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PULMONARY RECEPTOR CHEMOSENSITIVITY AND THE VENTILATORY RESPONSE TO INHALED CO_2 IN THE TURTLE*

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Abstract. Ventilatory responses of unanaesthetized turtles to changes in the intrapulmonary CO_2 content of a vascularly isolated lung and an intact lung were measured during spontaneous breathing. The hyperpnea associated with inhalation of CO_2 by the vascularly isolated lung was 19% of that associated with inhalation of CO_2 by the intact lung. Transection of the vagus nerve supplying the isolated lung abolished this response. We conclude that both inhibition of pulmonary stretch receptor discharge with increasing levels of $F_{I_{CO_2}}$ and a functional increase in central inspiratory volume threshold during hypercapnia contribute to tidal volume increases following CO_2 inhalation in normal animals. The major component of the ventilatory response of intact turtles to increasing levels of $F_{I_{CO_2}}$, however, was an increase in respiratory frequency. When CO_2 was inspired only by the vascularly isolated lung the increase in respiratory frequency was only 21% of that recorded when the same levels of CO_2 were inspired by the intact lung. Thus the ventilatory response of turtles to increasing levels of $F_{I_{CO_2}}$ is primarily dependent upon concomitant hypercapnia.

Breathing pattern	Pulmonary stretch receptor
Carbon dioxide	Vagus nerve
Control of breathing	Ventilatory reactions to CO_2

In vertebrates, pulmonary receptors with discharge correlated to the rate and degree of lung ventilation are of two major types: intrapulmonary chemoreceptors and intrapulmonary stretch receptors. The intrapulmonary chemoreceptors which have been found in the lungs of birds (Fedde and Peterson, 1970; Osborne and Burger, 1974) and lizards (Fedde *et al.*, 1977) are primarily sensitive to changes in airway CO_2 concentration. Intrapulmonary stretch receptors have been described in the lungs of mammals (Adrian, 1933), turtles (Milsom and Jones, 1976), lizards (Fedde *et al.*,

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1977) and frogs (Taglietti and Cassela, 1966; McKean, 1969) and although the adequate stimulus for these receptors is mechanical deformation, they have been shown to possess varying degrees of CO_2 sensitivity (Mustafa and Purves, 1972; Milsom and Jones, 1976; Fedde *et al.*, 1977; Milsom and Jones, 1977; Jones and Milsom, 1979). The primary function suggested for these receptors in mammals (Widdicombe, 1964) and birds (Osborne and Mitchell, 1977) is to reflexly control the tidal volume to respiratory frequency ratio and minimize the work of breathing (Otis *et al.*, 1950) or force of contraction of the respiratory muscles (Mead, 1960). Recently, however, due to their CO_2 sensitivity, pulmonary receptors have been implicated in the regulation of Pa_{CO_2} during muscular exercise, intravenous CO_2 loading experiments and CO_2 inhalation experiments (Wasserman *et al.*, 1967, 1975; Osborne and Mitchell, 1977). To date, this proposed role for intrapulmonary receptors remains unresolved. The following study is a comparison of the ventilatory responses of turtles to changes in the intrapulmonary CO_2 content of vascularly isolated lungs with the responses to changes in the intrapulmonary CO_2 content of intact lungs and an assessment of the role of CO_2 related receptor information, carried from pulmonary receptors within the vagus nerve, in this animal's response to hypercapnia.

Methods

A series of chronic experiments were performed on unanaesthetized, lightly restrained turtles (*Chrysemys picta*, 500–1500 g) at room temperature (22–23 °C). Surgery was performed under either a combination of cold (1–4 hr at −20 °C) and local anaesthesia (2% Lidocaine hydrochloride), or general anaesthesia (1.5–2 ml · 100 g^{-1} 10% MS222 injected I.P.). A window was removed from the plastron above the area of the heart using a necropsy saw allowing both primary bronchi to be cannulated separately. An occlusion cuff was also placed around the left pulmonary artery and the left vagus was exposed and loosely snared for sectioning at a later time. All catheters were led out through the skin at the base of the neck and the window was replaced and sealed with cotton wool and dental acrylic cement. Pneumotachographs and sidearms for tracheal pressure measurement and gas sampling were attached to the bronchial cannulae and the distal ends of these cannulae were attached to T connections. One arm of each T connection was open to atmosphere and the remaining arm was attached to a respiratory gas supply. Using a system of gas flow meters, the composition of the respiratory gas flowing past the end of each bronchial cannula could be independently altered thus separately controlling the composition of the inspired gas going to each lung when the turtle breathed.

The pressure drop across a pneumotachograph during tracheal air flow was recorded with a Hewlett-Packard 268 BC differential pressure transducer and intratracheal pressure generated during ventilation was measured with a Statham

P23V pressure transducer. The air flow signal was fed through a Hewlett-Packard 350-3700A integrating preamplifier to give tidal volume and all measurements, pressure, flow and volume were continuously recorded on a Sanborn 7700 chart recorder. The ventilating gas mixture was altered from 0 to 10% CO_2 in air using premixed gases and the O_2 and CO_2 composition of the inspired and expired gases was determined either on samples taken through the sidearm of the tracheal cannula and measured on a Fisher-Hamilton gas partitioner or by continuous sampling with a Centronic 200 MGA clinical mass spectrometer (sample rate < 10 ml $\cdot$ min^{-1}).

Animals were allowed to recover fully from anaesthesia (usually 24 hr) before experimentation began. The turtles were shielded from all activities of the experimenters and while resting quietly, the occlusion cuff was inflated isolating the blood supply of the left lung. The breathing pattern was then allowed to stabilize before presentation of the test gas samples. Left pulmonary artery occlusion, in itself, did not usually alter the normal breathing pattern. The animals were presented with air to both lungs and 10% CO_2 in air to each lung while the other received room air. Each combination was presented for a one-hour period and the presentation sequence was varied. All variables were recorded continuously during this time but data were selected for analysis only after the responses to each gas mixture had stabilized. Following this series of experiments the left vagus was sectioned under local anaesthesia and on the following day the above protocol was repeated.

Results

When 10% CO_2 was presented to the vascularly isolated left lung, there was a 47% increase in minute ventilation ($\dot{V}E$), a 20% increase in tidal volume (VT) and a 23% increase in respiratory frequency (f) (fig. 1, table 1). Following unilateral (left) vagotomy there was an increase in resting minute ventilation, however, there was no longer any respiratory response following introduction of 10% CO_2 to the vascularly, neurally isolated left lung. When the same levels of CO_2 were introduced to the vascularly intact right lung there was a 251% increase in $\dot{V}E$, a 56% increase in VT and a 108% increase in f. Although unilateral left vagotomy altered the relative roles of VT and f, there was no reduction in the response in minute ventilation to increasing levels of CO_2 in the intact right lung.

Discussion

Our results indicate that turtles increase their minute ventilation slightly in response to increases in the intrapulmonary CO_2 concentration of a vascularly isolated lung. The lack of any response to increased levels of CO_2 in this lung following vagotomy indicates that the afferent limb of this response arises from receptors within the lungs or pulmonary circulation.

TABLE 1

Comparison of effects of CO_2 inhalation by vascularly isolated and intact lungs on ventilation

Inhaled gas	n	f	V_T	$\dot{V}_E$
Prevagotomy	15			
Air to both lungs		100	100	100
10% CO_2 to isolated lung, air to intact lung		123	120	147
10% CO_2 to intact lung, air to isolated lung		208	156	351
Postvagotomy	9			
Air to both lungs		98	195	195
10% CO_2 to isolated lung, air to intact lung		105	196	202
10% CO_2 to intact lung, air to isolated lung		141	285	383

All values are expressed as a percentage of those recorded for animals breathing air in both lungs, before unilateral left vagotomy.

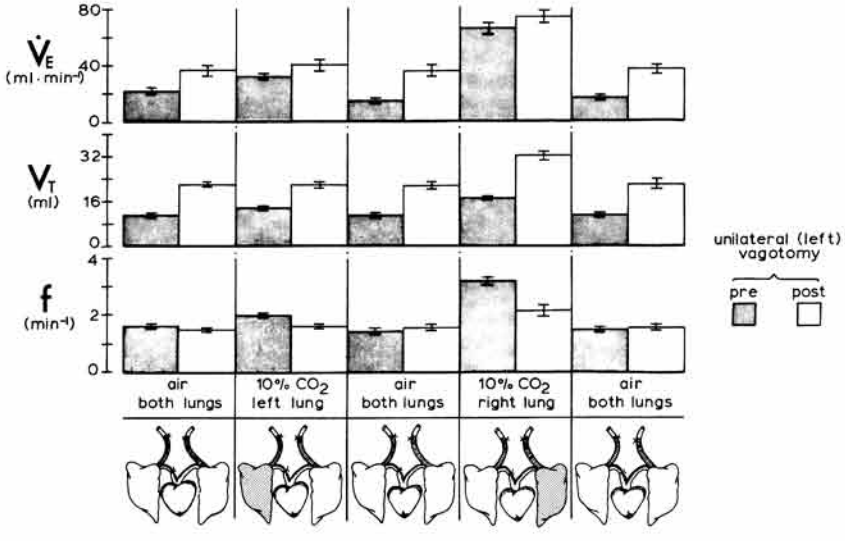

Fig. 1. Levels of minute ventilation ($\dot{V}_E$), tidal volume (V_T) and respiratory frequency (f) of turtles with blood flow to one lung occluded and both primary bronchi cannulated. Responses are shown of turtles breathing air in both lungs or 10% CO_2 in air in one lung and air in the other, before and after vagotomy. Values are the means ± SE.

Ventilatory responses to hypoxia have been shown to arise from chemoreceptors perfused by the pulmonary arteries and innervated by the vagi in the tortoise (Benchetrit *et al.*, 1977). There is as yet, however, no evidence to indicate whether these receptors are located in the lungs or pulmonary circulation. Intrapulmonary chemoreceptors have not been demonstrated in turtles but the discharge rates of pulmonary stretch receptors are inhibited by increasing levels of intrapulmonary CO_2 (Milsom and Jones, 1976; Jones and Milsom, 1979). At any level of lung inflation receptor discharge is reduced by approximately 55% when 10% CO_2 is added to air in the ventilating gas (Jones and Milsom, 1979). In spontaneously breathing, intact animals these receptors serve to regulate tidal volume within narrow limits and place the emphasis of respiratory responses to CO_2 inhalation on changes in respiratory frequency (Milsom and Jones, in preparation). This is clearly illustrated by the major role played by changes in tidal volume in the respiratory response caused by ventilation of the vascularly intact lung with 10% CO_2 following vagotomy. Consequently, the inhibition of the discharge of pulmonary stretch receptors during CO_2 inhalation will necessitate greater tidal volumes to provide the same volume information to the brain. The increase in tidal volume when CO_2 was inspired by the vascularly isolated lung is probably a reflection of this. The effect of CO_2 in increasing tidal volume in this situation will be partially offset by the increased discharge coming from receptors in the intact lung which is being ventilated with air at these increased tidal volumes and the slight decrease in Pa_{CO_2} which is bound to occur. However, when the same levels of CO_2 were inspired by the intact lung while the vascularly isolated lung inspired air, providing a similar offsetting pulmonary receptor input as in the reverse situation, the tidal volume response was more than twice as great. This increased response may be due to central or peripheral effects of the increase in blood P_{CO_2} and indicates that CO_2 may also act to functionally raise the inspiratory volume threshold in turtles as postulated in the cat (Bradley *et al.*, 1975).

It has been reported in mammals that pulmonary stretch receptor discharge during expiration prolongs the duration of expiration (Knox, 1973; Miserocchi and Milic-Emili, 1975; D'Angelo and Agostoni, 1975). It has therefore been suggested that the inhibition of end-expiratory discharge of pulmonary stretch receptors by CO_2 is responsible for the increased frequency of breathing obtained when CO_2 is inhaled by dogs on cardiopulmonary bypass (Bartoli *et al.*, 1974; Bradley *et al.*, 1976). This is achieved primarily by shortening the expiratory interval (Bartoli *et al.*, 1974). It is possible that the small increase in breathing frequency observed in the turtle when CO_2 was inhaled into a vascularly isolated lung also resulted from depression of end-expiratory discharge in pulmonary stretch receptors. In the turtle, however, the breathing frequency is increased through a shortening of the periods of intermittent breath holding rather than changes in the rate of active ventilation (Milsom and Jones, in preparation), thus if the changes in breathing frequency are the result of decreased pulmonary receptor discharge, the central integration of this information must be slightly different in the turtle and mammal.

The most important component of the ventilatory response of intact turtles to increasing levels of FI_{CO_2} is the increase in respiratory frequency. Since this component of the ventilatory response to CO_2 is greatly reduced when CO_2 is inspired only by the vascularly isolated lung, the total ventilatory response to CO_2 under these conditions is relatively small. Thus it would appear that the CO_2 sensitivity of pulmonary receptors does contribute to the increase in tidal volume seen during CO_2 inhalation but is of little significance to the overall response of the turtle to increased FI_{CO_2}. There is no evidence that turtles possess intrapulmonary chemoreceptors but if they are present in turtles as in lizards (Fedde *et al.*, 1977) they would appear to have only the same reflex functions as intrapulmonary mechanoreceptors.

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