On chemoreceptor control of ventilatory responses to CO_2 in unanesthetized ducks

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MILSOM, WILLIAM K., DAVID R. JONES, AND GEOFFREY R. J. GABBOTT. *On chemoreceptor control of ventilatory responses to CO_2 in unanesthetized ducks.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 50(6): 1121–1128, 1981.—Using cross perfusion between pairs of animals we examined the effects of increases in arterial CO_2 tension (Pa_{CO_2}) at pulmonary, carotid body, and central chemoreceptors on ventilation in unanesthetized, spontaneously breathing White Pekin ducks. By adjusting the level of inspired CO_2 of either the experimental or donor animals it was possible to manipulate Pa_{CO_2} at any one or combination of the receptor groups. Stimulation of central chemosensitive areas alone was three to four times more effective in increasing both frequency and minute ventilation ($\dot{V}E$) than stimulation of the carotid bodies alone. Increases in tidal volume were small in both instances. Increasing P_{CO_2} in the pulmonary circulation of an innervated lung, independent of changes in Pa_{CO_2} at the carotid body or head, had no effect on $\dot{V}E$. We conclude that central chemoreceptors play the major role in the steady-state response of awake ducks to CO_2 while the carotid bodies play a smaller but still significant role. The intrapulmonary chemoreceptors play no role in the response per se although their inhibition by high airway CO_2 causes marked effects on the pattern of breathing.

hypercapnia; central chemoreceptors; intrapulmonary chemoreceptors; carotid bodies; hyperpnea; birds

CO_2 PRODUCES A STRONG RESPIRATORY DRIVE in both unanesthetized and anesthetized birds (3). At least three receptor groups may potentially be involved in this response. Systemic arterial chemoreceptors (carotid bodies) in ducks (4, 17) and chickens (5) play an important role in the ventilatory response to transient changes in arterial CO_2 tension (Pa_{CO_2}) but play no apparent role in the steady-state response to step changes in Pa_{CO_2}. Intrapulmonary chemoreceptors increase activity when partial pressure of CO_2 (P_{CO_2}) decreases in the lungs and consequently their discharge will be correlated to the rate and extent of CO_2 accumulation and washout during breathing (10, 13, 21). It is possible that they may also contribute to the steady-state response to hypercapnia. A third group of receptors situated upstream of the carotid bodies, most likely in the brain (central chemoreceptors), have been implicated in the steady-state response to step changes in Pa_{CO_2} (16, 17, 32).

The present study was undertaken to characterize the respiratory response of the unanesthetized duck to hypercapnia. In one series of experiments we investigated the relative roles of peripheral and central chemoreceptors in the steady-state response to hypercapnia. In a second series we analyzed the effects of increasing mixed venous CO_2 tension ($P\bar{v}_{CO_2}$) versus mixed inspired CO_2 fraction ($F_{I_{CO_2}}$) on ventilation and in a third series of experiments we assessed the roles and relative contributions of the three potential receptor groups, alone and in combination, to the steady-state response to changes in Pa_{CO_2}.

METHODS

Experiments were performed on White Pekin ducks weighing between 2.5 and 4.5 kg. All general surgery was performed under general anesthesia (pentobarbital sodium, 30 mg·kg^{-1}) 1–2 days before experiments were run. Cross perfusion requires extensive use of heparin to prevent blood clotting in the perfusion cannulas making it important that all wounds have had time to heal. In all experiments the animals were unanesthetized but lightly restrained ventral side down on operating tables. This form of restraint had no noticeable effect on the breathing pattern of the ducks. The body temperatures of all birds were constantly monitored and maintained at 41 ± 1.0°C with heating lamps mounted above the birds.

Series I

Surgical preparation. During initial surgery, one ischiatic artery was exposed in a donor animal and a sealed cannula was inserted into the interclavicular air sac. In a second animal, here designated the recipient, the common carotid arteries, jugular veins, and vagi were exposed bilaterally, high in the neck, and one vagus was sectioned denervating the lung and carotid body on that side. The skin and superficial muscle of the neck in this region were clamped, cut completely around, and sewn back together disrupting all blood flow up and down the neck in these tissues. A cord was passed beneath the exposed carotids, jugulars, vagi, and trachea, encircling the esophagus, vertebral column, and associated muscles and the ends were led to the surface at the back of the neck. Tightening this cord on the day of the experiment occluded any blood flow to the head through these tissues. The vertebral and cervical arteries were exposed low in the neck by opening the interclavicular air sac and were ligated bilaterally and the common carotid arteries on both sides were exposed. The walls of the air sac were sewn back together and the skin closed over the wound.

On the day of the experiment the donor duck was

intubated, the interclavicular cannula opened, and the animal placed on unidirectional ventilation. The ischiatic artery was carefully reexposed, under local anesthesia, and cannulated in both upstream and downstream directions. In the recipient duck, also under local anesthesia, the neck was carefully reopened and the right carotid artery cannulated as shown in Fig. 1. The cannula labeled as arising from the donor was connected to the upstream ischiatic cannula of the donor (flow from ischiatic artery of donor to head of recipient); that labeled as going to the donor was connected to the downstream ischiatic cannula (flow from carotid artery of recipient to hindlimb of donor) (Fig. 1). A pneumatic occluder was placed on the left carotid artery (occluder C, Fig. 1) in this preparation.

The flow through the two cannulas marked E and F was driven by a Harvard model 1210 peristaltic pump. With this preparation, it was possible to occlude blood flow at any of the sites labeled A, C, E, or F in Fig. 1 and it was also possible to occlude all flow between donor and recipient animals (E and F) by halting the pump. The cord previously placed around the vertebral column and associated musculature was tightened, after first infiltrating the area with local anesthetic, leaving the carotid arteries and jugular veins as the only vessels servicing the head. Both lungs were cannulated separately, the right lung just below the bifurcation of the primary bronchi and the left via the trachea just anterior to the bifurcation. The distal ends of these cannulas were attached to T connections. One arm of each T was open to atmosphere and the remaining arm was attached to a gas supply. Using a system of gas flowmeters the composition of the gas flowing past the end of each cannula could be independently altered thus separately controlling the composition of the inspiratory gas going to each lung when the duck breathed. The difference in dead space of the two lungs cannulated in this way was ≃2 ml or 3% of V_T and the added resistance to airflow imposed by the pneumotachograph on the one lung was less than 0.5 mm $H_2O \cdot l^{-1} \cdot min^{-1}$. These may have introduced some small error into our measurements of V_T. Also because the interclavicular air sac is shared by both lung circuits it was possible for gases to exchange between the two lung circuits. However, there was no evidence from end-tidal CO_2 fraction ($F_{ET_{CO_2}}$) measurements to suggest that this occurred.

Series I

Condition	Pumps	Occluders A	C	E	F
1. autoperfused	off	o	o	c	c
2. cross-perfusion to head only	on	c	c	o	o

Series III

Condition	Pumps	Occluders A	B	C	D	E	F	G
1. autoperfused	off	o	o	o	o	c	c	c
2. cross-perfusion to head only	on	c	o	c	o	o	o	c
3. cross-perfusion to head and innervated carotid body	on	c	c	o	c	o	o	o
4. cross-perfusion to innervated carotid body only	on	o	c	o	c	c	o	o

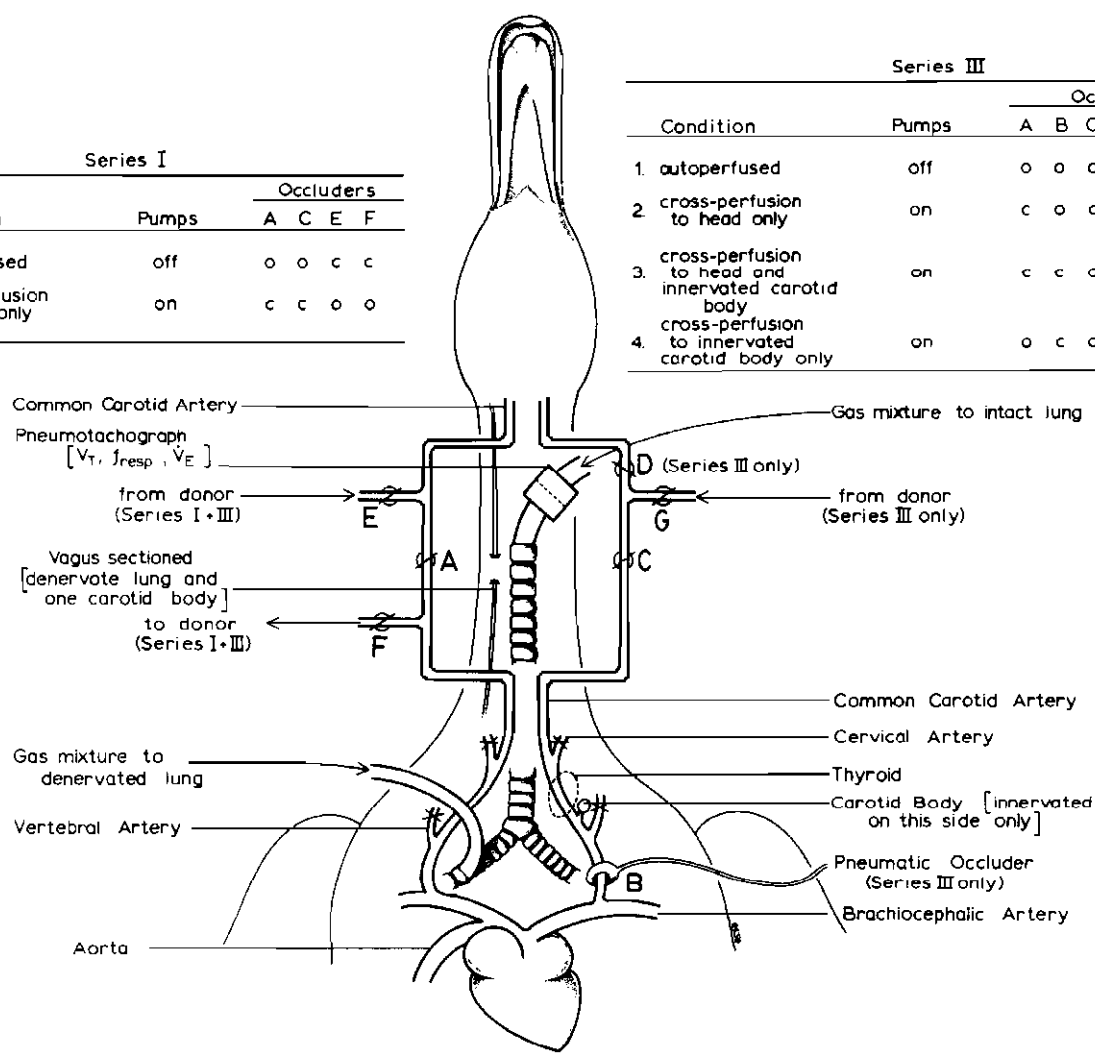

FIG. 1. Schematic diagram of preparation used for cross perfusion between pairs of animals to produce selective hypercapnia at individual receptor sites. For further explanation, see text (in tables: o, open; c, closed).

Protocol. The donor duck was placed on unidirectional ventilation (gas administered via the trachea and vented via the interclavicular air sac) to allow rapid conditioning of arterial blood gases for cross perfusion to the recipient duck that breathed spontaneously.

Autoperfusion (perfusion pump off, occluders A and C open, Fig. 1, left-hand table) represents the normal condition where the recipient animal is in total control of its own blood flow. Under this condition, a series of CO_2 response tests were carried out. Gas of $F_{I_{O_2}}$ of 0.50 and variable $F_{I_{CO_2}}$ (0–0.10) was given the recipient animal to breath via both lungs to produce step changes in Pa_{CO_2}. These tests were administered randomly, interspersed with the cross-perfusion CO_2 response tests.

Cross perfusion was established by occluding sites A and C and switching on the perfusion pump (occluders E and F open) (Fig. 1; left-hand table). Once cross perfusion was established, 1,000 IU heparin was administered every 2 h throughout the remainder of the experiments. By producing identical changes in Pa_{CO_2} of the recipient and donor during cross perfusion and comparing the ventilatory responses with those obtained at similar levels of Pa_{CO_2} during autoperfusion (Fig. 2), it was possible to confirm that cross perfusion per se had no effect on the ventilatory responses of the recipient to CO_2.

At the end of the day's experiments the recipient animal was anesthetized, placed on cross perfusion, and then the perfusion pump was turned off. Only if the recipient animal died within 1 min (as judged by respiratory failure) was cross perfusion considered to be supplying all of the recipient's cerebral blood flow and the experimental results acceptable.

In *series I*, during cross perfusion, P_{CO_2} of blood perfusing the head could be changed independent of levels of systemic Pa_{CO_2} or vice versa. The innervated carotid body was always perfused by blood from the recipient animal. All test gases presented to the recipient to change systemic Pa_{CO_2} were inhaled by both lungs. The effect on ventilation of changes in P_{CO_2} of blood perfusing the head could be tested against a background of systemic hypo-, normo-, and hypercapnia.

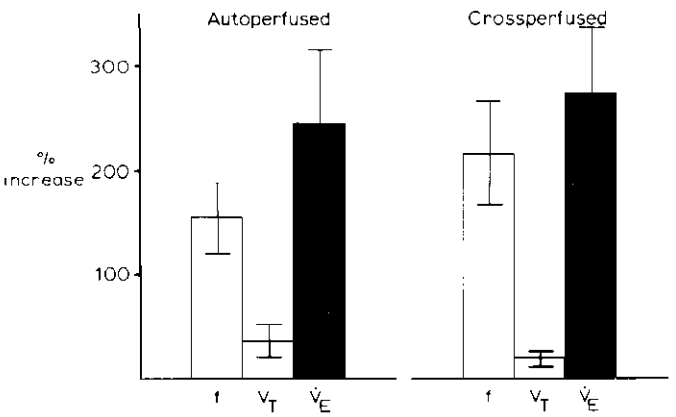

FIG. 2. Comparison of changes in respiratory frequency (f), tidal volume (V_T), and minute ventilation ($\dot{V}_E$) following elevation of systemic Pa_{CO_2} from 3.8 ± 0.2 to 7.0 ± 0.2 kPa during autoperfusion and cross perfusion (n = 5) (resting values, f = 8.0 ± 1.0; V_T = 56.6 ± 5.2; $\dot{V}_E$ = 456 ± 77).

Series II

Surgical preparation. For this series of experiments a unilateral right cervical vagotomy was performed under local anesthesia and both lungs cannulated as in *series I.* A branchial artery was also cannulated to take samples for blood gas measurements.

Protocol. With this preparation, pulmonary P_{CO_2} could be altered either by the expedient route of increasing $F_{I_{CO_2}}$ or the physiological but more difficult route of increasing $P\bar{v}_{CO_2}$. Ventilatory responses were recorded while the animals inhaled CO_2 through either the innervated lung, increasing $F_{I_{CO_2}}$ at the intact intrapulmonary chemoreceptors, or the denervated lung, increasing the Pa_{CO_2} of blood perfusing the body and hence increasing $P\bar{v}_{CO_2}$ at the intact intrapulmonary chemoreceptors while $F_{I_{CO_2}}$ in this lung remained at zero.

Series III

Surgical preparation. This series of experiments also involved cross perfusion between pairs of animals. The donor animals were prepared as in *series I* as were the recipient animals with the following additions. On the day of the initial surgery the common carotid artery on the side with the intact vagus nerve (left carotid artery) was exposed low in the neck and a pneumatic flow occluder was placed around this vessel below the level of the carotid body (occluder B). On the day of the experiment this carotid artery was also cannulated as shown in Fig. 1 and also connected to the upstream ischiatic cannula of the donor along with the right carotid artery cannula. Flow through cannula G was driven by a Watson-Marlow H3 flow inducer. With this preparation it was possible to occlude blood flow at any of the sites labeled A–G in Fig. 1 and it was also possible to occlude all flow between donor and recipient animals by halting the pumps. Otherwise, all surgical procedure was exactly as outlined in *series I.*

Protocol. In the *series III* experiments it was possible to alter the Pa_{CO_2} independently at any one or combination of potential receptor groups (central, carotid body, and intrapulmonary chemoreceptors) (Fig. 1, right-hand table) while maintaining P_{CO_2} constant at any chosen level at the other receptor sites. Pulmonary P_{CO_2} of the recipient animal was altered by increasing $P\bar{v}_{CO_2}$ reaching the innervated lung.

The donor animal was again placed on unidirectional ventilation to condition blood for the recipient. Autoperfusion was established by turning off the perfusion pumps (occluding sites E–G) and opening occluders A–D. Cross perfusion to the head alone was established by switching on the perfusion pump (opening occluders E and F) and occluding sites A and C. Cross perfusion of the head and carotid body or carotid body alone could be effected as outlined in the right-hand table of Fig. 1. Once cross perfusion was established heparin was administered as in *series I.* Again tests were performed to confirm that cross perfusion per se had no effect on the ventilatory response of the recipient to CO_2 and that our control of blood flow to the central circulation by cross perfusion was complete.

It should be noted that blood cross-perfused to the recipient will be drained by the jugular veins in the case of head perfusion and by the carotid venous drainage in the case of the carotid body perfusion and thus affect $P\bar{v}_{CO_2}$ of the recipient, interfering with attempts to control independently the P_{CO_2} of the various receptor groups. The effects of this contamination, however, were very small due to mixing of the venous return via the jugular veins with the remainder of the systemic venous drainage (cross-perfusion flow/cardiac output ≈0.10), and could be offset by altering $F_{I_{CO_2}}$ to the denervated lung to maintain a constant $F_{ET_{CO_2}}$ in the innervated lung.

The various CO_2 response tests possible with the arrangement in *series III* were applied in a totally arbitrary fashion. As in *series I*, these tests were interspersed with CO_2 response tests while the recipient animal was autoperfused.

Measurements

Arterial blood pressure was monitored in the upstream segments of the carotid and ischiatic cannulas in the recipient and donor ducks respectively (*series I* and *III*) and perfusion pressure was measured in the cross-perfusion cannulas supplying the recipient (using Biotec BT-70 pressure transducers). When cross perfusion was established, flow was adjusted so that the perfusion pressure to the head or carotid body of the recipient duck was equal to the arterial blood pressure of the recipient duck. Breathing was monitored in the recipient duck with a pneumotachograph attached to the tracheal cannula feeding the innervated lung (Hewlett-Packard pneumotachograph no. A547). The pressure drop across the pneumotachograph during tracheal airflow was recorded with a Hewlett-Packard model 270 differential pressure transducer and the airflow signal was fed through a Hewlett-Packard 350–3700 A integrating preamplifier to give tidal volume. Frequently during an experiment the pneumotachograph was switched to the tracheal cannula feeding the denervated lung ensuring that flow to each lung during spontaneous breathing was the same. All tidal volume measurements were multiplied by two to account for the fact that volumes were recorded from only one lung. The gas composition of the respiratory gases going to each lung in the recipient duck and in the expired air of the donor duck were monitored with a Centronic 200 MGA clinical mass spectrometer. All signals were amplified using conventional means and the blood pressure, tracheal airflow, electrocardiogram, and O_2 and CO_2 compositions were displayed on a Technirite eight-channel thermal pen recorder writing on rectilinear coordinates and stored on an eight-channel FM tape system for later analysis by computer. The stored data were analyzed using a specially prepared computer program for a Digital PDP Lab 8e computer. This program yielded mean and maximum inflation and deflation airflow rates, tidal volume, and respiratory frequency (f).

Arterial blood samples taken immediately before the measurement of all respiratory variables in each experimental run were analyzed using an Instrumentation Laboratories IL micro-13 blood gas analyzer maintained at 41 ± 1°C.

All measurements are reported as mean ± SE.

RESULTS

Series I. Peripheral and Central Effects of CO_2 on Ventilation

The respiratory responses to alterations in P_{CO_2} of blood perfusing the head at various levels of peripheral Pa_{CO_2} were studied in 10 pairs of animals. These results are shown in Table 1. Low Pa_{CO_2} in the blood perfusing the body of the recipient was produced as a consequence of hyperventilation in the recipient animal when the Pa_{CO_2} was elevated in the blood perfusing the head. Producing a low Pa_{CO_2} in blood perfusing the body while the Pa_{CO_2} was low in the blood perfusing the head was extremely difficult, as low central Pa_{CO_2} normally produced hypoventilation and systemic hypercapnia in our spontaneously breathing birds. We have only one set of measurements under these conditions and we include these values with caution.

There was a trend of increasing f, V_T, and $\dot{V}_E$ when central Pa_{CO_2} was elevated from 2.5 to 8.0 kPa (1 kPa = 7.5 Torr) regardless of the level of Pa_{CO_2} in the blood perfusing the body. The largest relative increases in $\dot{V}_E$ accompanying this increase in central Pa_{CO_2} occurred when peripheral Pa_{CO_2} was low. Elevating peripheral Pa_{CO_2} at constant central Pa_{CO_2} produced mixed results. When central Pa_{CO_2} was high (8.0 kPa), elevating peripheral Pa_{CO_2} (3.0–8.0 kPa) had little or no effect on ventilation. If the central Pa_{CO_2} was low (2.5 kPa) a trend of decreasing f and increasing V_T and $\dot{V}_E$ was produced as peripheral Pa_{CO_2} was elevated from 3.0 to 8.0 kPa.

The increases in V_T accompanying changes in Pa_{CO_2} from 2.5 or 3.0 to 8.0 kPa were similar regardless of whether the CO_2 was acting peripherally or centrally. However, because the effects on f were not similar, stimulation of receptors by blood perfusing the head produced much greater increases in $\dot{V}_E$ than stimulation of peripheral chemoreceptors. It would appear from these results that not only is there a group of receptors situated upstream of the carotid bodies, but that under these experimental conditions they contribute the vast majority of the ventilatory response of ducks to hypercapnia.

Series II. Effects of Increasing $P\bar{v}_{CO_2}$ versus $F_{I_{CO_2}}$ on Ventilation

The ventilatory responses to inhalation of CO_2 by an innervated and by a denervated lung to produce similar

TABLE 1. *Effect of changes in systemic and central Pa_{CO_2} on ventilation (series I)*

	Pa_{CO_2} of Blood Perfusing Body, kPa	Pa_{CO_2} of Blood Perfusing Head, kPa	
		2.5 ± 0.2	8.0 ± 0.3
f	3.0 ± 0.2	10.0 (n = 1)	12.7 ± 1.8 (n = 11)
V_T		84.0	137.0 ± 14.0
$\dot{V}_E$		840.0	1,760.0 ± 210.0
f	4.6 ± 0.1	9.7 ± 1.6 (n = 7)	10.0 ± 2.0 (n = 8)
V_T		120.0 ± 21.0	135.0 ± 9.0
$\dot{V}_E$		1,065.0 ± 225.0	1,372.0 ± 337.0
f	8.0 ± 0.5	8.6 ± 1.2 (n = 7)	12.5 ± 1.7 (n = 8)
V_T		131.0 ± 25.0	137.0 ± 19.0
$\dot{V}_E$		1,148.0 ± 306.0	1,726.0 ± 392.0

n, No. of observations on 5 pairs of animals; f, respiratory frequency in min^{-1}; V_T, tidal volume in ml; $\dot{V}_E$, minute ventilation in $ml \cdot min^{-1}$.

increases in Pa_{CO_2} to the head and carotid body were studied in five ducks. Changes in $\dot{V}_E$ resulting from changes in Pa_{CO_2} within the range of 4.0–5.3 kPa ($F_{I_{CO_2}}$ = 3–6%) were similar regardless of whether the CO_2 was inhaled through the innervated lung alone or the denervated lung alone (Fig. 3). When the CO_2 was inhaled through the innervated lung, however, the changes in $\dot{V}_E$ stemmed primarily from changes in V_T whereas when the CO_2 was inhaled by the denervated lung alone changes in f were primarily responsihle for the changes in $\dot{V}_E$ (Fig. 3).

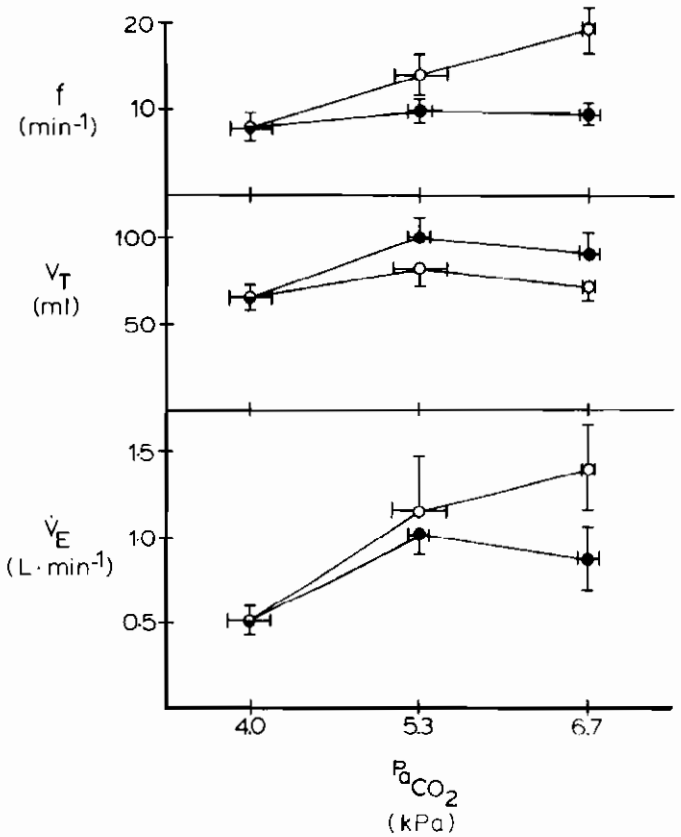

FIG. 3. Respiratory frequency (f), tidal volume (V_T), and minute ventilation ($\dot{V}_E$) as functions of Pa_{CO_2} produced by inhalation of CO_2 by an innervated lung (●) or denervated lung (○) alone.

With both routes of CO_2 administration, Pa_{CO_2}, $F_{E_{CO_2}}$ (at the intact lung), and presumably $P\bar{v}_{CO_2}$ were equal. The difference between $F_{I_{CO_2}}$ and $F_{E_{CO_2}}$ [$(F_I\text{-}F_E)_{CO_2}$] at the intact pulmonary chemoreceptors, however, would decrease as increasing levels of CO_2 were inhaled through the innervated lung (increasing $F_{I_{CO_2}}$ in this lung) and would increase when increasing levels of CO_2 were inhaled through the denervated lung. In the latter case, $F_{I_{CO_2}}$ remained at zero while $F_{E_{CO_2}}$ increased as a result of increases in $P\bar{v}_{CO_2}$. The effect of changes in this phasic input are shown in Fig. 4. While the duck was inhaling CO_2 into both lungs the CO_2 was removed from the gas going to the innervated lung and increased in the gas going to the denervated lung so that Pa_{CO_2} remained constant (7.1 kPa) and $F_{E_{CO_2}}$ remained constant or decreased slightly but $(F_I\text{-}F_E)_{CO_2}$ increased in the innervated lung producing an immediate change (within a single breath) in the breathing pattern. The breathing frequency increased, and the V_T decreased while $\dot{V}_E$ remained constant. Return to breathing the initial levels of CO_2 through both lungs, still maintaining the same Pa_{CO_2} and $F_{E_{CO_2}}$, decreased $(F_I\text{-}F_E)_{CO_2}$ in the innervated lung and immediately reversed the changes in the breathing pattern. Respiratory frequency was reduced and V_T increased.

When higher levels of CO_2 were inhaled (Pa_{CO_2} > 5.3 kPa) through the innervated lung alone $\dot{V}_E$ began to decline whereas when higher levels of CO_2 were inhaled by the denervated lung alone $\dot{V}_E$ continued to increase (Fig. 3). At these higher levels of inhaled CO_2 there appeared to be little difference between the ventilatory response to CO_2 inhalation through the innervated lung and CO_2 inhalation by bilaterally vagotomized ducks (Fig. 4).

Series III. Role of Intrapulmonary, Carotid Body, and Central Chemoreceptors in Ventilatory Response to CO_2

The roles and relative contributions of the three po-

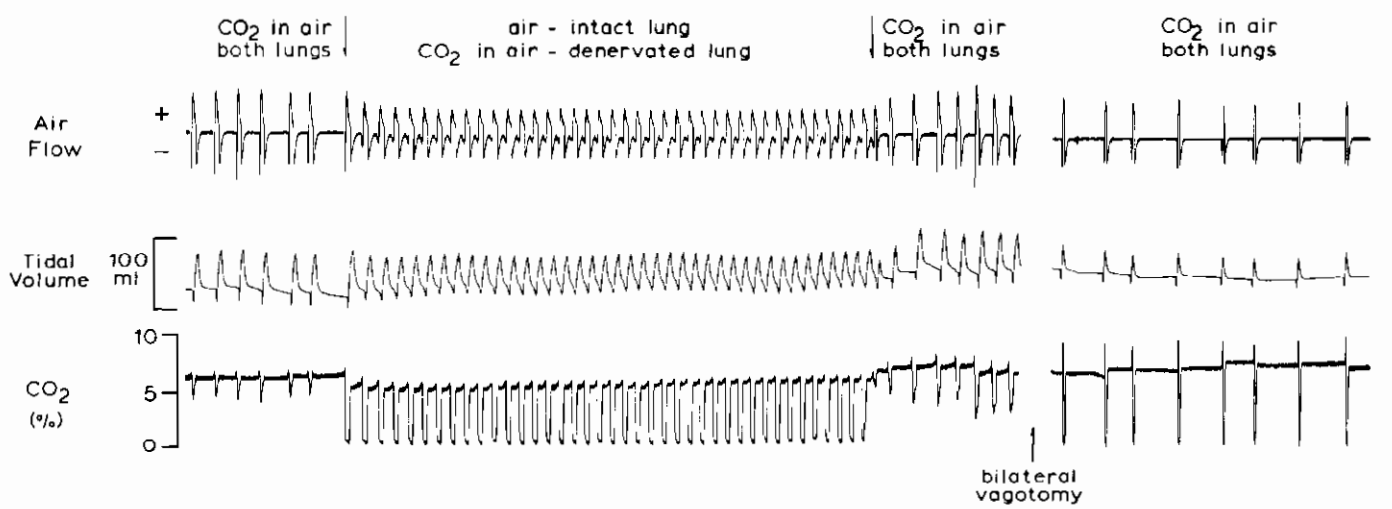

FIG. 4. Recordings of airflow [inspiration is up (+)], tidal volume, and tracheal CO_2 concentration during spontaneous ventilation of 5% CO_2 in air through both lungs in an unanesthetized duck. At *1st arrow*, CO_2 is removed from gas inhaled by innervated lung and doubled in gas inhaled by denervated lung. At *2nd arrow* gas compositions are reestablished at original levels. *Final panel* shows effects of bilateral vagotomy on breathing pattern.

tential receptor groups, alone and in combination, in the steady-state ventilatory response to changes in P_{CO_2} were studied in six pairs of ducks during cross perfusion. CO_2 was always administered to the recipient animal through inhalation by the denervated lung, that is, by increasing $P\bar{v}_{CO_2}$. When the P_{CO_2} of blood perfusing the intrapulmonary chemoreceptors was elevated by increasing Pa_{CO_2} from 3.8 ± 0.2 to 7.0 ± 0.2 kPa, while Pa_{CO_2} perfusing the head and carotid body remained normocapnic (Fig. 5), f increased slightly, V_T decreased slightly, and $\dot{V}_E$ remained unchanged. Presenting CO_2 to the carotid bodies while head and lung P_{CO_2} were maintained at normocapnic levels led to an 80% increase in $\dot{V}_E$ due primarily to a large increase in f with only a small increase in V_T (Fig. 5). If the blood perfusing the lungs and carotid body was kept normocapnic while the Pa_{CO_2} of blood perfusing the head was elevated to 7.0 kPa, $\dot{V}_E$ increased 194% almost exclusively due to an increase in f (Fig. 5). We have been unable to detect any synergism between the responses mediated by either the carotid body or central chemoreceptors and the intrapulmonary chemoreceptors. There was a slight interaction between the carotid body and central chemoreceptors, but this contributed no more than an extra 10% to the total response (Fig. 6).

DISCUSSION

This study indicates that the steady-state response to hyperoxic hypercapnia in birds is predominantly mediated by central chemoreceptors as is the case in mammals (14). Although we cannot make any statements about the exact location of the receptors involved, this evidence confirms that birds are able to hyperventilate in response to hypercapnia through a central mechanism alone (16, 32). This response was not dependent on CO_2 administration by venous CO_2 loading as the central chemoreceptors contributed most of the ventilatory response to increasing $F_{I_{CO_2}}$ (*series I*) and approximately 80% of the response to CO_2 following venous CO_2 loading (*series III*). It should be noted that the percentage responses given here may overestimate the real situation, since the responses were generated by stimulation of only one carotid body and one-half of the lung afferents.

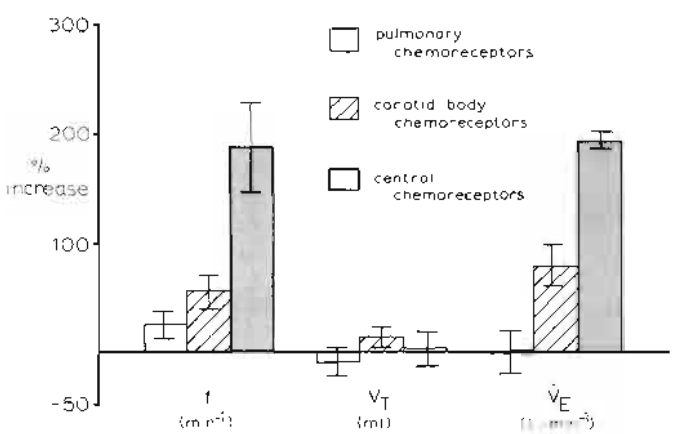

FIG. 5. Changes in respiratory frequency (f), tidal volume (V_T), and minute ventilation ($\dot{V}_E$) following elevation of Pa_{CO_2} from 3.8 ± 0.2 to 7.0 ± 0.2 kPa in blood perfusing various receptor groups (resting values in Figs. 5 and 6 are same as in Fig. 2).

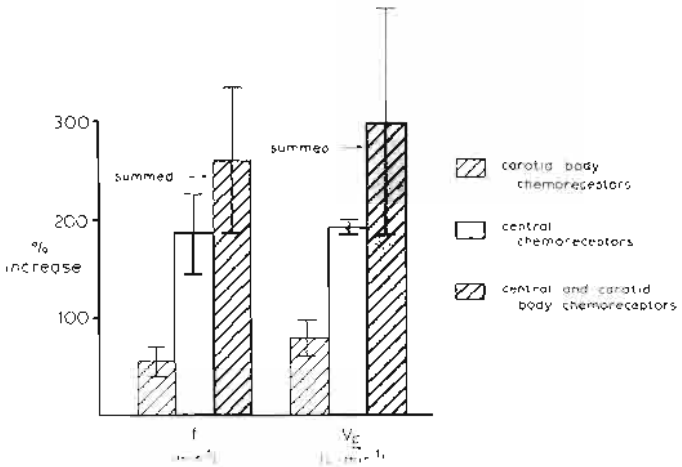

FIG. 6. Changes in respiratory frequency (f) and minute ventilation ($\dot{V}_E$) following elevation of Pa_{CO_2} from 3.8 ± 0.2 to 7.0 ± 0.2 kPa in blood perfusing central and carotid body chemoreceptors. *Arrows* indicate additive effects of perfusing 2 receptor groups individually for comparison with the effects of perfusing both groups simultaneously.

The remainder of the ventilatory response to hypercapnia arises from stimulation of the carotid body chemoreceptors alone although a small degree of synergism seems to exist when carotid body and central chemoreceptors are stimulated in unison.

We have found that the intrapulmonary chemoreceptors do not contribute to the ventilatory response to hypercapnia although stimulation of intrapulmonary chemoreceptors by venous CO_2 loading does lead to a very small change in the breathing pattern. Despite these changes, $\dot{V}_E$ remains roughly constant and effective gas exchange area ventilation will, if anything, be reduced as a result of the small increases in f and concomitant decreases in V_T (increased dead-space ventilation). The magnitude of these changes, however, is very small. These results are in agreement with the work of Burger et al. (8) who could not demonstrate any ventilatory stimulation by intrapulmonary chemoreceptors during increased metabolic rate in chickens.

This is in contrast with much of the literature. There have been recent suggestions by several authors (20, 23–26, 29, 30) that avian intrapulmonary chemoreceptors play a dominant role in ventilatory responses to CO_2 under isocapnic or hypocapnic conditions. There are reports of equivalent but nonadditive influences of intrapulmonary chemoreceptors and systemic chemoreceptors in the ventilatory response to CO_2 of chickens (9) as well as reports of synergism between these receptor groups (24). These particular studies, however, use unidirectionally ventilated anesthetized animals with split chests, recording respiratory amplitude (9, 24) and, in one case, reporting resting breathing frequencies of over $150 \cdot min^{-1}$ (24). Further, in all of these studies (9, 20, 23–26, 29, 30), regardless of differences in approach and technique, CO_2 was administered to the birds by increasing $F_{I_{CO_2}}$ to innervated lungs. In our experiments, if systemic Pa_{CO_2} was kept below 5.3 kPa, similar increases in $\dot{V}_E$ were observed regardless of whether CO_2 was inhaled by innervated or denervated lungs. The changes in breathing pattern responsible for increasing $\dot{V}_E$, however, were very different. Inhalation of CO_2 by the inner-

vated lung resulted primarily in an increase in V_T while inhalation of CO_2 by the denervated lung resulted primarily in an increase in f. Furthermore, as $F_{I_{CO_2}}$ was elevated above 5.3 kPa, if the CO_2 was inhaled by the innervated lung, $\dot{V}_E$ began to decrease, whereas if inhaled by the denervated lung $\dot{V}_E$ continued to increase. As the same steady-state $P_{a_{CO_2}}$ was attained regardless of which way the CO_2 was administered, these differences must stem from differences in the timing and magnitude of P_{CO_2} changes at the intrapulmonary chemoreceptors. Other authors (29, 33) have also noted similar decreases in $\dot{V}_E$ and general intolerance of CO_2 by birds with elevation of $F_{I_{CO_2}}$ above 0.06.

Avian intrapulmonary chemoreceptors are primarily located in the caudal portion of the paleopulmonic parabronchi, the major gas exchange region of the avian lung (31). Their discharge frequency decreases with increasing P_{CO_2} at the receptor sites (13). If the bird inhales CO_2 through the denervated lung, under steady-state conditions (constant $P_{a_{CO_2}}$), the P_{CO_2} at most intrapulmonary chemoreceptors in the innervated lung increases only as a result of increases in $P\bar{v}_{CO_2}$. Thus tonic intrapulmonary chemoreceptor discharge is reduced, since airway CO_2 increases in expiration, while the phasic discharge accompanying each breath, when fresh air crosses the lung, is increased. Increases in $\dot{V}_E$ stem primarily from changes in f. When the bird breathes CO_2 through the innervated lung, tonic discharge is again reduced by the rise in venous CO_2, but phasic discharge is greatly reduced or eliminated and now increases in $\dot{V}_E$ stem primarily from changes in V_T. If the animal is bilaterally vagotomized, eliminating all tonic and phasic discharge arising from the intrapulmonary chemoreceptors, f is further reduced. These results are fully consistent with predictions from models of the central integration of pulmonary stretch receptor input in mammals (6, 12, 18) supporting suggestions (2, 8, 10, 13, 21) that avian intrapulmonary chemoreceptors are the afferent limb of an inspiratory-inhibitory reflex, which uses the rate and extent of CO_2 washout during inspiration as the sensory signal rather than rate and extent of lung expansion.

Experimental hypercapnia is routinely produced in mammals by increasing $F_{I_{CO_2}}$. Since mammalian intrapulmonary stretch receptors respond to the rate and degree of change in lung volume or transpulmonary pressure and show only a slight sensitivity to CO_2 (7, 17, 21), changes in the composition of the ventilatory gas will have little direct effect on their discharge. However, as avian intrapulmonary receptors are chemo- rather than mechanoreceptors, using CO_2 as their sensory modality, increasing $F_{I_{CO_2}}$ has the mixed effect of producing arterial hypercapnia while eliminating pulmonary afferent information thus producing ventilatory responses superimposed on an abnormal breathing pattern. As a consequence, all studies of avian ventilatory responses to inhalation of CO_2 have been performed under varying degrees of "functional pulmonary vagotomy," and it is not surprising that the changes recorded in breathing pattern (particularly breathing frequency) have been inconsistent (1, 4, 11, 15, 17, 20, 26, 29). These studies have given rise to the view that the major respiratory response of birds to hypercapnia is a rapid and powerful increase in V_T with little change in f. It would now appear, at least in ducks, that this is a response to a nonphysiological stimulus (increasing $F_{I_{CO_2}}$) which interferes with pulmonary afferent input altering the breathing pattern and masking the true nature of the normal ventilatory response to hypercapnia. Although not conclusive on its own, the results of the single instance in *series I* where both peripheral and central hypocapnia were produced simultaneously further suggests that the relative roles of the chemoreceptors, in ducks at least, are similar during hypo- and hypercapnia.

We conclude that due to the nature and stimulus specificity of the intrapulmonary chemoreceptors in birds, a true ventilatory response to CO_2 can only be elicited by venous CO_2 loading. The ventilatory response, when elicited in this fashion, consists of a large increase in respiratory frequency accompanied by only a modest increase in tidal volume. Under steady-state conditions central chemosensitive areas play the dominant role in evoking the ventilatory response (approximately 80%) with some contribution from the carotid body chemoreceptors. There is also a slight synergistic effect on $\dot{V}_E$, arising from stimulation of central and carotid body chemoreceptors simultaneously. The intrapulmonary chemoreceptors, although contributing significantly to regulation of the breathing pattern, do not contribute to the ventilatory response to hypercapnia.

This study was supported by grants from the British Columbia Heart Fund and the Natural Sciences and Engineering Research Council of Canada awarded to D. R. Jones, and the Natural, Applied and Health Sciences Research Fund of the University of British Columbia to D. R. Jones and W. K. Milsom.

Received 5 May 1980; accepted in final form 7 January 1981.

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