INTRAPULMONARY CHEMORECEPTORS IN *GALLUS DOMESTICUS*: ADEQUATE STIMULUS AND FUNCTIONAL LOCALIZATION

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Abstract. Experiments were performed in anesthetized chickens in order to determine functional characteristics and localization of intrapulmonary chemoreceptors. Each lung of the bird was separately unidirectionally ventilated with controlled gas mixtures at controlled flow rates; pulmonary blood flow to one lung could be occluded. Action potentials in vagal afferents from the receptors as well as ventilatory responses to stimulation of the receptors were recorded. Main findings include: (a) Presence of two groups of afferent fibers carrying activity from intrapulmonary chemoreceptors, one in the vagus, the other in the cardiac sympathetic nerve, (b) Intrapulmonary chemoreceptors show high sensitivity to CO₂ concentration in lung gas and blood; they show little sensitivity to arterial pH, to most drugs which stimulate arterial chemoreceptors, to mild mechanical distortion of the lung, or to substances in bronchial arterial blood, (c) The receptors are located in the parabronchi. It is suggested that physiological processes monitored by intrapulmonary CO₂ receptors are those which modify parabronchial P_{CO₂}, i.e. ventilation, venous CO₂ load, and mixed venous P_{CO₂}.

Avian respiration
Intrapulmonary chemoreceptors
Pulmonary afferents

Respiratory control
Vagal afferents

Two definitive anatomical studies of the avian respiratory system have appeared recently (King, 1966; Duncker, 1972). Furthermore, understanding of the function of this system has been increased by recent studies of air-flow patterns (Bretz and Schmidt-Nielsen, 1971; Scheid and Piiper, 1971; Bouverot and Dejours, 1971; Brackenbury, 1971a) respiratory gas exchange (Scheid and Piiper, 1970), respiration during flight and altitude (Tucker, 1972) and respiratory mechanics (Brackenbury, 1971b).

1 This paper was presented at the workshop on "Receptors and Control of Respiration in Birds" held May 24-25, 1974, at the Max-Planck-Institut für experimentelle Medizin in Göttingen, Federal Republic of Germany.
2 Supported, in part, by research grants NB05125 and HSAA Award RR-06138.
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The control of avian respiration has also received increasing attention in recent years. The intact bird's respiratory responses to most stimuli are similar to those of mammals. Thus, in both classes of vertebrates, inflation of the ventilatory system with air produces apnea (Eaton et al., 1971), hyperventilation is seen at high body temperature (Frankel et al., 1962; Linsley and Burger, 1964), inhaled carbon dioxide increases minute ventilation (Jukes, 1971), hypoxia stimulates ventilation (Butler and Taylor, 1964) and vagotomy disturbs respiration (Fedde et al., 1963a).

High ventilatory reflex sensitivity in birds to airway \( F_{CO_2} \) (Burger, 1968; Peterson and Fedde, 1968) as well as the recent demonstration of similar reflexes in lizards (Gatz et al., 1974) and in mammals (Bartoli et al., 1973) suggests that chemosensitivity to \( CO_2 \) appeared early in the evolutionary development of tetrapods. Fedde and Peters (1970) demonstrated pulmonary receptors in chickens whose activity is inversely related to \( CO_2 \) over the physiological range. These receptors provide a sensing system for pulmonary chemosensitivity. The receptors will be referred to here as intrapulmonary chemoreceptors (IPC) because they meet the definition of chemoreceptors (Dawes and Comroe, 1954): they respond to the level of a chemical in their environment in the physiological range, and their response is correlated with a reflex (Osborne, 1971).

This paper will summarize studies concerning two major questions about IPC: (1) To what stimuli do they respond? Do they respond only to variations in \( P_{CO_2} \) or do they respond to \( H^+ \), hypoxia, mechanical distortion, or other stimuli? (2) Where in the lung are they located? These questions are important to the understanding of physiological processes monitored by IPC and the role of IPC in the control of avian respiration.

Brief reports of some of the work have already been published (Burger, 1968; Osborne and Burger, 1971; Stoll et al., 1971; Banzett and Burger, 1974).

Methods

A series of different experiments was conducted on White Leghorn males, 16–20 weeks of age. Sodium pentobarbital (35 mg \( \cdot \) kg\(^{-1} \)) was administered intravenously, and supplemented as necessary. The technique of unidirectional ventilation (Burger and Lorenz, 1960; Fedde, 1970) was used in a variety of ways to ventilate the birds. In experiments 1C and 1F below, the trachea was cannulated, the air sacs incised and the same gas concentration was passed through both lungs. In experiments 1A, 1B and 1E two independent gas streams were employed to unidirectionally ventilate the two lungs independently. This was accomplished by inserting a catheter (PE 190 tubing) into the right extrapulmonary bronchus and sealing it gas-tight with skin adhesive. Gas to the right lung was delivered through that cannula while gas to the left lung was delivered through the trachea. This latter preparation will be subsequently referred to as "bronchial cannulation".

The procedure was further modified in experiments 1D, 2A and 2B by mesobronchial cannulation. In those experiments the primary bronchi were cannulated.
on both sides with modified Foley catheters (Bardex, Fr. #8) inserted to the level at which the inflated cuff occluded the cranio-medial series of secondary bronchi. The caudal group of air sacs (caudal thoracic and abdominal) was left intact, and all cranial sacs (interclavicular, cranial thoracic and cervical) were opened, insuring ventilation of the paleopulmo in the normal caudal to cranial direction. To measure P_Co_2 from the lung, all indirect and direct ostia in the cranial sacs were occluded except the direct ostium of the cranial thoracic sac. This procedure insured that an average sampling of the gas from all ventilated bronchial circuits was obtained at a flow sufficient to sample.

In the experiments shown in fig. 1A–E the circulation of the left lung was blocked by ligation of the left pulmonary artery and vein.

P0_Co_2 (P_Co_2 of gas exiting the ostium of the cranial thoracic air sac) was measured with an infrared analyzer (LB-1, Beckman Instruments, Inc.) having a pressure in the sample cell of one-half atmosphere. The sampling catheter was 15 cm long, 0.28 mm I.D. and restricted at the tip to give a flow of 30 ml·min⁻¹. With this type of catheter, the analyzing system had a 110-msec delay and required 350 msec to reach 90% of the final response following a step change in P_Co_2 in either direction. Step changes in P_Co_2 (P_Co_2 of ventilating gas) were monitored by a capillary flow meter in series with the CO₂ feedline.

In those experiments in which the discharge characteristics of IPC were studied, extracellular microelectrode recordings were made from cell bodies in the nodose ganglion (Estavillo and Burger, 1973; Burger et al., 1973) after denervation of all vagal branches except the pulmonary rami. The pulmonary rami are known to carry IPC fibers (Fedde et al., 1963b; Fedde and Peterson, 1970). IPC were identified by an increased discharge frequency within one second following a step decrease in P_Co_2 in the ventilating gas. No neural recordings were made in excess of 5 hr after induction of anesthesia.

Results

(1) ADEQUATE STIMULUS OF IPC

(A) Reflex sensitivity to airway F_Co_2
The purpose of this experiment was to compare the influence of IPC and extrapulmonary chemoreceptors on the respiratory reflex response to alterations in intrapulmonary CO₂ concentration. This was accomplished by varying airway F_Co_2 in one lung during vascular occlusion of that lung, while maintaining constant systemic P_A_Co_2 with the other lung.

Before occlusion of the circulation to the left lung, the systemic circulation received blood from the right lung (ventilated with constant F_A_Co_2) as well as blood from the left lung which was exposed to F_A_Co_2 varying from 0.0 to 0.2. Extrapulmonary CO₂-sensing receptors were thus exposed to increasing P_A_Co_2 as F_A_Co_2 to the left lung was increased. Thus, extrapulmonary chemoreceptors should have enhanced the ventilatory stimulation produced by IPC during changes in F_A_Co_2 to
Fig. 1. Relationships between amplitude of vertical sternal deflection and $F_{CO_2}$ in the unidirectional ventilating gas to the left lung (eight animals per group). Each lung was ventilated separately and in B, C, D and E, the pulmonary circulation to the left lung was occluded. In C, the left cardiac sympathetic nerve was sectioned. In D, the left vagus was sectioned. In E, both nerves were interrupted. The $F_{CO_2}$ to the right lung was adjusted to give about 2 mm sternal deflection when $F_{CO_2}$ in the left lung was zero. The average values of $F_{CO_2}$ to the right lung were: in A, 0.12; in B, 0.076; in C, 0.10; in D, 0.037 and in E, 0.047. Note that the response to $F_{CO_2}$ was larger with the pulmonary circulation occluded (vagus intact, B and C) than with the pulmonary circulation intact (A). The major contribution to pulmonary sensitivity to airway $F_{CO_2}$ of the afferents in the cardiac sympathetic nerve is in the range from 0 to 0.05 $F_{CO_2}$ as seen in D directly and indirectly by subtraction of C from B.

The amplitude of vertical sternal movements increased with increasing $F_{CO_2}$, delivered to the left lung of unidirectionally ventilated preparations with circulation intact (fig. 1A). In order to separate the reflex response due to IPC from that caused by extrapulmonary chemoreceptors, the pulmonary circulation to the left lung was occluded and $F_{CO_2}$ was changed to that lung (fig. 1B). The ventilatory response was significantly greater after occlusion of the pulmonary circulation than when blood was flowing through the lung. Since changes in $F_{CO_2}$ during circulatory occlusion could not influence systemic $P_{aCO_2}$, the ventilatory response could have only arisen from changes in receptor activity in the left lung.

The increased ventilatory response to changes in $F_{CO_2}$ during vascular occlusion could be explained by the elimination of the buffering effect that blood flowing
through the lung has on parabronchial $F_{CO_2}$. IPC in the left lung with intact circulation would have been exposed to reduced $F_{CO_2}$ variations, as compared to blocked circulation when the $F_{CO_2}$ at the receptor site would have been very nearly the same as $F_{CO_2}$.

These data indicate that changes in IPC activity were more important than changes in arterial chemoreceptor activity in the reflex response to $F_{CO_2}$.

The afferent pathways from the IPC were also studied in these birds. When the left "cardiac sympathetic nerve" (supplying the left lung and heart with afferents as well as autonomic efferents) was severed in birds in which the pulmonary artery was blocked (fig. 1C) a significant reduction in sternal movements was seen at airway $F_{CO_2}$ of 0.03 ($P < 0.01$) compared to group B with intact innervation. At 0.1 and 0.2 $F_{CO_2}$ sternal movements were not affected by the sympathetic nerve section. When the left mid-cervical vagus was sectioned in birds with blocked pulmonary circulation, leaving only the pulmonary afferents in the cardiac sympathetic nerve intact, considerable reflex sensitivity was observed from 0.0 to 0.05 $F_{CO_2}$; from 0.05 to 0.2 $F_{CO_2}$ only a small additional increase in sternal movements occurred (fig. 1D). Thus, two groups of IPC are clearly present: one group contains afferent neurons carried in the vagus and is effective over a range of $F_{CO_2}$ from 0.0 to 0.2; the other group contains afferent neurons carried in sympathetic nerves and is most effective from 0.0 to 0.05 $F_{CO_2}$.

When both the left cardiac sympathetic nerve and left vagus were interrupted in animals with blocked pulmonary circulation, no significant reflex sensitivity to changes in left $F_{CO_2}$ from 0.0 to 0.2 were seen (fig. 1E).

(B) Reflex sensitivity to $F_{O_2}$ and its interaction with $F_{CO_2}$

In birds with blocked pulmonary circulation to the left lung and with $F_{CO_2}$ of 0.0 to that lung, the amplitude of vertical sternal movements increased by 140% ($P < 0.01$) when $F_{O_2}$ was reduced from 0.4 to 0.0. However, when $F_{O_2}$ was changed back to 0.4 the amplitude of vertical sternal movements decreased by only 10%. Reflex response to $F_{O_2}$ was insignificant when $F_{CO_2}$ was 0.05 and 0.1, in contrast to the increased responsiveness of mammalian carotid chemoreceptor cells to hypoxic hypoxia at higher $P_{aCO_2}$. Reflex responsiveness to hypoxic hypoxia at 0.0 $F_{CO_2}$ became insignificant above 0.08 $F_{O_2}$. Nerve section experiments indicated that afferents in the sympathetic cardiac nerve were responsible for much of the sensitivity to hypoxic hypoxia.

(C) Reflex sensitivity to injection of various substances into the pulmonary artery (ligated central to injection site)

Injections of lactic acid (0.1–0.5 ml of 0.1 N), sodium cyanide (10 to 50 $\mu$g) and procaine (30 mg) carried in 2 ml blood were not followed by alterations in sternal movement. Infusion of 20 ml of hypocapnic arterialized blood into the cannulated left pulmonary artery at 0.08 $F_{CO_2}$ caused a marked short-term reduction of ventilatory movement.
Effect of bronchial circulation on IPC discharge frequency
Variations in $P_{aCO_2}$ or intravenous injections of acetazolamide (a drug which increases IPC activity) resulted in no change in IPC discharge frequency over several minutes if pulmonary circulation was blocked, indicating that the bronchial circulation does not affect IPC.

Reflex sensitivity to variations of intrapulmonary pressure
We found no significant alterations in the amplitude of vertical sternal deflections when intrapulmonary pressure was altered by changing gas flow over a range of 0.25–2.0 l·min$^{-1}$ in the left lung with the pulmonary circulation blocked. Fedde and Peterson (1970) found similar results in preparations with intact pulmonary circulation.

Correlation of IPC discharge frequency to changes in $F_{CO_2}$ and pH
In six 16-week-old cocks, discharge frequency of six IPC were correlated to changes in pH via induced by airway $F_{CO_2}$ (fig. 2). After isolation of a receptor, $F_{CO_2}$ was adjusted so that the IPC was nearly inhibited, and pH was measured. The $F_{CO_2}$ level was then lowered in steps, and pH and discharge rates measured 2–4 min after each step. The adjustment of $F_{CO_2}$ and subsequent measurements of pH and IPC activity were serially repeated until either 0.008 or 0.0 $F_{CO_2}$ was reached. Then, with $F_{CO_2}$ held constant at either 0.008 or 0.0, glass distilled 1.0 $N$ HCl was then slowly infused via a bronchial vein cannula. pH and receptor activity measurements were repeated every 4 min until pH approximated the value found at the initial inhibitory level of $F_{CO_2}$. After cessation of the HCl infusion, pH was allowed to increase spontaneously in three animals and pH and discharge frequency measured subsequently every 4 min.

IPC discharge frequency and pH were highly correlated when pH was altered by $F_{CO_2}$ but were poorly correlated when pH was altered by HCl infusion or when pH changed spontaneously following HCl infusion. IPC discharge frequency was 8.5 times more sensitive to respiratory than to metabolic changes in these six experiments.

In summary, two groups of pulmonary afferents in the chicken contribute to the ventilatory sensitivity to airway $F_{CO_2}$. One group is carried in the vagus, the other in the cardiac sympathetic nerve. The adequate stimulus for vagal IPC is $P_{CO_2}$. Bronchial arterial blood seems not to affect IPC. Sensitivity of IPC to agents stimulatory to carotid body chemoreceptors, such as lobeline, cyanide, veratridine, $H^+$ ion or hypoxic hypoxia in hypercapnia, is low.

FUNCTIONAL LOCALIZATION OF IPC
Osborne (1971) clamped the pulmonary artery in order to vary airway $F_{CO_2}$ at the receptor site without altering $F_{CO_2}$. Seven IPC responded with small increases in discharge frequency. Subsequent to that study, 13 additional IPC have been tested in mesobronchial cannulated preparations: all IPC have responded by accelerating
Fig. 2. Relation of IPC discharge frequency to arterial pH as influenced by $F_{\text{CO}_2}$ (solid line) and as influenced by infusion of HCl (dashed line). $F_{\text{CO}_2}$ was increased until discharge frequency was low, after which $p$Ha and discharge frequency were measured. $F_{\text{CO}_2}$ was then lowered by a small step, and then measurements were repeated until zero (A) or 0.008 $F_{\text{CO}_2}$ (B through F) after which $F_{\text{CO}_2}$ remained constant at that value. HCl (1 N) was infused, and $p$Ha and discharge frequencies were measured every 4 min until $p$Ha approximated the initial value at high $F_{\text{CO}_2}$. A-C dash and dot line: discharge frequency and $p$Ha were measured every 4 min after cessation of HCl infusion. Note that the two methods of changing $p$Ha are not equally effective in altering discharge frequency.

discharge when the pulmonary artery was clamped at low $F_{\text{CO}_2}$ suggesting that most if not all IPC are affected by CO$_2$ in the pulmonary artery. Two lines of evidence are offered here in support of parabronchial location of these receptors.
(A) Contribution of $P_{co_2}$ from the pulmonary artery to the $P_{co_2}$ at the IPC receptor site

$P_{co_2}$ at a given location in the lung is mainly determined by the $F_{co_2}$ and by the amount of $CO_2$ derived from exchange with the pulmonary arterial blood. Therefore, the concentration of $CO_2$ at the receptor site during pulmonary arterial occlusion should closely approximate $F_{co_2}$. If the discharge frequency of the IPC is determined at various $F_{co_2}$ in the absence of blood flow, the IPC can then be used as a calibrated sensing system to provide an estimate of the $P_{co_2}$ at the receptor site during blood flow. The receptor site $P_{co_2}$ can then be compared to the input and output $P_{co_2}$ of the pulmonary gas during blood flow and thus, the location of the IPC along the parabronchus can be predicted.

In those preparations in which the cranio-medial series of secondary bronchi were occluded, a large bore silastic cannula, 2 mm I.D., was used to connect the innominate artery to the left pulmonary artery. The left lung was thereby supplied with systemic arterial blood at the rate of about one-third of the cardiac output. Perfusion of the lung with arterialized blood obviated the release of $CO_2$ via the Haldane effect. Ventilation was 0.7 l·min⁻¹; hence, the lung was overventilated.

**Fig. 3.** IPC discharge frequencies ordinate at $P_{co_2}$ from 7 to 35 torr (abscissa). Broken line, with pulmonary blood flow; solid line, without blood flow. The solid line provides an estimate of the effect of receptor site $P_{co_2}$ on IPC discharge rate. During pulmonary blood flow, receptor site $P_{co_2}$ is estimated to be 11, 16.5, 22 and 28 mm Hg at $P_{co_2}$, of 7, 14, 21 and 28 mm Hg, respectively, from the solid curve (see text for details).
and underperfused. The $P_{\text{CO}_2}$ of arterial blood was adjusted to 28 torr and IPC discharge frequency measured at various $P_{\text{CO}_2}$ with and without pulmonary blood flow (fig. 3). Higher discharge frequencies (i.e., lower receptor site $P_{\text{CO}_2}$) were seen when the pulmonary artery was clamped than when unclamped at $P_{\text{CO}_2}$ levels below 28 torr. When $P_{\text{CO}_2}$ was 7 torr and the output $P_{\text{CO}_2}$ in the pulmonary gas was 17 torr, the $P_{\text{CO}_2}$ at various receptor sites was estimated to be from less than 8 torr to 11 torr. Thus, some receptor sites appear to be close to the origin of the parabronchus while others may be located more toward the middle of the parabronchus.

**Discussion**

The partial pressure of carbon dioxide at the receptor is the primary determinant
of IPC activity. The physiological processes which can be monitored by IPC depend upon what parameter or combination of parameters determine receptor site $P_{CO_2}$.

Inspired $P_{CO_2}$ has a profound influence on IPC activity; however, it is unlikely that this is normally important, as significant variations in ambient $P_{CO_2}$ are seldom encountered.

Our results, and those of Osborne and Burger (1971), suggest that IPC are distributed in the parabronchi. Therefore, due to the axial gradient of $CO_2$ developed by cross-current exchange, individual IPC may sense $P_{CO_2}$ which is near to that of incoming air or that of mixed venous blood. Venous $CO_2$ load, the product of cardiac output times pulmonary arterial $P_{CO_2}$, affected IPC, as shown by increasing discharge frequency when the pulmonary circulation was blocked and by sensitivity to pulmonary arterial $P_{CO_2}$. Thus, venous $CO_2$ load was an important determinant of IPC activity in our experimental preparations. In the intact animal, IPC probably monitor metabolic $CO_2$ production and initiate homeostatic reflexes. Ventilatory flow dilutes $CO_2$ delivery by the pulmonary artery and thus is indirectly monitored by IPC. The dynamic response of an individual IPC to a change in ventilation (or $P_{AVCO_2}$) is further modified by the complex physical and chemical properties of the parabronchus. The dynamic response characteristics may nevertheless permit IPC to monitor individual breath size and ventilatory flow rate.

New models of the receptor–lung complex as well as new levels of experimentation will be needed for resolution of the role of IPC control of respiration.

References


