min. Five animals were used.

Fig. 5. Inhibitory effects of acetylcholine on the dose response curves in perfusion of isolated tracheal lungs of guinea pigs of various strains. Each point with vertical bars represents mean ± S. E. of 7 animals. A: Strain 2 B: Strain 13 C: JY-1 D: Hartley (Japan SLC) E: Hartley (Hitachi)

presented in Fig. 5. It was found that ACh dose response curves in inbred strain guinea pigs (Strain 2, Strain 13, JY-1) clearly shifted to the left compared with those in Hartley strains (Japan SLC, Hitachi). All of these results indicated that inbred strains of guineapigs had higher tracheobronchial sensitivity than Hartley strains.

Discussion

In humans, methods to test bronchial sensitivity have been established and clinically applied [12]. For these tests, ACh, histamine and methacholine have been generally used. It is still unclear whether bronchial sensitivity is congenitally determined or acquired after birth, but bronchial sensitivity has developed upon exposure to ozone and cold air, in viral infections and in cases of chronic bronchiolitis [13-16].

Although some studies on bronchial sensitivity have been carried out, none has dealt with strain differences in the bronchial sensitivity of guinea pigs, which are widely used as allergic animal models.

Clinically, bronchial sensitivity has been estimated by forced expiratory volume (FEV), FEV/forced vital capacity (FVC) %, volume (V) max, vital capacity (VC), etc. [17]. However, in basic studies, animals have been treated generally with anesthesia or cannula. In our experiments, guinea pigs with cannula and anesthesia were not used because the possible influence of anesthesia would have had to be taken into consideration. Therefore, we employed TNPF and dose threshold by recording tcPO2 as methods of testing strain differences in bronchial sensitivity, and isolated trachea-lungs to assess direct respon-
ses to ACh.

The TNPFD method clearly showed that animals of the inbred strains had higher sensitivity to ACh than those of the non-inbred strain (Hartley). In our previous studies, when 8-week-old male SPF Hartley guinea pigs (Japan SLC) of non-inbred strain were exposed to 0.08% ACh, TNPFD varied from 221±32 sec (n=8) to 359±34 sec (n=25).

Next, we assessed bronchoconstriction by measuring tcPO2. Generally, tcPO2 is used to monitor babies in the field of pediatrics [6], in histamine and other inhalation tests to observe dyspnea.

In this study, it was possible to do the tcPO2 test in a more natural state since animals were not subjected to anesthesia or cannulation. Lowered tcPO2 in guinea pigs correlated with pediatric symptoms like labored respiration, cyanosis etc., in animals exposed to ACh.

It was found that inbred Strain 13, Strain 2 and JY-1 guinea pigs had higher sensitivity than Hartley strains. Neither inbred strains nor non-inbred strains showed significant differences in their dose thresholds. The results obtained from the dose threshold study were consistent with those from TNPFD.

The perfusion rate in isolated trachealungs was also measured in this study. These experiments suggested that the animals of inbred strains had higher bronchial sensitivity to ACh than those of the Hartley strain since the dose response curves of isolated trachealungs of the inbred strains shifted to the left (lower concentrations of ACh), unlike those of the Hartley strain.

Thus, when the bronchial sensitivity of guinea pigs of various strains to ACh was assessed by the three methods mentioned above, it was found that the sensitivity of inbred strains was higher than that of non-inbred strains in all three tests.

In dogs, Hirshman et al. [18] reported that bronchial sensitivity tested by inhalation challenge with methacholine was high in Basenji-Greyhound (BG) cross-bred dogs. A bronchial methacholine response was elicited by a 10- to 30-fold lower concentration in BG dogs than in mongrels. They concluded that the BG dogs must have nonspecific bronchial hyperreactivity resembling human asthma. They also reported that the bronchial sensitivity of BG dogs was partially determined by heredity.

The mechanisms and causes of bronchial hypersensitivity have not been clarified but disorders of the autonomic nervous system have been implicated [19].

At present, we are breeding guinea pigs with high and low bronchial sensitivity to ACh and histamine exposure, and these animals have reached the F2 generation, separating roughly into two lines. We think that these animals will be used as asthmatic or nasal allergic models in the future.

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References


