

Histamine-induced bronchoconstriction in conscious guinea pigs measured by strain-gauge transduction

A new method

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A new objective method for measuring histaminedihydrochloride-induced bronchoconstriction in conscious guinea pigs has been developed. The aim of the present work was to investigate whether identical results could be obtained when the clinical observation of conscious guinea pigs with symptoms of respiratory distress (bronchoconstriction) was compared to an objective measuring technique of this parameter. An evaluation of the repeatability of the method was made and the respiration frequency (fR) and histaminedihydrochloride challenge concentration were compared to see whether there was a correlation between the two. Consequently, an apparatus was built that allowed simultaneous recording of breathing pattern and clinical observation of the animal being challenged. The breathing pattern was recorded by a strain-gauge transducer, connected to a measuring bridge, and the curves obtained on a jet ink x-y writer were used for calculating changes in duration of expiratory phase (T_e) and fR. During the attacks of histaminedihydrochloride-induced bronchoconstriction a significantly prolonged T_e could be calculated from the respiration curves. A high degree of agreement was found between this objective measure and clinically observed respiratory distress. The repeatability of the method was comparable to that of corresponding methods used for histaminedihydrochloride challenge in man. No obvious correlation was found between changes in fR and histaminedihydrochloride challenge concentration.

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In man, bronchial reactivity to nonspecific mediators or pharmac, such as histaminedihydrochloride and methacholine has been established as a good and reproducible measure of the degree of disturbance in the airways, particularly in connection with bronchial asthma (4, 11, 15).

In asthmatics, one of the effect parameters most often used for evaluating the degree of asthma or effect of treatment, is the PC_{20} or PD_{20} , referring to the provocation concentration (PC) or provocation dose (PD) of irritant eliciting a 20% decrease in the patient's FEV₁ (forced expiratory volume in 1 s) in proportion to the baseline value (4, 11, 15).

In animal models, however, it is not possible to use corresponding measurements as they require co-operation from the "patient". Nevertheless, airway responsiveness to non-specific irritants or specific allergens is often used as a measure in animal mod-

els when studying different aspects of asthma, e.g. degree of airways inflammation or effect of treatment. Most of the methods previously used are "invasive", e.g. tracheal intubation under general anaesthesia, or they appear to cause the animal discomfort, and could thus influence the results of the experiments.

The aims of this study were:

- 1) To establish a non-invasive objective method for recording respiratory distress in conscious guinea pigs and to evaluate the possible correlation between this method and the trained observers observation of onset of asthma in order to determine a "PC_{asthma}"-value. PC_{asthma} is defined as the provocation concentration of histaminedihydrochloride causing clinically obvious respiratory distress in the animals, i.e. an animal model counterpart to the above mentioned PC_{20} or

PD₂₀. The objective parameter chosen for testing was the development of a prolonged T_e in challenged animals as measured from the respiration curve. This would be easy to calculate and is a well-known symptom of bronchial obstruction. In this animal study, changes in T_e were therefore used as a parallel measure to the FEV₁ changes measured in bronchial challenge in man (4, 11, 15).

- 2) To evaluate the repeatability of the method.
- 3) To see whether there was an obvious correlation between increasing challenge histaminedihydrochloride concentration and changes in fR.

Material and methods

Experimental animals

The study was performed with 2 strains of guinea pigs, selectively bred for either high (IMM/S 209) or low (IMM/R 201-16) reactivity to inhalation of aerosolized aqueous ovalbumin as measured by the respiratory anaphylaxis induced (3, 6, 10). To calculate whether there was a correlation between clinical observations and the objective registration of asthma, 6 animals (3 males and 3 females) of the 209 IMM/S-strain were used. The respiration curves of these 6 animals were also used for the analysis of respiration frequency (fR). To calculate the repeatability of the method, 18 guinea pigs were used, 10 males and 8 females, 12 of the IMM/S 209 strain and 6 of the IMM/R 201-16 strain. They were all approx. 4½ months of age with an average weight of 0.7 kg.

Each animal was challenged with aerosolized histaminedihydrochloride on 3 different days with an interval of at least 7 days. In 6 of the 209-animals, one of the provocations was combined with a 15-min inhalation of physiological saline prior to the histaminedihydrochloride challenge. The baseline value was measured prior to the inhalation of saline, and the histaminedihydrochloride challenge was not performed until 15 min after the inhalation had ended. There was no significant difference between pre- and post-saline measuring for either T_e or fR.

Procedure for histaminedihydrochloride challenge

The tidal breathing method for bronchial histaminedihydrochloride challenge in man (4) was adapted to the guinea pig model. We thus used doubling doses of histaminedihydrochloride, with an initial concentration of 30 µg/ml. A diagram of the apparatus is shown in Fig. 1.

The aerosol was produced by a Wright Nebulizer (M.G. Medical, Colchester, Essex, UK), driven by compressed air at a flow of 6 l/min and a pressure of 1.2 bar, giving an output of 150 µg/min (SD 7 µg/min). The resulting aerosol of nebulized saline had

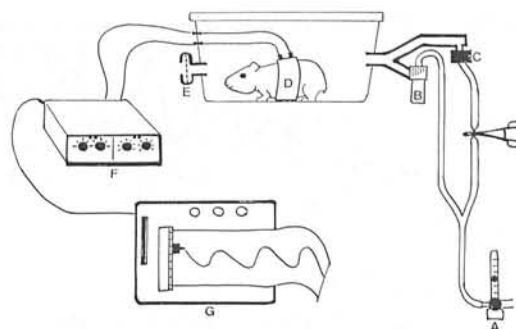


Fig. 1. Diagram of apparatus used for histaminedihydrochloride challenge. Further description in text. (A) Flowmeter. (B) Wright nebulizer (for histaminedihydrochloride). (C) Acorn nebulizer (for Terbutalin). (D) Strain-gauge transducer. (E) Respigard filter. (F) Measuring bridge. (G) Jet ink x-y writer.

a mass median diameter of 3.10 µm, GSD 1.37 (primary particles) (8). The duration of each histaminedihydrochloride inhalation was 2 min and afterwards the animals were clinically evaluated. At the same time, a recording was made of their breathing pattern. If there were no clinical signs of asthma/respiratory distress, the challenge was continued after another 2 min (the time used for observation and obtaining the curve) using a 2-fold concentration difference between each of the challenges.

Recording method

Clinical observation of the animal, using the clinical parameters of Lundberg (forced breathing, cough, gasping for air) was done (6) and breathing pattern and fR were recorded simultaneously by obtaining a respiration curve via a strain-gauge transduction system (Fig. 1). The observer was blinded with regards to the appearance of the respiration curve, i.e. the decision of "asthma" or "not asthma" was based solely on the clinical parameters of Lundberg (6), and the histaminedihydrochloride concentration eliciting clinical asthma was noted. Only after the provocation session had ended were the curve data compared to the clinical data.

The degree of bronchial constriction was measured by calculating the duration of the expiration (T_e) in % of the duration of the total respiration cycle (T_{tot}) and comparing the baseline T_e to that of the provocation concentration which elicited clinical symptoms of respiratory distress in the animals (PC_{asthma}). An example of a respiration curve is shown in Fig. 2. Baseline and PC_{asthma}-values for the 54-histaminedihydrochloride challenges are listed in Table 1. Table 4 shows the respiration curve counts from Fig. 2.

The guinea pig was placed in a transparent provocation box, which has a volume of approx. 5 l. Through a tube in one end of the box, the aerosolised

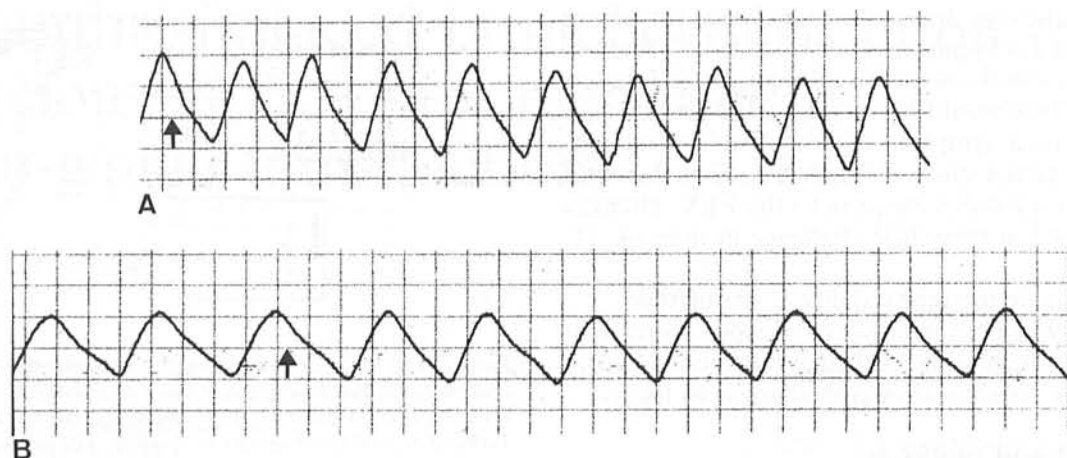


Fig. 2. Example of respiration curves (RC). A: Baseline RC. B: RC obtained after histaminedihydrochloride challenge with 30 µg/ml histaminedihydrochloride, which here was the PC_{asthma} . Arrows indicate T_e . Counts of T_i , T_e and T_{tot} are shown for these 2 curves in Table 4.

histaminedihydrochloride was introduced. As can be seen in Fig. 1, another nebulizer chamber (c) can be connected to the provocation box. This was an Acorn-nebulizer (Medic-Aid, Hook Lane, Pagham, Sussex, UK) containing a beta-2-agonist (Terbutalin 2.5 mg/ml, 2 ml). By moving the clamp from one tube to the other, it was possible to treat the animals immediately with terbutalin and at the same time stop the histaminedihydrochloride aerosol. This precaution was taken as it was not known how the animals would react to this kind of histaminedihydrochloride challenge; we did, but never had to use

it during this project. In the opposite end of the provocation box, a Respigard filter (Vital Signs Inc. Totowa, NJ, USA) was placed in the "exhaust-pipe". The whole procedure took place in a fume cupboard in order to keep the histaminedihydrochloride aerosol away from the investigators and to remove it quickly from the provocation area.

The strain-gauge, a silicone catheter containing mercury, protected by ordinary "elastic" gauze bandage and closed by velcro-tape, was placed around the abdomen of the animal and adjusted so that it fitted tightly but was not uncomfortable. The mea-

Table 1. T_e -values in % of T_{tot}

Animal No.	Provoca-tion day	Baseline value % of T_{tot}	30 µg/ml histamine value % of T_{tot}	60 µg/ml histamine value % of T_{tot}
1	1	68.2	*	69.5
—	2	67.5	73.5	*
—	3	60.2	71.5	*
2	1	67.6	76.4	*
—	2	62.9	*	68.2
—	3	59.8	*	66.1
3	1	61.3	*	70.3
—	2	58.0	*	73.9
—	3	60.9	65.8	*
4	1	68.7	78.6	*
—	2	62.3	72.3	*
—	3	60.4	*	68.3
5	1	63.3	65.0	*
—	2	58.4	71.7	*
—	3	60.5	*	62.6
6	1	62.7	*	73.4
—	2	64.9	72.4	*
—	3	63.2	64.7	*

* " PC_{asthma} " reached at higher/lower provocation concentration of histamine.

Table 2. " PC_{asthma} "-values obtained from 18 GP

Animals	PC_{20} -asthma Day 1 µg/ml	PC_{20} -asthma Day 2 µg/ml	PC_{20} -asthma Day 3 µg/ml
1	60	30	30
2	30	60	60
3	60	60	30
4	30	30	60
5	30	30	60
6	60	30	30
7	30	30	30
8	30	30	30
9	30	30	30
10	30	30	30
11	30	30	30
12	60	30	60
13	500	500	500
14	250	250	250
15	250	250	250
16	500	250	500
17	250	250	250
18	125	250	250

Statistics: One way analysis of variance.

($t=2.10$, $n=18$, $RSD=0.245$, $2p=0.05$). (RSD=residual standard deviation.)

Reproducibility at 95% tolerance limits = $\pm t_{\alpha/2} \times SD / \log 2$

= ± 0.74 2-fold concentration differences.

Table 3. Correlation between histamine concentration and changes in frequency of respiration

Animal No.	Provocation day	Base-line value n=18	30 µg/ml histamine value n=18	60 µg/ml histamine value n=10
1	1	129	143	106
—	2	128	149	*
—	3	108	128	*
2	1	127	147	*
—	2	127	106	134
—	3	153	103	106
3	1	142	125	140
—	2	146	158	146
—	3	121	108	*
4	1	146	135	*
—	2	115	149	*
—	3	133	92	124
5	1	158	92	*
—	2	119	140	*
—	3	116	94	85
6	1	116	119	152
—	2	119	89	*
—	3	146	101	*
		Median	Median	Median
		127.5	122.0	129.0

* Value not obtained because "PC_{asthma}" was reached at a lower concentration of histamine.

Statistics: Wilcoxon's test

Diff BfR-30fR p=0.24

Diff BfR-60fR p=0.35

Diff 30fR-60fR p=0.53

BfR=baseline frequency of resp; 30fR=frequency of resp. at 30 µg/ml histamine;

60fR=frequency of resp. at 60 µg/ml histamine.

suring bridge was adjusted so that it was in equilibrium (both strain-gauge transducer and measuring bridge were constructed for this experiment by Spiropharma Ltd, Denmark). The measuring bridge is based on the principle of Wheatstone's bridge (13). The signals from the measuring bridge were transmitted to the jet ink x-y writer, an ECG writer using a jet of ink instead of an ordinary pen so as to minimize the loss of amplitude on the respiration curve caused by friction between the paper and an ordinary pen. The respiration curve was recorded on ECG paper divided into mm². The paper was transported through the jet ink x-y writer with a speed of 25 mm/s (Fig. 2).

Analysis of respiration curves

When the curves were analysed, a section of 10 consecutive respirations was chosen from each measurement. Each respiration was divided into in- and expiration by drawing a line through the top point of the curve. The durations of inspiration (T_i) and T_e,

Table 4. T_i and T_e respectively, in % of T_{tot} (10 consecutive respirations counted and average calculated)

A: Counting of baseline RC				
	T _i (mm)	T _e (mm)	T _{tot} (mm)	T _i /T _e % of T _{tot}
	4	7	11	36/64
	5	7	12	42/58
	4	8	12	33/67
	5	8	13	38/62
	5	8	13	38/62
	5	8	13	38/62
	4	8	12	33/67
	5	9	14	36/64
	5	9	14	36/64
	5	8	13	38/62
Average	4.7mm	8.0mm	12.7mm	36.8/63.2
B: Counting of "PC _{asthma} " RC (in this case reached at 30 µg/ml histaminedihydrochloride)				
	T _i (mm)	T _e (mm)	T _{tot} (mm)	T _i /T _e % of T _{tot}
	6	11	17	35/65
	6	11	17	35/65
	7	12	19	37/63
	6	10	16	38/62
	5	12	17	29/71
	5	10	15	33/67
	6	10	16	38/62
	6	11	17	35/65
	6	10	16	38/62
	6	11	17	35/65
Average	5.9mm	10.8mm	16.7mm	35.3/64.7

respectively, as well as T_{tot} were counted in mm (Table 4).

The mean T_i and T_e respectively, in percentage of T_{tot}, was calculated (T_i/T_{tot} × 100% and T_e/T_{tot} × 100% resp.). The fR was calculated from the same parts of the curves.

When comparing the curves from different animals, the amplitude of the respiration curves could not be used as a measure of in- or expiratory volumes as it changed when the measuring bridge was adjusted to fit the individual animal.

Statistical analysis

The repeatability of the method was calculated according to Altmann & Bland (1). Since results varied over several orders of magnitude, logarithmic transformation of results was performed. Data on fR and degree of bronchoconstriction were compared by Wilcoxon's 2-sample test.

Results

The clinical observation method for detecting bronchial constriction was used and the breathing pat-

tern was recorded simultaneously. A high degree of agreement between the 2 methods of detecting bronchoconstriction was observed. There was a 100% agreement between the 2 methods at 95% tolerance limits.

Repeatability

A total of 54 histaminedihydrochloride challenges were performed using the method and materials described above. Table 2 shows that the "PC-_{asthma}" of the animals tended to fall into 2 separate groups. This difference is probably due to the animals being of 2 different strains, and will be the topic of a later investigation. At 95% tolerance limits, the mean difference between replicated observations was ± 0.74 2-fold concentration differences (doublings). This is a high repeatability compared to corresponding human studies (15).

Correlation between challenge histaminedihydrochloride concentration and changes in fR

In Table 3 the respiration frequencies are listed before histaminedihydrochloride inhalation (baseline) and after challenge with 30 or 60 $\mu\text{g/ml}$ histamine. The differences between baseline frequency and 30 $\mu\text{g/ml}$ and 60 $\mu\text{g/ml}$ frequencies, respectively, were calculated. When tested with Wilcoxon's test, no obvious correlation was found between increasing concentrations of histaminedihydrochloride (and thus increasing respiratory distress) and changes in fR.

Discussion

In this study a new method for measuring histaminedihydrochloride-induced bronchoconstriction in conscious guinea pigs was developed.

In previous studies involving animal models for bronchial hyperreactivity (2, 7, 12), the animals have been anaesthetized and intubated with a tracheal cannula, thus making it possible to measure exchanged gas volumes and at the same time perform broncho-alveolar lavage (BAL).

Other investigations conducted in conscious animals have used different kinds of whole body plethysmographs measuring gas-exchange volumes, fR and a number of other parameters (5, 9). In the work of Snyder et al. (14) the guinea pigs were challenged with a variety of compounds, all established as possessing bronchoconstrictor effect. The "end point of dyspnoea" (the point just before the animal collapsed) was estimated by observing the animals' respiratory symptoms. In several papers on airway responsiveness in inbred guinea pigs, Lundberg and

co-workers recorded degree of respiratory distress by observing clinical symptoms only (3, 6, 10).

The method introduced in this paper combines a non-invasive objective measurement of the guinea pigs' breathing pattern with the conventional clinical observation. A high degree of agreement was found between the 2 measures. The repeatability of the method is as good as that of histaminedihydrochloride challenges in man (Table 2). No obvious correlation was found between changes in fR and increase in challenge histaminedihydrochloride concentration and, thus, degree of respiratory distress (Table 3).

Our starting concentration of histaminedihydrochloride was 30 $\mu\text{g/ml}$. Table 1 shows that many of the animals developed clinically and objectively measurable bronchial obstruction already at the first provocation concentration. This might camouflage the fact that some of the animals could have developed symptoms at even lower concentrations. The correct procedure in later projects with this method would therefore be to start at a lower histaminedihydrochloride concentration, e.g. 7.5 $\mu\text{g/ml}$, increasing to 15 $\mu\text{g/ml}$, 30 $\mu\text{g/ml}$, and so on.

The method described here is non-invasive, and fairly gentle, an important factor, as guinea pigs are sensitive to handling and obviously feel uncomfortable when "locked" into a position for too long and this may interfere with the observations during experiments with conscious guinea pigs. Furthermore, the non-invasive nature of the method allows numerous observations in each animal, thus establishing an animal model for the investigation of chronic asthma.

With this study we have: 1) developed an objective, non-invasive method for recording bronchoconstriction in conscious guinea pigs; 2) established a high degree of agreement between the clinical method, used as a basis of recording of respiratory distress, and the objective method for recording prolonged T_c ; and 3) found no obvious connection between changes in fR and histaminedihydrochloride challenge concentration.

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