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HISTAMINE-INDUCED BRONCHOSPASM IN UNANAESTHETIZED GUINEA-PIGS

I. Aerosol Technique

By

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In screening drugs for bronchospasmolytic activity, histamine aerosol techniques are commonly used to induce bronchial constriction in unanaesthetized animals. The principle of this method was originally described by *Kallos & Pagel* (7) and has ever since then been recognized as valuable in pharmacological work, where preferably the histaminesensitive guinea-pigs have been used (1). In studies accounted for in the literature, different stages in the changing pattern of respiration in aerosolized histamine have been selected as an indication of "bronchial asthma" (13, 5, 6, 9). The amounts of histamine in the solutions in the used nebulizers have also varied considerably; sometimes concentrations as high as 10 mg/ml have been mentioned. However, correlations between the amount of histamine used and the adequacy of the stages of breathing influence have practically never been presented. Various equipments for generating the histamine mist have further been used, and the amount of histamine necessary to obtain bronchoconstriction seems to a great deal to depend on the physical properties of the aerosol produced (4). Consequently, the importance of the procedure of generating the aerosol have indicated the need to realize these problems before choosing nebulizers for an aerosol study. A construction described by *Rooth* (12), which produces a mist of high density and even quality, was chosen in the present study. The

nebulizers originally adapted for clinical therapy have not been used in animal experiments before, but the construction seems to form a suitable basis for *a study of the relation between the histamine concentrations and the breathing behaviour* of unanaesthetized guinea-pigs in an aerosol. The obtained data were intended to result in a defined and sensitive method for screening bronchospasmolytic compounds of the sympathomimetic type.

METHODS

Usually two nebulizers were run parallel during the experiments (Fig. 1 B). The air was delivered from a compressor (2 kg/cm^2) and the volume of aerosol produced by each nebulizer was about 8 litres per minute requiring 0.3 ml of solution. The diameters of the aerosol droplets were $1-2 \mu$ with the adjustable baffle 4-5 cm from the nozzle in the nebulizer (12). Two macrolon cages (Fig. 1 A) measuring $12 \times 12 \times 20 \text{ cm}$ were connected to the nebulizers by a

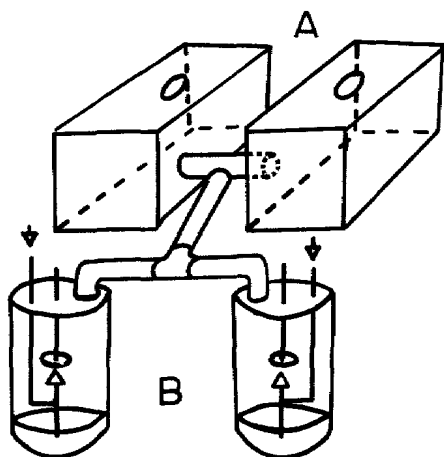


Fig. 1.

The drawing illustrates the components and the arrangement of the apparatus for testing guinea-pigs in histamine aerosol. At (A) two macrolon cages for the animals. The cages are connected to two nebulizers of Rooth-Petersen type (B) by wide glass and PVC tubes.

short and wide polyethylene tube. Outlets for the excess of generated mist were available through slits in the back of the boxes. The arrangement gives a slight excess of pressure and an even flow of mist through the cages. Untreated (saline treated) male guinea-pigs, each weighing between 200–400 g, were used. One animal placed in each box. This arrangement made a direct comparison possible, e.g., a drug-treated animal and a saline-treated control in a screening. Each animal could be removed separately without affecting the concentration of aerosol in the other box. The solution used to produce the aerosol was prepared from histamine dihydrochloride and dissolved in distilled water containing 10 per cent glycerol by volume. Histamine concentrations referred to the base form were varied between 0.2 and 0.8 mg/ml. These amounts of histamine correspond to concentrations between 8 μ g/litre and 35 μ g/litre, respectively, in the aerosol.

For an objective registration of the successive alterations of the breathing rhythm in the histamine milieu, a strain-gauge transducer on a flexible plate (5 $\times$ 20 mm) was fixed to the thorax. The frequencies of breathing were registered by a Grass polygraph. This objective recording was combined with a direct observation of the animals.

RESULTS

Normal breathing frequency of guinea-pigs is about 100–130 per minute. Under the influence of histamine, the breathing will at first increase by sometimes more than 100 breaths per minute. This increase is reversed in a sudden drop and is followed by irregular and laboured breathing. The disturbance will finally result in a respiratory collapse and death. These observations were also made and described by e.g. *Landay & Gay* (8), *Amdur* (2) and, after s.c. histamine, by *Westling* (14).

The first part of this study was intended to demonstrate quantitative and qualitative influence on breathing behaviour from a stay in histamine aerosols of different concentrations. Two respiratory stages, which could be indicated with sufficient certainty by registration and observation, were selected as points of influence (POI) and analysed. The first stage (POI-1) was related to the above-mentioned distinct drop in frequency, after an increase. The second stage (POI-2) was related to an advanced respiratory spasm where the animals were "kneeling".

TABLE 1

Histamine Concentration in the Aerosol and Influence on Respiration.

Histamine concentration in the solution nebulized (mg/ml)	Time in minutes in the aerosol to reach		Number of guinea-pigs
	POI-1	POI-2	
0.4	2.2 $\pm$ 0.2	(6 $\pm$ 2)	20
0.8	1.6 $\pm$ 0.4	1.8 $\pm$ 0.5	20

The effect of two histamine concentrations (0.4 and 0.8 mg/ml) compared with regard to time of treated guinea-pigs to reach two different stages (POI-1 and POI-2) of histamine-influenced respiration (see text).

Table 1 gives a representative series from this part of the investigation. The time from introduction of the animal in the aerosol to the appearance of the mentioned respiratory stages (POI-1 and POI-2) was noted. The histamine concentrations 0.4 mg/ml and 0.8 mg/ml were used, and 20 animals were tested in each dose.

When using 0.4 mg/ml of histamine, POI-1 was reached after a stay of 2.2 minutes in the aerosol. The stage was usually registered by strain gauge without difficulties and disturbance and was also easily observed. Fig. 2 illustrates typical strain-gauge registrations from two different animals in this series of experiment. In the registrations, POI-1 was reached after about 90 sec and 114 sec, respectively, where the breathing frequency had dropped to about 120 per minute after an increase to about 220 and 180 per minute. In Table 1 the number of minutes to reach POI-2 in 0.4 mg/ml are put in parentheses, because several animals exceeded 10 minutes in the aerosol. After this time, the guinea-pigs seems to begin to adapt to histamine, resulting in less adequate responses. When animals were put in an aerosol emanating from 0.8 mg/ml of histamine, POI-1 was reached after about 1.6 minutes. However, the point was often difficult to localize because the changes in respiration appeared in quick succession. Further, many animals, after the introduction in hista-

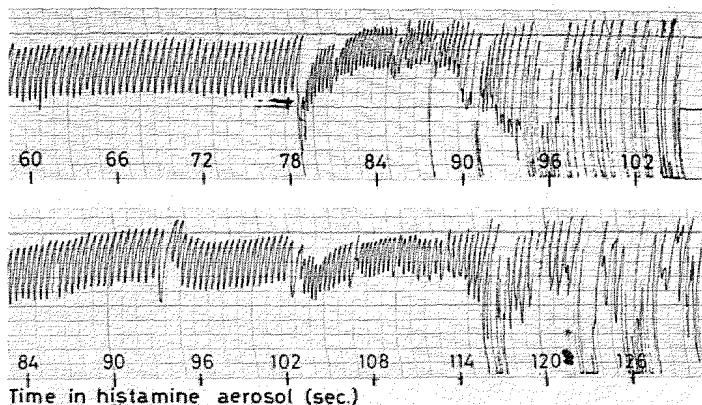


Fig. 2.

The figure demonstrates two representative registrations from different guinea-pigs in aerosol by use of a strain-gauge on the thorax. The aerosol was generated from a solution containing 0.4 mg/ml histamine. The initial changes of the breath are registered and an increase in frequency from about 120 breaths per minute to about 200 is noted after about 84 and 108 sec, respectively, in histamine. The rise in frequency is a warning sign indicating the appearance of the drop selected as POI-1. This stage for the guinea-pigs shown in the figure is reached after about 90 and 114 sec. At this time in histamine, the frequency is about the same or lower than before the initial rise; an increasing irregularity of the amplitude of breathing also appears.

mine, immediately started irregular breathing as a sort of shock response to this higher concentration. POI-2 was reached after about 1.8 minutes, but this indication stage too was less reproducible. Registration by use of strain-gauge transducer gave results difficult to interpret, and a direct observation of the animals was preferable. Indication by use of POI-2 and 0.8 mg/ml of histamine must be characterized as less specific; great individual differences were revealed in the time it took to reach an adequate response.

As a consequence of the presented analysis, the POI-1 has been selected as a suitable indication stage, where registration by strain-gauge as well as observation gave a clear indication. Further studies of breathing behaviour were performed, using

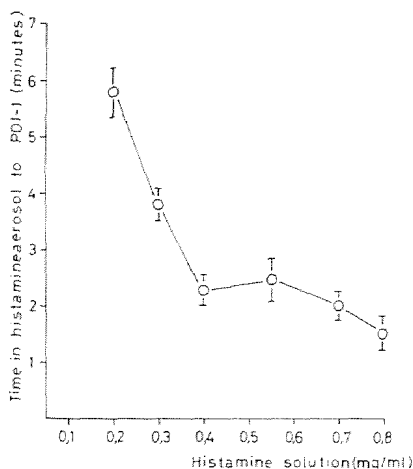


Fig. 3.

The figure illustrates the relation between histamine concentration in the nebulizer solution (abscissa) and the time possible for guinea-pigs to withstand the generated aerosol before the appearance of POI-1. Each point represents 30 animals and the vertical bars indicate S.E. of the mean.

POI-1 and histamine concentrations between 0.2 and 0.8 mg/ml in the solution. Fig. 3 records the results from this part of the studies. The doses 0.2, 0.3, and 0.4 mg/ml seem to give a linear dose response relation. In this relation, 0.4 mg/ml was the most effective, taking the animals to POI-1 after about 2.3 minutes of histamine treatment, i.e. within a safe time-distance from adaptation phenomenon. According to the figure, higher concentrations of histamine were of no advantage. From observations of a large number of animals, in aerosol from 0.4 mg/ml of histamine, it was further obvious that only 2 or 3 per cent could withstand the aerosol for more than 4 minutes. Most animals (about 80 per cent) reached the POI-1 between the first and third minute. The distribution of the number of animals with respect to time to POI-1 is given in the diagram in Fig. 4.

When the method was used for screening purpose, 4 min-

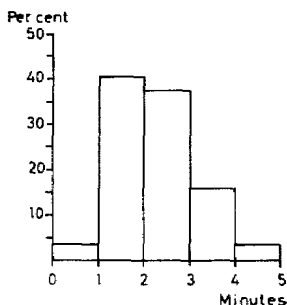


Fig. 4.

The distribution of guinea-pigs in per cent (ordinate) in relation to time (abscissa) to reach a defined influence on the respiration (POI-I) is illustrated. Most animals reach this stage of respiration during the 2nd and 3rd minute; only 4 per cent withstand the histamine aerosol for more than 4 minutes. The histamine concentration was 0.4 mg/ml. The presented data are based on 146 animals.

utes was the limit. The guinea-pigs treated with a bronchospasmolytic agent and exceeding 4 minutes in the aerosol were considered to be protected against the influence of histamine by the administered drug.

DISCUSSION

The relation obtained between concentration of histamine (0.2 and 0.4 mg/ml) in the aerosol and time to POI-I seems to indicate the necessity of raising a certain concentration of histamine in the respiratory tract before the spasmic stage appears (Fig. 3). When using POI-I no improvement seems to be gained from using higher concentrations of histamine than 0.4 mg/ml. On the contrary, in the next higher amount the stay in the aerosol tends to be prolonged. A possible explanation of the phenomenon is perhaps a feeling in the animals of irritation induced by histamine, i.e. in concentrations up to 0.4 mg/ml, no obvious irritation might be noted by the animals and no hesitation in breathing occur, whereas at 0.5–0.6 mg/ml an repulsive reaction might begin which makes the

animal unwilling to breathe deeply, which is why they can endure for a longer time than in 0.4 mg/ml. In spite of the discomfort at histamine, with a tendency to superficial breathing, still higher histamine concentration will rapidly induce bronchial constriction. It may be pointed out that the results presented in Fig. 3 are obtained from experiments running during the same period of time and with animals from the same stock.

To make the best use of unanesthetized animals in experiments of the presented type, a basic principle is to try to eliminate inadequate disturbance of the breathing reflexes. An important step is to select aerosols of suitable quality and concentration. Not to "force down" but to "slip" the histamine into the lungs is the ideal way.

If an initial influence of respiration (POI-1) is registered as indication in an adequate histamine concentration, the animals show a slight individual difference in the response. As a consequence, in this case there is no improvement by pre-treating the animals in histamine to determine the individual sensitivity. On the contrary, a pretest may be advisable and of some advantage when an advanced respiratory spasm (POI-2) is selected because of the great variability in response in this situation. However, it is important to remember that also a short exposure to histamine can decrease the compliance of the lungs for days. Furthermore, if the animals are put back in histamine only a few hours after treatment, they may also, in response to this earlier experience in the histamine cage, react with a type of retarded breathing. Moreover it seems natural to "place" the point of influence as early as possible in the chain of breathing events, because, as mentioned, a form of tolerance seems to appear after some time in histamine. In this connexion, an investigation by *Atkinson, Pun & Rand* (3) is relevant. These authors have ventilated guinea-pigs with 10 per cent CO₂ in air and noted a rapid decrease in the response to histamine. Changed response by the animals to administered sympathomimetic agents was also mentioned. Consequently, a prolongation of disturbed

breathing could produce the same result, i.e. insensitivity to histamine and inadequate response to tested bronchodilating agents.

A registration of the behaviour of the animal in the mist by observation has, of course, no influence on the motility, and this is of importance for an undisturbed breathing reflex. However, an objective registration of the breathing is always desirable. The author, in some experiments, has tried to analyse the respiratory change in histamine aerosol by using body plethysmographs. The method was time-consuming and the results mostly hard to interpret. The same experience of a thoracography by use of pneumatic systems has been reported by *Noelpp & Noelpp-Eschenhagen* (10). These authors have also tried optical and electrical devices for registration in order not to hamper the free movement and to influence the breathing reflexes as little as possible. As mentioned, the author has found the use of a small strain-gauge, connected to the writing unit by a thin wire, suitable as an undisturbing but effective way of registering the breathing.

SUMMARY

A correlation between different histamine concentrations, administered as aerosol, and the influence on the respiration of guinea-pigs was made in the present study. The intention was to obtain basic data for developing a sensitive method for screening bronchospasmolytic agents.

The most suitable histamine concentration for use in the nebulizer proved to be 0.4 mg/ml. This was the highest in a linear dose response between amount of histamine and time to reach a defined influence on the respiration. The most reproducible stage without obvious individual variations in the changing pattern of breathing and consequently suitable as a definition of histamine influence, was the marked increase in frequency followed by a rapid drop. This stage appeared as an early sign of histamine influence and was called POI-1. The stage was easily localized by use of a strain-gauge on the

thorax of the animals or by a direct observation. POI-1 was reached in 96 per cent of untreated guinea-pigs within 4 minutes when the aerosol was generated from a solution containing 0.4 mg/ml histamine.

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