Bronchial Reactivities in Guinea Pigs to Acetylcholine or Histamine Exposure

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The bronchial reactivities in Hartley guinea pigs to acetylcholine (ACh) or histamine (Hist) were investigated, and the following results obtained; 1. Eight-week-old animals were exposed to ACh and Hist. A significant relationship was observed between the concentrations of the chemicals and the time needed to produce falling down (TNPFDF) due to the asthmatic reaction to ACh and Hist. 2. Eight-week-old animals were exposed to 0.1% ACh and 0.05% Hist, for which the mean TNPFDF $\pm$ standard error were 377 ± 33 sec and 122 ± 5 sec respectively. However, no difference in reactivity between male and female animals was noted. 3. Eight- and 9-week-old animals were exposed to 0.01% ACh and 0.025% Hist. A positive correlation was observed ($r=0.736$, $p<0.01$) between the TNPFDF for ACh and that for Hist. 4. Growing animals from 2 weeks to 20 weeks old were exposed to 0.08% ACh and 0.025% Hist. After inhalation of both chemicals, 6-week-old animals showed the greatest prolongation of mean TNPFDF (lowest sensitivity). 5. Eight-week-old animals were exposed to 0.08% ACh and 0.025% Hist. With both of these chemicals, a positive correlation was observed between TNPFDF and dose threshold (ACh $r=0.886$, $p<0.001$; Hist $r=0.891$, $p<0.001$).

It is known that guinea pigs have a high bronchial sensitivity upon exposure to various chemicals [1], and have been used as asthmatic and allergic animal models [2].

It was previously reported that guinea pigs have thicker bronchial smooth muscle at the terminal and respiratory bronchioles than rabbits, hamsters, rats or mice, and that this is responsible for their high bronchial sensitivity [3].

At present, we are trying to breed guinea pigs with high or low bronchial sensitivities by using the time needed to produce falling down (TNPFDF) due to acetylcholine or histamine exposure as an indicator. For this purpose, various fundamental experiments are in progress.

In many previous studies using guinea pigs as an experimental model, no attention was paid to the age of the animals or to the

experimental environment.

In our experiments reported here, we tried to take the above factors into consideration as far as possible.

Materials and Methods

Experimental animals: 2-week-old (male, weighing between 187 and 224 g) and 8-week-old (male, weighing between 462 and 547 g, female, weighing between 410 and 489 g) SPF Hartley-strain guinea pigs purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Japan) were used. Eight-week-old guinea pigs were used (apart from the 2-week-old guinea pigs employed to observe changes in sensitivity with age) because after this age, the animals showed a constant TNPFDF when exposed to acetylcholine or histamine, and thus 8-week-old animals were thought to

be the youngest of young adults.

Throughout the experiments, these animals were kept in polycarbonate cages ($740 \times 450 \times 230$ mm) with wood shavings and hay on the floor.

The animals were maintained in an air-conditioned clean animal room ($22 \pm 1^\circ\text{C}$ room temperature and $55 \pm 10\%$ relative humidity) with artificial lighting for 12 hours from 7 a. m. to 7 p. m., and a continuous flow of fresh air filtered by microfilters was supplied horizontally from the ceiling. Animals were given a commercial diet (RC-4: Oriental Yeast Co., Tokyo) and tap-water *ad libitum* throughout the experimental term.

Drugs: Acetylcholine chloride (ACh, Ovisot, Daiichi Seiyaku Co., Tokyo) and histamine dihydrochloride (Hist, Nakarai Chemical Co., Kyoto) were dissolved in physiological saline just before use in this experiment.

Inhalation: The previously reported method usually employed in our laboratory was used [3]. Each guinea pig was placed in a glass receptacle (volume, 10 l), and exposed to ACh or Hist using a glass nebulizer nebulizing volume 0.1ml/min, Nippon Shoji Co., Osaka) connected to a compressor (delivered air 61/min, Nippon Shoji Co., Osaka), and the aerosol in the glass receptacle leaked out constantly from the bottom. The air concentrations of ACh and Hist were obtained by calculation from the volume of drug solution consumed (0.1ml/min) and the remitting air (61/min) of the nebulizer. Nominal concentrations of 0.03, 0.05, 0.08 and 0.01% ACh used were 5.0, 8.4, 13.4 and $16.7 \mu\text{g/l}$ air, and those of 0.025, 0.05 and 0.1% Hist used were 1.0, 2.1 and $4.2 \mu\text{g/l}$ air in the glassreceptacle, respectively. Particle size of the aerosol was measured by the method of Murayama *et al.* [16]. Briefly the surface of a slide glass (76×26 mm) was spread with Tefron (Iuchi Seieidou, Osaka). Such slides placed at 5 sites, as shown in Fig. 1, were exposed to physiological saline for 15 sec, and photographed by light microscopy. From the spots of aerosol seen on these photomicrographs, the particle size was measured (particle size, $6.2\text{--}21.8 \mu\text{m}$, mean diameter $14.7 \mu\text{m}$), and the distribution of particle size (5 slides $\times$ 100 particle sizes) is shown in Fig. 2. Inhalation was performed once a week in a room under the same environmental conditions as those in the animal room, (in order to avoid

effects of environmental changes), and when the animals were to be exposed to both of the chemicals, repeated inhalation was performed once a week, first with ACh (8 weeks old) and then with Hist (9 weeks old), as shown in Fig. 7.

Assessment: The sensitivity was expressed by the time interval from the start of inhalation to TNPFD caused by the chemicals. The lowest concentration causing mild dyspnea (respiration with mild sinking of the intercostal chest wall) was taken as the threshold ($\mu\text{g/ml}$) of this response [4].

Fig. 1. Sampling sites in the bottom ($\phi 120$ cm) of glass receptacle for measurement of particle size of the aerosol. Glass slides (5 sites, 76×26 mm) were exposed for 15 sec.

Fig. 2. Distribution of particle size ($N=500$) of aerosol in the glass receptacle

Results

1. Dose dependency for exposure to ACh and Hist : The results are shown in Figs. 3 and 4. The 8-week-old male guinea pigs were exposed to 0.03%, 0.05% and 0.1% (W/V) ACh or 0.025%, 0.05% and 0.1% (W/V) Hist. Between the concentrations of each of the chemicals used and the TNPFD caused by the asthmatic reaction, dose dependency was observed.

Fig. 3. Correlation between the dose and the time needed to produce falling down (TNPFD) of guinea pigs by exposure to acetylcholine. Each column with vertical bar represents the mean $\pm$ S. E. of 10 guinea pigs.

2. Distribution of TNPFD upon exposure to ACh and Hist : The results are shown in Figs. 5 and 6. The 8-week-old male and female guinea pigs were exposed to 0.1% ACh or 0.05% Hist. With both of the chemicals, the mean TNPFD $\pm$ standard error of the animals was 377 ± 33 sec and 122 ± 5 sec, respectively. No significant difference in the reaction between male and female animals was observed.

3. Correlation of the reactions to ACh and Hist : The results are shown of Fig. 7. The 8-week-old male guinea pigs were exposed to 0.08% ACh or 0.025% Hist. A positive correlation ($r = 0.736$, $p < 0.001$) was observed between the TNPFD for ACh and that for Hist.

4. Change in sensitivity with age : The results are shown in Fig. 8. Growing male guinea pigs from 2 weeks to 20 weeks old were exposed to 0.08% ACh or 0.025% Hist. As statistical methods for testing the sensitivity (TNPFD), Bartlett's test and Dunnett's

Fig. 4. Correlation between the dose and the time needed to produce falling down (TNPFD) of guinea pigs by exposure to histamine. Each column with vertical bar represents the mean $\pm$ S. E. of 10 guinea pigs.

Fig. 5. Distribution of the time needed to produce falling down (TNPFD) of guinea pigs by exposure to 0.1% acetylcholine. Bars represent the mean values.

multiple comparison were used. From the results, obtained with both of the chemicals, an almost constant level of sensitivity was observed from 8 weeks to 20 weeks of age, and animals 6 weeks old showed a distinctly lower sensitivity to Hist. On the other hand, animals 2-6 weeks old showed similar sensitivity to ACh, although the animals in the 6-week-old

Fig. 6. Distribution of the time needed to produce falling down (TNPF) of guinea pigs by exposure to 0.05% histamine. Bars represent the mean values.

Fig. 7. Correlation between the values of the time needed to produce falling down (TNPF) of guinea pigs by exposure to 0.08% acetylcholine and that by 0.025% histamine

Fig. 8. Changes in the bronchial sensitivity with age of guinea pigs determined by exposure to 0.08% acetylcholine (ACh) or 0.025% histamine (Hist). TNPF means the time needed to produce falling down. Each point vertical bar represents the means $\pm$ S. E. *: $p < 0.05$, as compared to 7-10 week old animals with ACh and to 2-4, 8, 12-20 week old animals with Hist, using Bartlett's test and Dunnett's multiple comparison

group had the lowest sensitivity of all ($p < 0.05$, at 6 weeks of age as compared with 7-20-week-old animals for ACh and 2-4, 8, 12-20-week-old animals for Hist).

5. Correlation between TNPF and doses of ACh and Hist : The results are shown in Figs. 9 and 10. The 8-week-old male guinea pigs

were exposed to 0.08% ACh or 0.025% Hist to determine the TNPF and 312-10,000 $\mu\text{g/ml}$ of ACh or 63-500 $\mu\text{g/ml}$ of Hist (2 min exposure) were used in order to determine each threshold of response. With both of the chemicals, a positive correlation was observed between TNPF and each threshold (ACh $r = 0.886$, $p <$

Fig. 9. Correlation between the time needed to produce falling down (TNPFd) and the doses of acetylcholine exposed to guinea pigs

Fig. 10. Correlation between the time needed to produce falling down (TNPFd) and the doses of histamine exposed to guinea pigs

0.001, Hist $r = 0.891$, $p < 0.001$).

Discussion

In humans, methods of testing bronchial sensitivity have been established and applied clinically [5, 6, 7]. These methods are basically modifications of Tiffeneau's method [8], in which patients were asked to inhale for a limited period of time serially doubled doses from lower to higher concentrations of ACh, Hist or methacholine. The dose threshold was determined by the concentration of the

chemicals ($\mu\text{g/ml}$) that triggered bronchoconstriction. Patients with a lower dose threshold were judged to have an asthmatic disposition [9].

In experiments with guinea pigs, the same method is applied and the dose threshold is used as an indicator of bronchial sensitivity [4]. However, the reported values have not been constant due to the use of different instruments. It was thought that if the TNPFd caused by the asthma-like reaction after exposure to ACh and Hist is constant, then it might be used as an indicator of bronchial sensitivity. Accordingly, the TNPFd induced by various concentrations of the chemicals was measured, and dose dependency between the concentration of the chemicals and TNPFd was noted.

Furthermore, in order to clarify the normal range, commercially purchased guinea pigs were exposed to 0.1% ACh or 0.05% Hist. With both of these chemicals, the mean values of $\text{TNPFd} \pm \text{standard error}$ for the animals were 377 ± 33 sec for ACh and 122 ± 5 sec for Hist, respectively, and no difference in reactivity was observed between male and female animals. A wide range of individual differences was observed in the bronchial sensitivity of guinea pigs, and thus selection of animals for experimental purposes was thought to be essential.

In experiments for testing the bronchial sensitivity of humans, a correlation between the dose threshold of ACh and that of Hist has been observed [5], but there has been no report of such a correlation in experimental animals.

In this experiment, when guinea pigs were exposed to 0.08% ACh or 0.025% Hist, a positive correlation was found between the values of TNPFd for each of the chemicals. ACh and Hist were used for testing bronchial sensitivity, and the reactive mechanisms of the two chemicals are thought to be different [13, 14], although the positive correlation seen in the experimental animals was considered to support clinical findings.

When guinea pigs are exposed to high concentrations of these chemicals, all the animals show a strong reaction, and thus individual differences in their sensitivity are obscured. Therefore, it is necessary to select an adequate concentration of each chemical to be used.

In humans, bronchial sensitivity and first

age at onset of bronchial asthma have been reported to be either uncorrelated or positively correlated with each other [10, 11].

Therefore, as guinea pigs were weaned at the age of 2 weeks (body weight 160–170 g) [12], we started to expose animals of that age to ACh and Hist, and found a peak prolongation of TNPF (lowest bronchial sensitivity) at the age of 6 weeks.

Thereafter the animals showed constant bronchial sensitivity from 8 weeks to 20 weeks of age. From these experimental results, changes in sensitivity with age should be taken into consideration when testing the bronchial sensitivity of guinea pigs.

The mechanism of bronchoconstriction induced by ACh is known to occur directly through muscarinic acetylcholine receptors [13], while Hist is known to act through the vagus nerve in addition to its direct action on H_1 -receptors [14]. It is assumed that changes in these receptors and nerves might take place with age.

It is reported that guinea pigs gain tolerance through repeated exposure to ACh [15]. However, there is a possibility that later testing for bronchial sensitivity could have been carried out at the age of lowered sensitivity, as we ourselves discovered. In our experiments, the animals did not show any tolerance to the chemicals.

In clinical or fundamental studies, the bronchial dose threshold of response to chemical exposure has been determined. Generally, bronchoconstriction caused by bronchial asthma has been estimated by forced expiratory volume (FEV), FEV/forced vital capacity (FVC) %, volume (V) max and vital capacity (VC), etc. [17]. Therefore, we looked for the existence of any relation between TNPF due to asthmatic reaction and the dose threshold to ACh and Hist in guinea pigs.

With both of the chemicals, a positive correlation was observed. However, this method of using dose threshold as an indicator, in which a large number of animals is required, is thought unsuitable because of the long time and enormous efforts involved. TNPF was used in our assessment as an indicator of bronchial sensitivity because it is a simple and practical parameter to estimate. However, the actions of ACh and Hist are known not to be confined to bronchoconstriction, and other

actions may also operate [18, 19]. Symptoms of prostration in guinea pigs upon exposure to ACh and Hist included cyanosis, wheezing and labored respiration, which were thought to be caused by bronchoconstriction. However, our assessment (TNPF) may require further investigation in order to confirm its propriety.

Recently, in various fields, guinea pigs have been used in experiments as asthmatic and allergic animal models. As we have observed, careful maintenance of experimental conditions such as animal age and chemical concentration should be taken into consideration.

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モルモットにおけるアセチルコリンおよび ヒスタミン吸入に対する気道反応性

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Hartley 系モルモットを用いアセチルコリン (ACh) およびヒスタミン (Hist) の吸入による気道の感受性について検討し、以下の結果が得られた。1. 8 週齢のモルモットを用い、ACh あるいは Hist の吸入によって生じる薬液濃度と呼吸困難による横転開始所要時間との関係について検討した結果、いずれの薬物の場合においても用量依存性が認められた。2. 8 週齢のモルモットを用い、0.1% ACh あるいは 0.05% Hist 吸入による平均横転開始所要時間 ± 標準誤差について検討した結果、それぞれ 377 ± 33 sec, 122 ± 5 sec であったが、性差は認められなかった。3. 8 および 9 週齢の同一モルモットを用い、0.08% ACh あるいは 0.025% Hist 吸入による

横転開始所要時間について相互関係を調べると、両者間には相関関係 ($r = 0.736$, $p < 0.01$) のあることが認められた。4. 生後 2 週齢から 20 週齢に至るまでのモルモットを用い、0.08% ACh あるいは 0.025% Hist 吸入による横転開始所要時間と週齢との関係について検討した結果、両薬物とも 6 週齢をピークとする平均横転開始所要時間の延長 (感受性の低下) が認められた。5. 8 週齢のモルモットを用い、0.08% ACh あるいは 0.025% Hist 吸入による横転開始所要時間とそれぞれの閾値との間には相関関係 (ACh $r = 0.886$, $p < 0.001$, Hist $r = 0.891$, $p < 0.001$) が認められた。