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Research Article

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Reflex Effects of Aerosolized Histamine on Phrenic Nerve Activity

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ABSTRACT Studies were conducted in anesthetized, paralyzed dogs on the effect of aerosolized histamine on phrenic nerve activity. The paralyzed dogs were ventilated in phase with their recorded phrenic nerve activity at a constant inspiratory flow-rate, using a cycle-triggered ventilator. Phrenic nerve activity was measured before and during administration of aerosolized histamine while the inspiratory flow-rate and arterial blood gases were kept constant. In addition, before and after histamine, phrenic nerve activity was recorded for single bursts during which the ventilator was switched off. The effects of histamine on respiratory resistance were prevented by prior administration of isoproterenol and atropine. Although no changes occurred in respiratory resistance, histamine increased the instantaneous magnitude of phrenic nerve activity. The effect was evident early in the inspiratory period and was found even when the lungs were not inflated. Inflation of the lungs excited phrenic nerve activity; this effect increased after histamine. All of these actions of histamine were abolished by vagotomy. We conclude that histamine increases phrenic nerve activity during inspiration by a vagal reflex.

INTRODUCTION

Recent studies from this (1) and other laboratories (2-4) have shown that increases in pulmonary vagal afferent activity secondary to lung inflation cause an increase in the neural output to inspiratory muscles. This reflex pathway has potential pathophysiological importance since stimulation of pulmonary vagal receptors occurs in many common pulmonary diseases (for summary see 5). In lung disease, therefore, changes in the efferent neural output to the respiratory muscles may be occasioned by changes in afferent ac-

tivity from the lungs and may not just be secondary to alterations in arterial blood gases. In this study we have examined the effect of the inhalation of aerosolized histamine on phrenic nerve activity while arterial blood gases were kept at constant levels. Histamine was studied since it is known to stimulate all neural receptors in the parenchyma and airways of the lung (6-9). Furthermore, since histamine is released in the lung in allergic asthma (10), reflex actions mediated by the effects of histamine on the activity of pulmonary vagal receptors could play a role in initiating the disturbances of neural control of ventilation in this disease (11, 12). Histamine also causes bronchoconstriction and an increase in airway resistance (13). Because increases in airway resistance from any cause can lead to reflex changes in inspiratory neural output (14), we avoided this potentially confounding variable by preventing the effect of histamine on airway resistance pharmacologically. Thus, in this study we examined the reflex consequences of the direct effects of histamine on pulmonary vagal receptors and not of the indirect effects produced by increased bronchial smooth muscle tone.

METHODS

Studies were carried out in 10 adult mongrel dogs (7.1-10.3 kg body wt). The dogs were anesthetized initially by administering pentobarbital sodium (20-30 mg/kg) intravenously; anesthesia was maintained by a constant intravenous infusion of pentobarbital (3-6 mg/kg per h). The trachea was exposed through a midline incision in the neck and cannulated below the larynx. Catheters were inserted into the femoral artery for measurements of blood pressure and obtaining blood samples and into the femoral vein for administering drugs intravenously. Body temperature was measured by a rectal thermometer and was maintained at $38.5 \pm 0.5^\circ\text{C}$ by a heating pad.

The C5 root of one phrenic nerve was isolated in the neck by a ventral approach, cut distally, and its sheath removed. The cervical vagus nerves were isolated through the same ventral incision on the ipsilateral side and through a similar ventral approach on the contralateral side, and prepared for

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later sectioning. The phrenic nerve signal was recorded using a bipolar platinum electrode suspended in a pool of warm mineral oil. The nerve signal was amplified by a Grass P15 AC differential pre-amplifier (Grass Instrument Co., Quincy, MA) then rectified and processed by a low-pass, third-order Paynter filter (averaging period 100 ms) to compute the moving average of the neural activity.

After preparations for phrenic nerve recording were completed, the dog was paralyzed by administering gallamine triethiodide intravenously (60 mg initially, followed by subsequent doses of 30 mg as required). The dog was then ventilated by the cycle triggered ventilator described by Pack et al. (1). Using this apparatus (see Fig. 1), the lungs were ventilated in phase with the animal's recorded phrenic nerve activity: immediately after the onset of the inspiratory burst of phrenic nerve activity, a solenoid valve in the ventilator (valve B, Fig. 1) was automatically closed and the lungs were inflated at a constant inspiratory airflow from a positive pressure source; as soon as the moving average phrenic neurogram began to decrease, the solenoid valve (valve B) opened automatically and the lungs deflated passively. The inspiratory flow-rate was set between 0.25 and 0.33 liter/s and was maintained constant in each animal throughout the experiment. This flow-rate, which was higher than the eupneic flow-rate of the animal, was chosen to provide sufficient volumes of expired gas for end-tidal samples despite the tachypnea produced by the administration of histamine. End-tidal CO_2 was held at a constant level between 6.5 and 7.0% by adding small amounts of CO_2 , as needed, to the inspire. This relatively high end-tidal CO_2 was chosen because we found, in preliminary experiments with this cycle-triggered ventilator using constant inspiratory airflow, that end-tidal CO_2 increased, on occasion, above normal levels because of the short inspiratory durations after the administration of histamine. Furthermore, after histamine administration, we controlled end-tidal CO_2 at slightly lower levels, i.e., between 6.0 and 6.5%, in order to obtain either the same, or

slightly lower, arterial blood gas values as before histamine; this reduction in end-tidal CO_2 presumably counteracted the widening arterial-alveolar difference for CO_2 caused by histamine. The end-tidal CO_2 concentration was monitored by means of a Godart capnograph (Utrecht, Holland) and constancy of blood gas composition was checked by frequent determinations of arterial blood gas composition. Arterial PO_2 was determined by a Clark Electrode, arterial PCO_2 by a Severinghaus electrode, and pH by a glass electrode (Radiometer Corp., Bucks, England). Isoproterenol was used in these experiments (see experimental protocol, below) to prevent the change in airway resistance produced by histamine. Because we wished to minimize the effects of isoproterenol on respiratory neural output, which are mediated largely by stimulation of the carotid body (15), the animals were ventilated throughout with 100% O_2 . This hyperoxic condition markedly reduces the stimulatory effect of isoproterenol on ventilation (15).

Airflow was measured using a pneumotachograph (Silverman) and differential pressure transducer (Validyne MP45, Validyne Engineering Corp., Northridge, CA). Tidal volume was computed by electrical integration. Blood pressure and tracheal pressure were measured using pressure transducers (Statham P23Db, Statham Instruments, Inc., Oxnard, CA) attached to appropriate cannulae (femoral artery and tracheal). These data, together with measurements of tracheal CO_2 concentration, were displayed on an oscilloscopic recorder (Electronics for Medicine, Inc., Pleasantville, NY, model DR 12).

Total respiratory resistance was determined by the forced oscillation method as modified by Goldman et al. (16) using a frequency of oscillation of 3 Hz. Prior to the determination of resistance, both solenoid valves of the ventilator, i.e., valves A and B in Fig. 1, were closed, with the animal's lung volume at functional residual capacity. During the subsequent period of apnea, 50 cycles of oscillatory flow were introduced to the lung. The maximum airflow of the forced

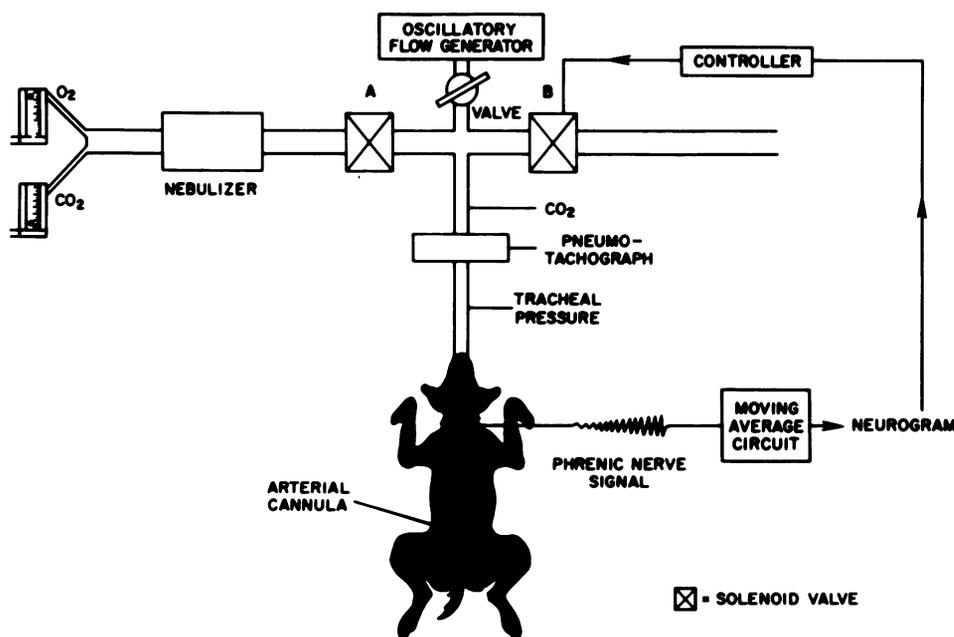


FIGURE 1 Experimental preparation used in these studies (for details see text).

oscillation was <0.25 liter/s. Thus the volume delivered and withdrawn from the lung during each cycle of oscillation was less than one-half of the animal's eupneic tidal volume. During the period of forced oscillation, the pressure proximal to the endotracheal tube was measured by a pressure transducer (Validyne MP45) and tracheal airflow by the pneumotachograph (Silverman) and associated differential pressure transducer (Validyne MP45). Data from the transducers were digitized on-line into a PDP-12 computer system at a sampling rate of 250 Hz for subsequent computerized calculation of total respiratory resistance (see section on data analysis).

Experimental protocol

Measurements before administration of histamine (control state). To block the change in respiratory resistance produced by histamine, isoproterenol was administered by aerosol (0.5 cm^3 of 0.05% solution of isoproterenol hydrochloride) and atropine intravenously (0.1 mg/kg). Isoproterenol was aerosolized using a DeVilbiss ultrasonic nebulizer (model 640) in series with the inspiratory side of the ventilator (see Fig. 1). Because isoproterenol is known to stimulate phrenic nerve activity (15), the control measurements of phrenic nerve activity were made after these drugs had been administered.

After administration of these drugs, phrenic nerve activity, as processed by the moving average method, was recorded during 10 consecutive breaths at the pre-set level of inspiratory flow-rate and end-tidal CO_2 concentration. After these measurements, phrenic nerve activity was also recorded in the absence of breathing movements. For this purpose, while the animal was paralyzed, the ventilator was turned off for a single breath during expiration so that the lungs were not inflated during the subsequent phrenic nerve burst. 10 such identical single breath tests (apnea) were carried out and the resulting phrenic neurograms were recorded. Since each of these tests produced a brief change in end-tidal CO_2 concentration, the next test was not carried out until the end-tidal CO_2 concentration was once again constant at the required level for at least five breaths. The phrenic neurograms of the breaths preceding each apneic test were also recorded. After these recordings of phrenic nerve activity were obtained, total respiratory resistance in the control state was measured by the forced oscillation method.

Administration of histamine. Following control measurements, histamine phosphate, dissolved in a buffered solution of 0.5% NaCl, 0.275% NaHCO_3 , and 0.4% phenol, was administered to the animal using the DeVilbiss nebulizer in the inspiratory line of the ventilator. Because the response to histamine is known to vary from dog to dog (17), aerosolized histamine phosphate was administered to each dog in increasing concentrations from 0.01 to 0.50% (0.01, 0.025, 0.05, 0.10, 0.25, 0.50%). During this initial period of histamine administration, the concentration was found that produced at least a 50% increase in respiratory rate above that in the control period. At each concentration, 1 ml of the solution of histamine phosphate was nebulized at a rate of ~ 1 ml/min. After delivery of a given concentration of histamine, the respiratory rate was monitored. If breathing frequency had increased by $>50\%$ of its control value, the concentration of histamine given to the animal was not further increased. If, on the other hand, respiratory rate had not so increased, 1 ml of the next higher concentration of histamine phosphate was nebulized and this process was repeated. Once the required concentration of histamine phosphate was determined, the nebulizer was adjusted to deliver this concen-

tration of histamine continuously but using a lower rate of nebulization (0.25 ml/min). This rate of continuous administration of histamine, in what is called here the maintenance period, resulted in a relatively constant level of stimulation as judged by measurements of respiratory rate.

The amounts of histamine delivered to each animal during the initial and maintenance periods were calculated retrospectively by the following method. Since the nebulizer was in series in the inspiratory line of the ventilator (see Fig. 1), the amount delivered to the animal depended on the fraction of time during which the lungs were being inflated. For the initial period, this ratio of inspiratory duration to total duration of the respiratory cycle was measured at each incremental concentration of histamine in each animal. These ratios, together with the corresponding concentrations, were then used to compute the total dose of nebulized histamine administered during the initial period. Similarly, the ratio of inspiratory duration to total cycle time, during the maintenance period, was measured and used to calculate the rate of delivery of nebulized histamine during this maintenance period.

Measurements during continuous administration of histamine. After the maintenance rate had been determined, delivery of nebulized histamine at this constant rate produced a relatively constant respiratory frequency and phrenic neurogram configuration. Phrenic nerve activity was then recorded during 10 consecutive breaths at the same inspiratory flow-rate as in the control state. To ascertain whether histamine altered phrenic nerve activity in the absence of lung inflation, an additional 10 neurograms were recorded, with the ventilator switched off for single breaths, as described above for the control (prehistamine) measurements. The neurograms of the breaths preceding these tests were also recorded. Between each of these tests sufficient time was allowed for end-tidal CO_2 concentration to return to its required level.

Following these recordings of phrenic nerve activity, respiratory resistance was determined by the forced oscillation method. Whenever there was a statistically significant increase in respiratory resistance between control and test levels ($P < 0.01$), the animal was allowed to return to the baseline state and the entire experiment was repeated after administering a 50% greater dose of isoproterenol.

Studies after bilateral vagotomy. In 5 of the 10 animals studied, the entire protocol was repeated following bilateral cervical vagotomy. For these studies, identical doses of isoproterenol, atropine, and histamine were administered as in the prevagotomy state. Phrenic neurograms were measured for 10 consecutive breaths, before and during administration of histamine (see above).

Data analysis

Measurements of phrenic nerve activity. To quantify phrenic nerve activity, the data were digitized from paper records using a digitizer of the cursar tablet type (Bitpad, Sumagraphics, Fairfield, CT) into a PDP-12 computer system. The effective sampling rate was 200 Hz. In the tests, at both constant and zero airflow, the height of the moving average phrenic neurogram was measured automatically at 50-ms intervals from the onset of the phrenic nerve burst and the data tabulated.

In the tests at constant airflow, comparison of the height of the phrenic neurograms, before and during administration of histamine, were made at two specific times from the onset of inspiration: firstly at 100 ms from the onset of phrenic nerve activity; secondly at the longest time from the onset

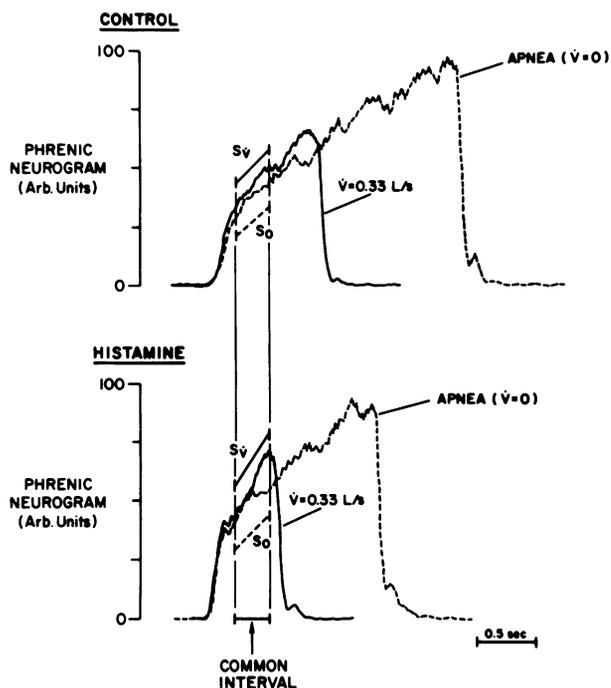


FIGURE 2 Effect of lung inflation on phrenic neural output before (top panel) and during (bottom panel) histamine administration. In each panel, the neurogram of a single test in which the lungs were not inflated for one phrenic nerve burst ($\dot{V} = 0$, - - -) is compared with the preceding breath at the same constant inspiratory airflow (0.33 liter/s, —). Before (control) and during histamine administration, the increased airflow induced augmentation of phrenic nerve activity. To compute the augmentation of phrenic nerve output by lung inflation, the slopes of the phrenic neurograms were compared in tests at zero (S_0) and increased (S_v) air-flow over a common time interval. (For details see text.) In the control period and during histamine, the arterial PCO_2 (41 and 39 mm Hg, respectively) and total respiratory resistance (1.41 and 1.43 cm H_2O /liter per s, respectively) were almost identical.

of inspiration which was common to all such tests in a given animal. To carry out this latter measurement we: (a) Measured the duration of the shortest phrenic nerve burst of any test in a given animal. In practice this was always a test during histamine administration. (b) Determined the 50-ms interval (50, 100, 150, 200 ms, etc.) that was closest to, but preceded, this measured time. (c) Obtained the height of each recorded phrenic neurogram at this specific 50-ms interval. Since the durations of inspiration varied between dogs, this time of measurement also varied between animals.

In a similar manner, measurements were made in the tests at zero airflow at the longest time after the onset of inspiration that was common to all of these tests in any given animal. The duration of inspiration in the tests at zero airflow were longer. Therefore, these latter measurements were made at a later time after the onset of inspiration.

To assess whether aerosolized histamine affected the amount of augmentation of phrenic nerve activity produced by lung inflation, the effect of lung inflation on phrenic nerve activity was measured before and during administration of

histamine. This effect was determined by comparing the slopes of the phrenic neurograms during phrenic nerve bursts in which the lungs were or were not inflated. The slopes of the neurograms were computed during the linear rise of phrenic neural activity following the initial abrupt onset (see examples, Fig. 2), by computerized least-squares linear regression. Specifically, the slopes were determined for tests at constant high airflow (S_v),¹ and at zero airflow (S_0), over a common time interval. This interval was set by the duration of inspiration in the tests at high airflow during histamine, since these phrenic bursts were shortest. In practice, the interval lasted from 200 ms after the start of phrenic activity to the shortest duration of inspiration, which always occurred in tests during histamine administration (see Fig. 2). This choice of interval ensured that, in each animal, the slopes of all neurograms were measured over a common time interval with respect to the onset of inspiration. The difference between the magnitude of the slopes of the neurograms in the tests at zero (S_0) and high airflow (S_v), i.e., $S_v - S_0$, is a measure of the effect of lung inflation on the magnitude of phrenic nerve activity (1). Therefore, by comparing the magnitude of this difference in neural slopes before and during histamine administration, the effect of histamine on the amount of augmentation of phrenic nerve activity due to lung inflation was determined (see example, Fig. 2).

For measurements of respiratory timing, inspiratory duration was measured from the onset of phrenic nerve activity until the beginning of the rapid terminal decline in activity. Expiratory duration was measured from the end of inspiration in one breath (see above) until the onset of phrenic nerve activity in the next breath.

Measurement of respiratory resistance. Respiratory resistance was computed from the digitized airflow and tracheal pressure signals during oscillatory flow using the method described by Goldman et al. (16). The digitized data were initially filtered using a nonrecursive digital filter that did not introduce phase shift and whose corner frequency was 75 Hz. The two points in each cycle of oscillation at which volume acceleration is zero were determined automatically by detecting the zero crossing of the numerically differentiated flow-signal. The filtered airflow signal was differentiated using a seven point central difference approximation. The automatic determination of the zero-crossings of this differentiated signal were checked by direct visual inspection. At these points on each cycle of oscillation the pressure related to inertia is zero and that related to the elastic properties of the system is identical (16). Thus, resistance was calculated directly by dividing the difference in the pressure between these two points on any one cycle of oscillation by the difference in airflow. Identical measurements were made on 50 cycles of oscillation and the mean and standard deviation of the measurements of total respiratory resistance computed.

Statistical analysis of data. In each animal measurements before and during administration of histamine were compared by a paired *t* test. Differences were said to be significant if $P < 0.01$.

RESULTS

Tests at constant inspiratory airflow. Aerosolized histamine increased phrenic nerve activity as judged

¹ Abbreviations used in this paper: S_0 , slope of phrenic neurogram at zero airflow; S_v , constant high airflow.

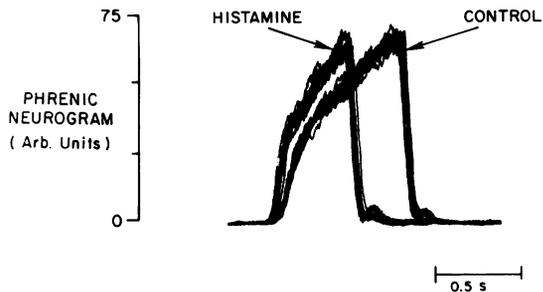


FIGURE 3 Augmentation of phrenic nerve activity produced by histamine. The record shows the phrenic neurograms of 10 single breaths at a constant inspiratory flow-rate (0.33 liter/s), before (control) and during histamine administration. The data are from the same animal as that in Fig. 2, and the arterial PCO_2 and respiratory resistance are given in the legend to that figure.

by comparison of neurograms measured at the same inspiratory airflow and arterial blood gases before and during its administration (see Fig. 3). This increase was observed even though changes in respiratory resistance were blocked by the prior administration of isoproterenol and atropine. (For results of measurements of blood gas tensions and respiratory resistance, see below.) This augmenting effect of histamine on inspiratory neural output was found in every animal studied. Phrenic nerve activity was compared in each animal by measuring the heights of the phrenic neurograms before and during histamine administration at an identical time after the start of inspiration. In each animal, at the longest time from the onset of inspiration that was common to all tests (for details of measurement, see data analysis), phrenic nerve activity was significantly greater during administration of histamine ($P < 0.001$). This is shown for each of the 10 dogs in the three right-hand columns of Table I in which the heights of the phrenic neurograms

(mean \pm SE) for 10 breaths at the same inspiratory airflow before and during histamine administration are given, together with the times from the onset of inspiration when these measurements were made. The time of this measurement varied between animals since it was dependent on the duration of inspiration during histamine administration.

The augmentation of phrenic nerve activity caused by histamine was present from early in inspiration. Thus, at 100 ms after the onset of phrenic nerve activity, the magnitude of the phrenic neurogram during histamine administration was significantly greater than prehistamine in each animal studied ($P < 0.001$). This is shown in the three left-hand columns of Table I in which the heights of the phrenic neurograms (mean \pm SE) at 100 ms after the onset of inspiration are given. Again, in each dog, these measurements were from 10 single breaths in which inspiratory airflow was the same before and during histamine administration.

Tests with no lung inflation (apnea). The excitatory effect of histamine on phrenic nerve activity occurred even in the absence of lung inflation (see Fig. 4). In each animal, the heights of the phrenic neurograms were measured during tests in which the lungs were not inflated for a single phrenic nerve burst, at the longest time from the onset of inspiration which was common to all such tests before and during histamine administration (for details of measurement, see data analysis). This latter time varied between animals since it depended on each animal's duration of inspiration after histamine. This augmentation of inspiratory neural output in the absence of lung inflation was found in all animals ($P < 0.001$). This is shown in Table II in which the heights of the phrenic neurograms (mean \pm SE) for 10 single test breaths when the lungs were not inflated, before and during histamine administration, are given, together with the times from

TABLE I
Magnitude of Phrenic Nerve Activity before and during Histamine Administration

Dog No.	Time of measurement (From onset of inspiration)	Height of phrenic neurogram		Time of measurement (From onset of inspiration)	Height of phrenic neurogram	
		Control	Histamine		Control	Histamine
	ms	arbitrary units		ms	arbitrary units	
1	100	16.8 \pm 0.2	29.0 \pm 0.6	400	102.0 \pm 0.4	134.2 \pm 0.7
2	100	17.3 \pm 0.1	24.2 \pm 0.3	500	36.0 \pm 0.2	48.2 \pm 0.2
3	100	17.8 \pm 0.2	30.3 \pm 0.2	300	34.8 \pm 0.2	48.2 \pm 0.3
4	100	17.2 \pm 0.4	28.3 \pm 0.4	500	64.0 \pm 0.5	82.2 \pm 0.3
5	100	18.0 \pm 0.3	30.0 \pm 0.3	300	58.0 \pm 0.3	66.8 \pm 0.3
6	100	16.8 \pm 0.2	30.2 \pm 0.3	400	53.2 \pm 0.3	67.0 \pm 0.2
7	100	17.0 \pm 0.3	51.0 \pm 0.5	450	96.5 \pm 0.7	118.8 \pm 0.5
8	100	16.8 \pm 0.2	30.3 \pm 0.6	500	63.7 \pm 0.4	90.8 \pm 0.6
9	100	16.7 \pm 0.3	25.0 \pm 0.7	450	42.0 \pm 0.4	93.7 \pm 0.6
10	100	17.0 \pm 0.3	29.3 \pm 0.4	450	50.2 \pm 0.3	66.0 \pm 0.3

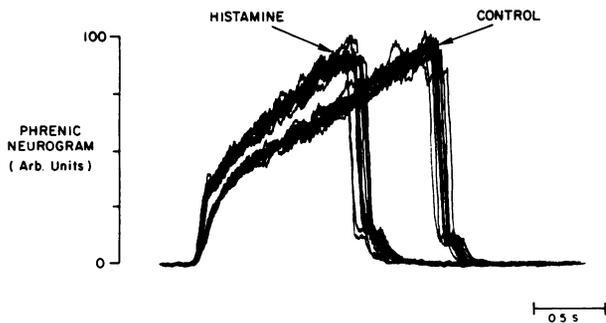


FIGURE 4 Augmenting effect of histamine on phrenic nerve activity in the absence of lung inflation ($\dot{V} = 0$). The record shows the neurograms of 10 single inspirations when $\dot{V} = 0$, in the control period and during histamine administration. These data are from the same animal as in Figs. 2 and 3. The arterial PCO_2 and respiratory resistance are given in the legend to Fig. 2.

the onset of phrenic nerve activity when the measurements were made.

Effect of increased airflow on phrenic nerve activity before and during histamine administration. As previously described (1), an increased rate of lung inflation caused an increase in the rate of rise of phrenic nerve activity as compared to that in the absence of lung inflation. In the present study this increase was observed in 9 of the 10 dogs before histamine, and in all 10 dogs during histamine administration. While lung inflation augmented phrenic nerve activity before and during histamine administration, histamine increased this augmenting effect of lung inflation on inspiratory efferent activity. This is illustrated in Fig. 2, in which the augmentation of phrenic nerve activity due to lung inflation during administration of histamine, measured by the difference between the slope of the neurogram during the increased rate of inflation (S_v) and that during no lung inflation (S_0) (see lower panel, Fig. 2), is greater than the augmentation caused

by the same rate of lung inflation before histamine administration (the difference, $S_v - S_0$, in the upper panel, Fig. 2). Over the 10 animals studied, the magnitude of the difference in the slopes of the neurograms of the breaths at high and zero airflow was significantly greater during histamine administration than in the control state ($P < 0.01$). Averaged over measurements in all the animals, the magnitude of this difference in slopes (arbitrary units/second) due to lung inflation was 68.0 ± 14.3 (mean \pm SE) during histamine administration compared with 17.7 ± 3.8 (mean \pm SE) before histamine.

Effects of histamine on respiratory timing. Aerosolized histamine also reduced both the duration of inspiration and the duration of expiration; the effect on expiratory duration, in absolute terms, was more marked. In the tests at constant airflow, there was a statistically significant reduction in these durations in each animal. In these tests inspiratory duration, averaged across the 10 measurements in the 10 animals studied, was reduced from 0.80 ± 0.02 s (mean \pm SE) to 0.46 ± 0.01 s by histamine, whereas expiratory duration similarly computed shortened from 1.59 ± 0.05 s to 0.86 ± 0.05 s [$n = 100(10 \times 10)$]. The inspiratory and expiratory durations were also reduced by histamine in the tests at zero airflow, these differences being statistically significant in each animal studied. During apnea, inspiratory duration, averaged across the 10 measurements in the 10 dogs, was reduced by histamine from 2.33 ± 0.05 s (mean \pm SE) to 1.67 ± 0.08 s, while expiratory duration shortened from 2.79 ± 0.12 s to 1.15 ± 0.06 s ($n = 100$).

Histamine dose, arterial blood gases, respiratory resistance and blood pressure. The doses of histamine that we used varied between dogs. This is shown in Table III (left-hand columns), in which the cumulative dose of histamine during the initial period of its administration, the final concentration used, and the rate of administration during the maintenance period are given for each animal. In each animal the augmentation of phrenic nerve activity described above was observed after the initial cumulative dose of histamine had been given. In all animals the maintenance period lasted for ~ 10 min during which measurements were made.

The augmentation of phrenic nerve activity, which we observed, was not explained by any alterations in arterial blood gas tensions. In all animals the arterial pCO_2 during the continuous administration of histamine was either identical to or slightly less than the arterial pCO_2 in the control state (see columns 4 and 5, Table III). Furthermore, as indicated in the Methods section, these experiments were all carried out under hyperoxic conditions. In these dogs the arterial pO_2 was 460 ± 14.7 mm Hg (mean \pm SE) before histamine and 457 ± 20.9 mm Hg during the maintenance period.

TABLE II

Effect of Histamine on Phrenic Nerve Activity during Apnea

Dog No.	Time of measurement (From onset of inspiration)	Height of phrenic neurogram	
		Control	Histamine
	ms	arbitrary units	
1	650	130.3 \pm 0.6	176.8 \pm 1.3
2	1,300	52.0 \pm 0.6	75.0 \pm 1.3
3	650	59.8 \pm 0.4	69.8 \pm 0.3
4	1,850	101.8 \pm 1.1	128.7 \pm 0.8
5	1,500	129.3 \pm 0.7	158.0 \pm 0.7
6	1,300	86.2 \pm 1.0	103.3 \pm 0.3
7	1,150	116.8 \pm 0.7	165.3 \pm 1.7
8	2,350	133.5 \pm 0.9	157.0 \pm 0.9
9	2,100	100.7 \pm 1.2	138.2 \pm 1.4
10	1,150	93.8 \pm 0.7	104.3 \pm 0.5

TABLE III
Histamine Dose, Arterial PCO₂, and Respiratory Resistance

Dog No.	Initial cumulative histamine dose	Final histamine concentration	Maintenance rate of delivery of histamine	Arterial PCO ₂		Total respiratory resistance	
				Control	Histamine	Control	Histamine
				mm Hg		cm H ₂ O/liter/s	
1	3.01	0.50	0.19	51	48	1.85±0.02	1.82±0.02
2	3.01	0.50	0.35	49	48	1.68±0.02	1.68±0.03
3	1.15	0.25	0.24	48	48	2.14±0.07	2.03±0.05
4	0.28	0.05	0.05	54	51	1.55±0.03	1.56±0.03
5	0.80	0.10	0.09	52	49	1.47±0.04	1.46±0.05
6	0.31	0.05	0.05	41	39	1.41±0.02	1.43±0.02
7	0.25	0.05	0.05	43	42	1.83±0.03	1.81±0.06
8	0.34	0.05	0.06	45	42	1.35±0.02	1.36±0.02
9	0.76	0.10	0.11	58	55	1.85±0.07	1.82±0.09
10	0.55	0.10	0.10	50	49	1.68±0.01	1.68±0.02

Similarly, the measured pH in arterial blood was stable. Averaged across all dogs, the pH was 7.23±0.01 (mean±SE) before histamine and 7.24±0.015 during the period of continuous administration of the drug.

Equally, the changes in phrenic nerve activity, which we observed, were not secondary to alterations in respiratory resistance. In these animals the effect of histamine on bronchial smooth muscle was prevented by administration of isoproterenol and atropine. Thus the measurements of respiratory resistance made over 50 consecutive breaths in the control period were not significantly different from measurements made over an equal number of breaths during the period of histamine administration (mean±SE are shown for each in columns 6 and 7, Table III).

Finally, at the doses of histamine that we used, there was no significant change in mean blood pressure. Averaged across all animals, mean blood pressure was 125±5.7 mm Hg (mean±SE) in the control period and 115±9.7 mm Hg during continuous administration of histamine.

Effects of histamine on phrenic nerve activity after vagotomy. After bilateral cervical vagotomy, all effects of histamine on both the instantaneous magnitude of phrenic nerve activity and respiratory timing were abolished (see Fig. 5). In no animal did we observe a statistically significant change in these variables following vagotomy using the same dose of histamine as that which produced changes before vagotomy.

DISCUSSION

The results from this study indicate that, although arterial blood gases and inspiratory flow-rate were controlled, aerosolized histamine caused an increase in the magnitude of phrenic nerve activity from early in inspiration. This increase was mediated by changes in vagal afferent activity since following bilateral vagotomy administration of the same dose of histamine did not produce excitation of inspiratory neural output.

The conclusion from our experiments differs from that of Winning and Widdicombe (18) and Davies et al. (19) who concluded that histamine had no excitatory effect on phrenic nerve activity. In their studies, however, arterial pCO₂ was not controlled. Since histamine can induce hyperventilation and a reduction in arterial pCO₂ (18), the excitatory effect demonstrated in the present study might not have been observed in the earlier studies because of the inhibition of phrenic nerve activity produced by relative hypocapnia. Miserocchi et al. (20) did control arterial blood gases but reached an opposite conclusion to that presented here, i.e., that histamine inhibited inspiratory neural output. Inspiratory neural output was, however, not measured directly but rather by changes in mechanical events, mean inspiratory flow-rate and mean rate of rise of tracheal pressure during an occluded inspiration. Since histamine is known to alter pulmonary mechanics (13), the results of Miserocchi et al.

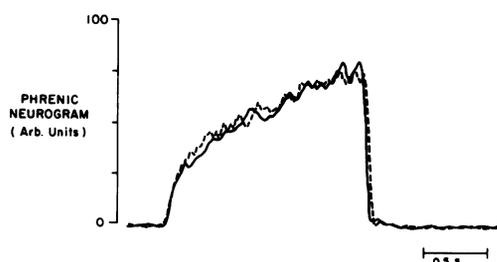


FIGURE 5 Effect of histamine on phrenic nerve activity after bilateral cervical vagotomy. The neurograms of single breaths recorded at a constant inspiratory flow-rate (0.33 liter/s), in the control period (---) and during histamine administration (—) are shown. As can be seen from the superimposition of the neurograms, histamine was without effect. Again, before and during histamine, the arterial PCO₂ (44 and 43 mm Hg, respectively) and respiratory resistance (1.38 and 1.36 cm H₂O/liter per s, respectively) were almost identical. These data are from the same animal as in Figs. 2-4.

(20) are difficult to interpret. Specifically, the reduction in mean inspiratory flow-rate produced by histamine does not imply that inspiratory neural output is decreased, since it could have been due to the increase in airway resistance. An increase in resistance cannot, however, explain the reduction in the rate of rise of the tracheal pressure that developed during the occluded inspiration. A possible explanation of their observations is that histamine caused changes in the configuration of the respiratory system which, by altering the length of the inspiratory muscles, would change the force developed by them. Indeed, in some animals, histamine increases functional residual capacity (13), which leads to a reduction in the pressure developed during airway occlusion (21). Thus, the measurement of these pressure and flow variables by Miserocchi et al. (20) does not provide convincing evidence that histamine reduces inspiratory neural output. Unfortunately in this regard, the measurements of phrenic nerve activity referred to in their study were not reported.

The augmentation of phrenic nerve activity by histamine reported here interacts with that produced by lung inflation (1–4). The same constant rate of lung inflation produced a more marked increase in the rate of rise of phrenic nerve activity following histamine administration, i.e., the gain of the recently described positive vagal feedback loop (1–4) was increased by histamine. (This is a positive feedback loop since lung inflation causes an increase in inspiratory neural output, which will cause further increases in the rate of lung inflation, etc.) However, the augmentation produced by histamine was not completely due to this effect since it was seen even in the absence of lung inflation. Furthermore, the augmentation produced by histamine was present early in the inspiratory period, whereas most (1–3), but not all (4) investigators find that the first 200–300 ms of phrenic nerve activity cannot be altered by changes in lung inflation.

With respect to the type of neural receptor mediating this effect of histamine, the available data do not allow us to define which pulmonary vagal receptor is responsible, since all pulmonary vagal receptors are stimulated by histamine, i.e. pulmonary stretch (6), rapidly-adapting (7), bronchial C (8) and juxta-capillary (J) (9). But of these receptors, the most likely to mediate the responses are the rapidly adapting receptors, which in dogs are markedly stimulated by histamine even when changes in tracheal pressure are virtually abolished by prior administration of isoproterenol (7). Histamine increases their firing rate during lung inflation, and with marked stimulation, may cause the receptors to fire tonically (22). Thus, this pattern of stimulation of the receptors by histamine correlates with the stimulation of phrenic nerve activity reported here. Receptors innervated by nonmyelinated fibers

(bronchial [8] and juxta-capillary [9]) are also stimulated by histamine, but this stimulation is less intense than for rapidly adapting receptors. Also, there is no evidence for a respiratory modulation of the firing rate of these receptors (8), which would be necessary if we were to explain the increased facilitatory effect of increased airflow after histamine by a change in the firing of these receptors. These receptors are unlikely, therefore, to mediate the response. The effects of lung inflation after histamine could conceivably be explained by stimulation of pulmonary stretch receptors by histamine (6). This stimulation is thought, however, to be secondary to contraction of bronchial smooth muscle and not a direct effect (6). Since we abolished changes in pulmonary resistance pharmacologically, it is unlikely that much contraction of smooth muscle occurred in the large airways where these receptors are concentrated (for review, see 23). Thus, in our experiments, there was probably little change in pulmonary stretch receptor activity. Nonetheless, we cannot exclude completely the possibility that these receptors might mediate part of the observed response.

In our studies, histamine produced changes in respiratory timing; respiratory frequency increased and both inspiratory and expiratory durations decreased, as has been previously reported (18, 19, 20). The magnitude of the decrease in expiratory duration was greater than that of inspiratory duration and these changes in respiratory timing were present even when the lungs were not inflated. (Both observations have been described previously [20].) Thus in this circumstance changes in vagal afferent activity can alter inspiratory duration even in the absence of lung inflation. This is not taken into account in current models of the neural mechanisms controlling inspiratory duration (24).

The increase in phrenic nerve activity caused by histamine is relevant to changes in control of ventilation in acute asthma, since histamine is released in the lung in allergic asthma (10). In animal models of this disease, the onset of asthma produces a change in respiratory pattern with the animals breathing faster with smaller tidal volumes. This acceleration of breathing may be related to the effects of histamine, which produces reductions in both inspiratory and expiratory durations. Furthermore, our observations may explain, at least in part, the increase in inspiratory neural output, as measured by occlusion pressure (P_{100}), during an acute asthmatic attack (25, 26). The increase in phrenic nerve activity with histamine, which we have reported, is presently sufficiently early in inspiration to lead to increases in the pressure developed 100 ms after the onset of inspiratory pressure change in the occluded airway. Since the effect we have demonstrated is mediated by vagal receptors, we hypothesize that a vagal reflex also underlies devel-

opment of the increased occlusion pressure in acute asthma. Support for this hypothesis is provided by the studies of Savoy et al. (27) who demonstrated that local anesthesia of the upper airways, which can block pulmonary vagal reflexes (28), markedly reduces the increase in occlusion pressure, but not in airway resistance produced by aerosolized methylcholine.

Our demonstration that changes in vagal afferent activity can alter the instantaneous magnitude of phrenic nerve activity may have more general pathophysiological implications. It is conceivable that in pulmonary diseases, particularly those involving the airways, there may be alterations in the magnitude of neural output to the inspiratory muscles that are secondary to pathological derangements in receptor function. This hypothesis remains, however, to be experimentally tested.

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REFERENCES

- Pack, A. I., R. G. DeLaney, and A. P. Fishman. 1981. Augmentation of phrenic neural activity by increased rates of lung inflation. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **50**: 149-161.
- Bartoli, A., B. A. Cross, A. Guz, A. Huszczuk, and R. Jefferies. 1975. The effect of varying tidal volume on the associated phrenic motoneuron out-put; studies of vagal and chemical feedback. *Respir. Physiol.* **25**: 135-155.
- Cross, B. A., P. W. Jones, and A. Guz, 1980. The role of vagal afferent information during inspiration in determining phrenic motoneuron output. *Respir. Physiol.* **39**: 149-167.
- DiMarco, A. F., C. von Euler, J. R. Romaniuk, and Y. Yamamoto. 1980. Low threshold facilitation of inspiration by lung volume increments. *Acta Physiol. Scand.* **109**: 343-344.
- Palecek, F. 1977. Control of breathing in diseases of the respiratory system. In *International Review of Physiology. Respiratory Physiology II*. J. G. Widdicombe, editor. University Park Press, Baltimore. **14**: 255-290.
- Widdicombe, J. G. 1961. The activity of pulmonary stretch receptors during bronchoconstriction, pulmonary oedema, atelectasis and breathing against a resistance. *J. Physiol. (Lond.)* **159**: 436-450.
- Vidruk, E. H., H. L. Hahn, J. A. Nadel, and S. R. Sampson. 1977. Mechanisms by which histamine stimulates rapidly adapting receptors in dog lungs. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **43**: 397-402.
- Coleridge, H. M., and J. C. G. Coleridge. 1977. Impulse activity in afferent vagal C-fibers with endings in the intrapulmonary airways of dogs. *Respir. Physiol.* **29**: 125-142.
- Paintal, A. S. 1974. Fluid pump of type J receptors of the cat. *J. Physiol. (Lond.)* **238**: 53P-54P.
- Chiesa, A., D. Dain, G. L. Meyers, G. F. Kessler, and W. M. Gold. 1975. Histamine release during antigen inhalation in experimental asthma in dogs. *Am. Rev. Respir. Dis.* **111**: 148-156.
- Cotton, D. J., E. R. Bleecker, S. P. Fischer, P. D. Graf, W. M. Gold, and J. A. Nadel. 1977. Rapid, shallow breathing after *Ascaris suum* antigen inhalation; role of vagus nerves. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **42**: 101-106.
- Michoud, M. C., P. D. Paré, R. Boucher, and J. C. Hogg. 1978. Airway responses to histamine and methacholine in *Ascaris suum*—allergic rhesus monkeys. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **45**: 846-851.
- Karczewski, W., and J. G. Widdicombe. 1960. The role of the vagus nerves in the respiratory and circulatory responses to intravenous histamine and phenyl diguanide in rabbits. *J. Physiol. (Lond.)* **201**: 271-291.
- Kryger, M. H., O. Yacoub, and N. R. Anthonisen. 1975. Effect of inspiratory flow resistance on occlusion pressure in hypoxia and hypercapnia. *Respir. Physiol.* **24**: 241-248.
- Winn, R., J. R. Hildebrandt, and J. Hildebrandt. 1979. Cardiorespiratory responses following isoproterenol injection in rabbits. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **47**: 352-359.
- Goldman, M., R. J. Knudson, J. Mead, N. Peterson, J. R. Schwaber, and M. E. Wohl. 1970. A simplified measurement of respiratory resistance by forced oscillation. *J. Appl. Physiol.* **28**: 113-116.
- Snapper, J. R., J. M. Drazen, S. H. Liring, W. Schneider, and R. H. Ingram, Jr. 1978. Distribution of pulmonary responsiveness to aerosol histamine in dogs. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **44**: 738-742.
- Winning, A. J., and J. G. Widdicombe. 1976. The effect of lung reflexes on the pattern of breathing in cats. *Respir. Physiol.* **27**: 253-266.
- Davies, A., M. Dixon, D. Callanan, A. Huszczuk, and J. G. Widdicombe. 1978. Lung reflexes in rabbits during pulmonary stretch receptor block by sulphur dioxide. *Respir. Physiol.* **34**: 83-101.
- Miserocchi, G., M. Mazzarelli, B. Quinn, and R. S. Mozes. 1979. Histamine and phenyldiguanide induced tachypnea in hypercapnia and hypoxia. *Respir. Physiol.* **36**: 217-229.
- Eldridge, F. L., and K. Z. Vaughn. 1977. Relationship of thoracic volume and airway occlusion pressure: muscular effects. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **43**: 312-321.
- Sampson, S. R., and E. H. Vidruk. 1975. Properties of 'irritant receptors' in canine lung. *Respir. Physiol.* **25**: 9-22.
- Pack, A. I. 1981. Sensory inputs to the medulla. *Annu. Rev. Physiol.* **43**: 73-90.
- von Euler, C. 1977. The functional organization of the respiratory phase-switching mechanisms. *Fed. Proc.* **36**: 2375-2380.
- Zackon, H., P. J. Despas, and N. R. Anthonisen. 1976. Occlusion pressure responses to asthma and chronic obstructive pulmonary disease. *Am. Rev. Respir. Dis.* **114**: 917-927.
- Kelsen, S. G., B. Fleegler, and M. D. Altose. 1979. The respiratory neuromuscular response to hypoxia, hypercapnia and obstruction to airflow in asthma. *Am. Rev. Respir. Dis.* **120**: 517-527.
- Savoy, J., J. A. Fleetham, M. E. Arnup, and N. R. Anthonisen. 1981. Airway anesthesia and respiratory response to methylcholine induced bronchoconstriction. *Respir. Physiol.* **43**: 59-68.
- Dain, D. S., H. A. Boushey, and W. M. Gold. 1975. Inhibition of respiratory reflexes by local anesthetic aerosols in dogs and rabbits. *J. Appl. Physiol.* **38**: 1045-1050.