Vagal Afferent Activities and Respiratory Reflexes during Drug-Induced Bronchoconstriction in the Guinea Pig

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(Received 16 April 1992/Accepted 16 June 1992)

ABSTRACT. Vagal afferent activities and respiratory reflexes during drug-induced bronchoconstriction were studied in 31 anesthetized, spontaneously breathing or artificially ventilated guinea pigs. Histamine (5, 10, 20 μg/kg), Ach (10, 20, 40 μg/kg) and endothelin-1 (2 μg/kg) were intravenously injected to the animals in order to induce the bronchoconstriction. In spontaneously breathing and vagi intact animals, a considerable respiratory change characterized by rapid-shallow breathing was elicited by histamine. Such respiratory change was abolished by bilateral vagotomy, indicating that the vagal pathway fairly participated in the respiratory change during bronchoconstriction. Indeed, recordings of electrical activities of single vagal afferent nerve fibers from pulmonary stretch and irritant receptors elucidated that the bronchoconstriction by the three drugs markedly influenced these receptor activities. The response of stretch receptors to bronchoconstriction was grouped into four types: two of those types showed a marked increase in their activities and the other two a decrease or no change. Such uneven response was assumed to be derived from heterogeneous contraction and aeration among the intrapulmonary small airway. On the other hand, irritant receptors were invariably stimulated by increased transmural pressure during bronchoconstriction. Administration of isoproterenol (20 μg/kg) which inhibited the smooth muscle contraction abolished stimulatory effect of the drugs to irritant receptors, suggesting that the effect was due to indirect action through the muscle contraction rather than their direct action to the nerve endings.—Key words: airway, bronchoconstriction, guinea pig, histamine, vagal receptor.

A variety of physiological and pathological stimuli to the airway can cause tracheobronchial constriction [11]. The excessive constriction elicits the abnormal respiratory pattern with an augmented respiratory effort, increased respiratory frequency, cough and wheezing which are concerned with the respiratory distress or dyspnea [23].

The autonomic nervous system supplying the airways has been assumed to contribute to the manifestation of these symptoms [2]. Physiological and morphological studies on the airway have evidenced the presence of three well defined vagal receptors, i.e., slowly adapting pulmonary stretch receptors, rapidly adapting irritant receptors and C-fiber endings (type J receptors). The stretch receptors are stimulated by increase in transmural pressure and reduction in lung compliance within the physiological range and provide inhibitory feedback to inspiratory process as the lung expands. The irritant receptors also respond to declining lung compliance as well as mechanical touch stimuli to the mucous membrane and various chemical substances such as histamine, prostaglandinF2α, ammonia vapor and cigarette smoke. C-fiber endings are located at bronchi and alveoli, and are stimulated by inflammatory or edematous change in addition to chemical substances such as capsaicin, phenyl diguanide and bradykinin [5, 12]. Since the abnormal activity of these receptors has produced significant changes in respiratory function [18, 23], it is reasonable to consider that the activity of these receptors is accelerated by the severe tracheobronchial constriction during respiratory abnormalities as in asthma.

On the other hand, there are several lines of evidence that many kinds of chemical mediators act to determine the airway caliber. Histamine, kinins, arachidonic acid metabolites, platelet-activating factor, substance P and a newly found bronchoactive substance, endothelin, are conceived to be released during asthmatic attack and cause bronchoconstriction [2, 19]. Nevertheless, the interaction between these two factors, i.e., nervous system and chemical mediators, has not been studied especially considering its time course. The time course of the contraction induced by histamine and ACh is similar although they differ physiologically: histamine is a representative of the mediators released from mast cells and ACh acts as a chemical mediator of autonomic nervous system. Compared with the time...
course of the contractile action of histamine and ACh, endothelin-1 is unique in regard to the intensity and duration of its effect on smooth muscle.

The main purpose of this study is to clarify how vagal afferent fibers from the airway are stimulated during bronchoconstriction induced by these chemical mediators, and how they are associated with the respiratory reflexes. We have discussed here the possible role of these receptor activities on abnormal respiration.

MATERIALS AND METHODS

Respiratory changes by histamine-induced bronchoconstriction in spontaneously breathing animals:
The experiments were performed on 5 male Hartley strain guinea pigs weighing 410-610 g, anesthetized by intraperitoneal injection of urethane (1.0–1.2 g/kg). The animals were placed in a supine position on a heated operating table. A midline incision was made in the neck to cannulate the right jugular vein for administration of histamine. Another catheter was placed into the esophagus at the cervical portion so as to monitor the intrathoracic pressure (Peso). The trachea was exposed and a cannula with two sidearms was caudally inserted from an incision made at the level of 8th tracheal cartilage: this cannula allowed the animal to breathe spontaneously and its arms were connected to a differential pressure transducer (TOYODA DD102S) to measure tracheal airflow (V).

Histamine (20 μg/kg) was administered into the jugular vein via the catheter mentioned above in a duration of 10 seconds. To block the vagus nerves, either surgical vagotomy or application of small cotton balls soaked with 4% xylocaine was performed to the cervical vagi which were desheathed the surrounding tissues carefully. The respiratory response before and after the blocking of vagus nerves was examined.

Changes in respiration rate and total respiratory resistance (Rres) were assessed. Control values were obtained from three succeeding breaths just prior to the histamine injection, and the values after the injection were measured where the respiration rate became the maximum. Rres was measured as the ratio of change in intratracheal pressure (cmH2O)/inspiratory air flow rate (ml/sec), both measured between points of zero to peak air flow in the inspiratory phase. These values are expressed as mean ±S.E. Differences were considered significant if P<0.05 based on Student's t-test.

Vagal afferent activities during drug-induced bronchoconstriction in artificially ventilated animals:
Afferent activity of vagus nerve was recorded from 26 guinea pigs. The animals were anesthetized and prepared for drug injections as described above. Arterial blood pressure was monitored by a pressure transducer via a catheter placed in the left carotid artery. A tracheal cannula was inserted into the lower trachea and connected to a positive-pressure ventilator (Summit Med. Model B-3) to ventilate artificially. The ventilation was set at 50 cycles/min in respiratory frequency and at 10 ml/kg in tidal volume. A pressure transducer was attached to the tracheal cannula in order to measure intratracheal pressure (Prr). A thoracotomy was performed and the chest was widely opened so that receptive field of the pulmonary receptors being recorded could be identified by gentle touch stimuli. The right vagus nerve was transected below the nodose ganglion and its peripheral nerve trunk was separated from the surrounding connective tissue in length of 2–3 cm in preparation for recording its electrical activity. The left vagus was left intact.

Afferent activity of the whole nerve or its thin filament was recorded with a pair of platinum electrodes. As for recording the single unit activity, the nerve trunk was dissected into several thin filaments until a single unit activity was clearly discriminated. These nerve preparations were performed within a pool of paraffin oil using fine forceps, with the aid of a binocular microscope. The experimental set up is diagrammatically depicted in Fig. 1. The signal was amplified by a low noise DC-amplifier (DIA Med., DPA201) and a biophysical amplifier (DIA Med., DPA-200), and displayed on an oscilloscope in parallel with a loud speaker. If necessary, the amplified signal of discharge was integrated by an integrator (time constant = 0.1 sec) simultaneously. All the signals were displayed on a thermal-array recorder (GRAPHTEC, WR7700) and recorded by a magnetic tape recorder (SONY, FE-30A).

Once an acceptable single unit was found the ventilator was turned off at collapsed lung level, and maintained pressure was delivered into the lung through the ventilation line to identify the receptor type. The location of the receptive field of the fiber and its excitation properties were determined by gently probing the external surface of the lungs with
In this study, the effects of three bronchoconstrictive agents, i.e., histamine dihydrochloride (Wako Pure Chemical Industries, Ltd.), acetylcholine chloride (ACh, Daiichi Seiyaku Co., Ltd.) and endothelin-1, (Sigma), on receptor activity were examined. Both histamine (5, 10, 20 μg/kg i.v.) and ACh (10, 20, 40 μg/kg) induced an marked increase in Pr and receptor activity was examined 3 min after isoproterenol administration and compared with the histamine challenge. All these drugs were dissolved in saline, in volume of 1 ml/kg.

RESULTS

A clear change in respiratory pattern after histamine challenge was observed, i.e., rapid-shallow breathing characterized by a high frequency of breathing accompanied by a gradual increment of intrathoracic negative pressure (Fig. 2A). In many cases such rapid-shallow breathing was interrupted by a transient occurrence of an augmented breath. The respiration rate increased more than 4 fold of control value, from 48.0±5.9 to 218.7±16.0/min (mean ± S.E., P<0.001, Fig. 3A). There was also a significant increase in Rres from 0.40±0.11 to 1.28±0.12 cmH2O/ml/sec (mean ± S.E., P<0.01, Fig. 3B).

Bilateral vagotomy invariably changed the spontaneous breathing to slower and deeper pattern (Fig. 2B). After the vagotomy, the control respiration rate decreased to 24.4±3.67/min and control Rres increased to 0.70±0.08 cmH2O/ml/sec, respectively (Fig. 3A, B). These were significantly dif-

Fig. 1. Diagrammatic representation of the experimental setup. Pr: intratracheal pressure, ENG: electroneurogram, BP: blood pressure.

Fig. 2. Effects of histamine (Hist) on respiration with vagi intact (A) or blocked (B). Peso: intrathoracic pressure, V: tracheal airflow. Note that the spontaneous breathing was changed to slower, deeper pattern after vagal blocking.
different (P<0.01) compared with those values before the vagotomy. On this condition, histamine administration no longer altered the respiratory pattern wherein the rapid-shallow breathing was absent (Fig. 2B) and the respiration rate was maintained at 26.4±3.9/min (Fig. 3) which was close to the level before administration. However, Rres significantly increased (1.22±0.13 cmH2O/ml/sec) compared with the control value (P<0.01).

In artificially ventilated animals, the peak response of Prr to histamine and ACh was observed at the 15th respiratory cycle (18.4 sec) on the average. Endothelin-1 (2 μg/kg) also increased Prr and the maximal response was obtained at 42.2 sec.

Spontaneous afferent activity of the whole nerve of the vagus exhibited an inspiratory augmented activity which was coincident with the respiratory cycle of artificial ventilation (Fig. 4). All of the drugs induced a pronounced increase in its activity especially in expiratory phase, resulting in a less evident inspiratory modulation at the initial stage just after the administration (Fig. 4). Such nerve response occurred almost in parallel with bronchoconstriction represented by a marked increase in Prr.

Single unit activities were recorded from a total of 16 pulmonary stretch and 15 irritant receptors. Figure 5 (upper panel) is an example of a pulmonary stretch receptor activity which was stimulated by a stepwise increase of lung inflation but virtually inhibited by collapsing pressure in the lung. This receptor activity was characterized by its slowly adapting discharge. On the other hand, the irritant receptor showed a rapidly adapting discharge to the inflation and/or deflation: in most cases more responsive to deflation than inflation (Fig. 5, lower panel). Such identification for the receptors was further confirmed by their response to mechanical touch stimuli onto the receptive field in the intrathoracic airway and lung: pulmonary stretch receptors commonly displayed a long-lasting regular discharge and irritant receptors only a short burst of irregular discharges in response to the mechanical probing.

Both stretch and irritant receptors were mainly localized at intrapulmonary small airways, while...
only twenty percent of irritant receptors and 14% of stretch receptors were localized in bronchus lobaris. The authors have not yet found the presence of irritant receptors in carina nor main bronchus, which were frequently observed in the cat, dog and rabbit [17, 18, 22].

All the stretch receptors regularly increased their discharge in the course of inspiration during normal respiration. A majority of the receptors had no discharge during expiration whereas some exhibited a small number of discharges also during expiration, and some had a cardiac modulation besides respiratory modulation. The representative response to histamine injection (20 µg/kg) is shown in Fig. 6.

**STRETCH RECEPTOR**

**IRRITANT RECEPTOR**

Fig. 5. Discharge patterns of the slowly adapting stretch (upper panel) and irritant (lower panel) receptors to maintained positive and negative pressure. Abbreviations as in Fig. 1.

In the upper panel, the slowly adapting stretch receptor shows the larger discharge, and the smaller discharge evoked by a strong deflation of the lung was of a rapidly adapting irritant receptor.

**STRETCH RECEPTOR**

**IRRITANT RECEPTOR**

Fig. 6. Representative records of single unit activities and PrT in response to histamine (Hist; 20 µg/kg). The upper and lower panels show the records from a slowly adapting stretch receptor and a rapidly adapting irritant receptor, respectively. Abbreviations as in Fig. 1.
(upper panel). In this case, the stretch receptor increased its discharge, especially during expiratory phase.

The response of stretch receptors to histamine, ACh and endothelin-1 were classified into four different patterns (Fig. 7). The first pattern showed a simply increased firing following administration of the drugs (type 1). The second pattern was biphasic, which transiently decreased and then increased the discharge (type 2). Simple decreasing discharge was the third pattern (type 3), and the fourth showed little or no change in firing frequency (type 4). Some fibers responded variously to different drugs. Of the six receptors tested for both ACh and histamine, three showed a similar response pattern between both drugs and the other three receptors different pattern. No specific distribution of the number of receptors was seen among the four types. The mean activity of stretch receptors was found to be almost parallel to the increase in Prt (Fig. 8).

A typical example of response of irritant receptor to histamine is shown in Fig. 6 (lower panel). Although the discharge was sparse before the administration, it became much more frequent by histamine injection where the increased activity was apparent in both inspiration and expiration. All the irritant receptors invariably increased their activity with the administration of each drug dissimilar to that in stretch receptors. The mean change of discharge frequency is summarized in Fig. 9. For

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**Fig. 7.** Different patterns of stretch receptor activity induced by bronchoconstrictive agents. Abscissa: respiratory cycle, ordinate: impulse number per respiratory cycle, as percent change from control value. ▲: type 1, simply increased. ●: type 2, biphasic. □: type 3, simply decreased. ■: type 4, little or no change. Hist: histamine, ACh: acetylcholine, ET-1: endothelin-1.

**Fig. 8.** Changes in discharge frequency of stretch receptors and Prt induced by three bronchoconstrictors. Upper panel: stretch receptor activity, as impulse number per respiratory cycle. Lower panel: Prt as percent change from control value. Results are expressed as mean ± SE (n=7). Abbreviations as in Fig. 7.
histamine and ACh, the peak activity of receptors was indicated at $11.0 \pm 0.28$ and $10.8 \pm 0.91$ (mean $\pm$ S.E.) of mean respiratory cycles, respectively. These values preceded to the respiratory cycle when the Prr reached to the maximum. Their response to endothelin-1 was rather variable in which the maximal activity were observed at 22nd cycle in three receptors, 33rd cycle in one, and the remaining one receptor had two peaks at 21st and 32nd cycle. In every drug, the activation of irritant receptors was limited in a shorter period compared with that of stretch receptors, though the change in Prr was almost the same in both.

Fig. 10. Effects of isoproterenol on response of irritant receptor to bronchomotor drugs. Upper panel: receptor activity, as impulse number per respiratory cycle. Lower panel: Prr. ○: response to histamine (20 $\mu$g/kg) in the absence of isoproterenol, △: histamine with pretreatment of isoproterenol, ●: ACh with pretreatment of isoproterenol, ■: endothelin-1 with pretreatment of isoproterenol.

A considerable change in respiratory pattern in spontaneously breathing guinea pigs was induced by histamine, one of the most potent bronchoconstrictors, where the respiration was characterized by rapid-shallow breathing interposed by an augmented breath. However, bilateral vagal blocking with local anesthesia or vagotomy thoroughly abolished this respiratory change, indicating that this abnormal respiratory pattern was mediated by
vagus nerves. Vagal blocking itself changed the respiration pattern to be slower and deeper, which has widely been known as related to inhibition of Hering-Breuer inflation reflex: the block of impulse conduction from pulmonary stretch receptors delays the central switching mechanism from inspiration to expiration [1]. Therefore, vagal afferent information arising from visceral organs, especially tracheobronchial tree, and/or vagal efferent activity might play an important role to induce the typical response to histamine. On the other hand, the respiratory resistance (Rres) was markedly increased by histamine administration irrespective of the vagus nerve. This result suggested that the bronchoconstriction was produced essentially by direct action of this drug to smooth muscle.

The electrophysiological study of the afferent vagus nerve suggests a significant alteration of nerve activity during the drug-induced bronchoconstriction. The authors could clearly identify the pulmonary stretch and irritant receptors in the guinea pig by their spontaneous discharge properties. The pulmonary stretch receptors had an inspiratory augmented activity, a slowly adapting discharge to maintained positive pressure and showed a decrease in any spontaneous activity with negative pressure of the lung. The irritant receptors had sparse activities during eupnea, and were stimulated by both positive and negative pressure in which they had a tendency to response more intensely to negative pressure.

In the present study, there found to be non-uniform response pattern in the activation of pulmonary stretch receptors by bronchoconstrictive agents. Some conflicting factors influence the discharge frequency of stretch receptors: the previous reports suggest that an increase in transmural pressure [16] and a decrease in compliance [16, 25] are adequate stimuli of stretch receptors rather than lung volume changes. On the contrary, if the bronchus proximal to the receptor site obstructs, the activity would be reduced in the case of air insufficiency [14] and otherwise it should be increased by a large amount of air trapping which reflects an increase in FRC. There is also a controversy on the effect of smooth muscle contraction on the activity of stretch receptors. Shortening of smooth muscle and thus relaxation of nerve endings would decrease the discharge frequency [22], but smooth muscle contraction does activate stretch receptors [15] because of the stiffness of receptor site [16] and the rise in inflation rate [6] which intensifies dynamic response of the receptor. The divergent response of stretch receptors to the drugs demonstrated in this study is in agreement with the previous report [9] and arises from the heterogenous contraction and aeration among the whole airway [16]. Considering the factors stated above, heterogeneity of the response shown by stretch receptors depends on their locations rather than their variation of physiological property. The slowly adapting stretch receptors are the afferents responsible for the Hering-Breuer inflation reflex; their mounting discharge in inspiration accelerates an inspiratory off-switch mechanisms which limits the depth and duration of inspiration [1]. Although the stretch receptors examined in this study showed heterogenous reaction to drug administration, their average activity tended to increase. During bronchoconstriction, therefore, stretch receptors may participate in shortening the inspiration time and consequently raise the respiration rate.

On the other hand, the duration of expiration is affected by a balance of input from stretch and irritant receptors to the central respiratory neurons in the medulla [7]. Unlike stretch receptors, irritant receptors were invariably stimulated by drug administration which was probably due to the localization of endings and their responsibility to mechanical deformation of the airway [17]. A larger percentage of these receptors (20%), compared with that of stretch receptors, were located in extrapulmonary airways, where intratracheal pressure adequately reflects the transmural pressure. In addition, irritant receptors have multibranched structures that can be distributed to both superficial and submucosal layer [17] and are stimulated by smooth muscle contraction [11, 21] and collapse of the lung [9]. Stimulation of irritant receptor by drugs could be partially ascribed to their direct action on the receptor besides a secondary effect of bronchoconstriction. The activation of irritant receptors by histamine was attributed to the contraction of the smooth muscle through an indirect action in guinea pigs [3] and in rabbits [11], but a direct action on the endings themselves was also indicated in dogs [21]. ACh seems capable of stimulating irritant receptors mostly through its action on airway smooth muscle [21]. Our data provided evidence that none of the drugs examined in this study, i.e., histamine, ACh and endothelin-1, acts directly on the irritant receptor in guinea pig: their action on the receptor were
completely abolished by administration of isoproterenol of which action prevents the smooth muscle contraction. Their stimulation reflexly initiates hyperventilation other than cough, mucous secretion and bronchoconstriction [7, 11]. There are also several lines of evidence to support the view that the augmented deep breath or 'gasp' reflex which appeared after the rapid-shallow breathing was a reflex effect of irritant receptors [7, 8, 14]. This reflex contributes to re-expand the lung and thereby reinstitute the normal pulmonary distensibility [8]. Once the lungs are hyperinflated the airways would open, and thereafter the receptors, especially pulmonary stretch receptors, would sense the overall pressure and change their discharge accordingly. Activation of pulmonary stretch receptors creates a negative feedback loop on the airway tone through the reflex bronchodilation which optimizes the constricted airway.

Since endothelin-1 produces the long lasting contraction of smooth muscle [20, 24], the response of pulmonary receptors to this drug was expected to be different from other drugs. Indeed, the duration of the activation of stretch receptors was much longer compared with the other drugs, which reacted in parallel to the intratracheal pressure. Furthermore, in the irritant receptors the time-dependent alteration of the discharge frequency varied from receptor to receptor. This might be due to the gradual contractile action on airway smooth muscle. In addition, bronchoconstriction by endothelin-1 might be complex since it is known to release the secondary mediators i.e. thromboxane A2, which can also affect the smooth muscle tone [10, 13].

With regard to bronchial and alveolar C-fibers (type J receptors), although we have not directly tested their activity during drug-induced bronchoconstriction, evidence has accumulated that these receptors are capable of inducing rapid-shallow breathing [12]. These endings are numerous in the vagus, but they are usually silent in the normal lung and become active by nociceptive stimuli such as inflammatory changes and large deflation of the lung [4]. Under severe bronchoconstrictive conditions, a great possibility exists that the C-fiber endings or type J receptors could recruit to fire in addition to the activation of irritant and stretch receptors [4]. Drug-induced contraction would then further accelerate the bronchoconstriction by means of a positive feedback mediated by irritant receptors and/or C-fibers. This would in turn stimulate the nerve endings again and develop the dyspnoea. According to the foregoing analysis on the reflex actions of these three kinds of receptors, the increase in these receptor activities would cause the increase in respiratory frequency.

Consequently, the present study confirmed that the vagal afferents arising from the bronchi are considerably stimulated by bronchoconstriction and strongly suggested that these receptor activation is one of the most potent sources in the induction of respiratory distress during bronchoconstrictive disorders.

REFERENCES