Ventilatory response to venous CO_2 loading by gut ventilation in ducks

DAVID R. JONES, WILLIAM K. MILSOM, AND PATRICK J. BUTLER[1]
Department of Zoology, University of British Columbia, Vancouver, B.C., Canada V6T 2A9

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Venous CO_2 loading was achieved in unanesthetized, spontaneously breathing white Pekin ducks by gut-ventilation to load CO_2 across the intestinal circulation. Gut ventilation with air had no effect on breathing, while venous CO_2 loading resulted in an increase in minute ventilation ($\dot{V}E$). Ventilation sensitivity ($\Delta\dot{V}E/\Delta PaCO_2$) when expressed as a percentage of $\dot{V}E$ obtained either without or during gut ventilation with air ($15\% \cdot mmHg^{-1}$; 1 mmHg = 133.322 Pa) was within the range of values previously recorded in birds by investigators using other techniques. An isocapnic hyperpnea, as observed in some other studies, was never seen using gut loading of CO_2. These experiments have shown the efficacy of loading CO_2 across the gut wall in ducks and this technique should prove useful in resolving the roles of the various CO_2-sensitive receptor groups in increasing $\dot{V}E$ in response to CO_2 loads.

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Nous avons procédé à l'engorgement de CO_2 veineux par ventilation du tube digestif de façon à charger de CO_2 la circulation intestinale chez des canards de Pékin blancs, non anesthésiés, à respiration spontanée. La ventilation du tube digestif au moyen d'air n'affecte pas la respiration, alors que l'arrivée de CO_2 veineux entraîne une augmentation de la ventilation à chaque minute ($\dot{V}E$). La sensibilité à la ventilation ($\Delta\dot{V}E/\Delta PaCO_2$) exprimée en pourcentage de la valeur de $\dot{V}E$ obtenue sans ventilation ou au cours de la ventilation du tube digestif au moyen d'air ($15\% \cdot mmHg^{-1}$; 1 mmHg = 133.322 Pa) se situe à l'intérieur de la gamme de valeurs obtenues chez des oiseaux par d'autres chercheurs, au moyen d'autres techniques. La ventilation du tube digestif au moyen de CO_2 n'a jamais entraîné d'hyperpnée isocapnique, comme ce fut le cas dans d'autres études. Ces expériences démontrent l'efficacité de la technique de ventilation au CO_2 à travers la paroi du tube digestif chez les canards; cette technique devrait s'avérer utile pour déterminer les rôles respectifs des différents groupes de récepteurs sensibles au CO_2 dans l'augmentation de la ventilation $\dot{V}E$ en réaction à des charges de CO_2.

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Introduction

Since the activity of intrapulmonary chemoreceptors in birds is inversely related to the CO_2 concentration in pulmonary gas, in studies of ventilatory responses to CO_2 flux achieved by increasing the CO_2 content of inspired gas their discharge will be reduced or even eliminated during inspiration (Fedde *et al.* 1974; Molony 1974; Fedde and Scheid 1976; Tallman and Grodins 1982*b*). This can produce ventilatory responses which are superimposed on an abnormal and highly variable breathing pattern (Milsom *et al.* 1981). As a consequence, venous CO_2 loading would appear to offer a more valid means of testing ventilatory responses to pulmonary CO_2 flux in birds. It is rather disturbing, therefore, that experiments employing blood infusion for venous CO_2 loading have occasionally given such equivocal results. Nightingale and Fedde (1971), for instance, found that venous infusion of CO_2-laden blood in birds caused an isocapnic hyperpnea, while Boon *et al.* (1980) found that infusion of hypocapnic blood produced a fall in minute ventilation ($\dot{V}E$) accompanied by arterial isocapnia (isocapnic hypopnea). In marked contrast to the above observations, Tallman and Grodins (1982*a*) reported that increases in $\dot{V}E$ stemming from venous CO_2 loading by an extracorporeal circuit were adequately accounted for by concomitant changes in $PaCO_2$ (hypercapnic hyperpnea). Certainly, we have seen some odd effects in a series of experiments we conducted employing venous CO_2 loading by cross-perfusion between pairs of animals (W. K. Milsom, D. R. Jones, and P. J. Butler, unpublished). In several instances, cross-perfusion *per se* significantly elevated minute ventilation reducing $PaCO_2$ and elevating $PaCO_2$ (hypocapnic hyperpnea). Similar results have also been reported in cats and dogs (Jones *et al.* 1982; Ponte and Purves 1978).

Recently, Nye and Marsh (1982) described an indirect method of venous CO_2 loading which obviates the need for cardiovascular interventions. Carbon dioxide is loaded by ventilating the gut with gas mixtures containing CO_2. We have adapted this technique in the present experiments for use in unanesthetised, intact ducks to compare ventilatory sensitivity with venous CO_2 loading by gut ventilation to CO_2 sensitivities obtained by others not only in experiments involving venous blood loading but also involving inhalation of low levels of CO_2.

Methods

Experiments were conducted at room temperature (20–22°C) on white Pekin ducks (*Anas platyrhynchos*). The ducks were acclimated to room temperature for at least 1 week before any experiment. The body mass of all ducks was in the range of 2–3 kg.

All major surgery was performed under general anesthesia (pentobarbital (30 mg/kg) or urethan (1 g/kg) i.v.) 2–8 days before experiments were run. Minor surgery on the day of the experiments, such as for the implantation of cannulae, was done under local anesthetic (2% Xylocaine). All wounds were periodically infiltrated with local anesthetic during the course of the experiment to minimize stress to the animals. These experiments conformed to the guidelines on the ethics of animal experiments of the Canada Council on Animal Care as adopted by the Animal Care Committee of the University of British Columbia.

Major surgery

Each duck was positioned, ventral side up, on an operating table and the central cardiovascular area was exposed through a ventral

[1]Present address: Department of Zoology and Comparative Physiology, University of Birmingham, Birmingham, B15 2TT United Kingdom.

incision in the skin and wall of the interclavicular air sac. A flow probe was implanted on the left pulmonary artery and a cuff with the same internal diameter as the flow probe was placed on the right pulmonary artery. This was done to equalize any restrictions to flow because of the presence of the flow probe and to maintain a roughly equal flow in both arteries (see Jones *et al.* 1983). At the end of these procedures, the cervical air sac and the overlying tissue and skin were sutured closed. Also, at this time, one ischiatic artery was exposed in the leg and the trachea was exposed in the neck. The skin over these incisions was closed using stainless steel wound clips.

Minor surgery

On the day of the experiment the ischiatic artery was carefully reexposed under local anesthesia and cannulated in an upstream direction with PE-160 tubing. At the same time, a cannula (either PE-90 or PE-160) was inserted into the right atrium via the left wing vein for sampling mixed venous blood. The position of the latter catheter was judged from the pressure trace at the time of placement and confirmed at autopsy. The trachea was reexposed, divided, and cannulated with soft, wide-bore, vinyl tubing.

A small incision (1.5−2 cm) was made in the ventral thoracoabdominal wall after first infiltrating the wall with local anesthetic. The gut was identified and the gut wall was heavily infiltrated with local anesthetic above and below the region of section. A transverse cut was made in the gut wall, just below the gizzard, to allow insertion of two cuffed endotracheal tubes, one pointing upstream and one downstream. Inflation of the cuffs held the tubes in the gut lumen. The upstream cannula allowed the upper gastrointestinal tract to drain. Another cannula was inserted through the cloaca into the rectum and the lower gastrointestinal tract was then flushed with warm saline.

Measurements

Arterial blood pressure was monitored from the upstream segment of the ischiatic artery using a Biotec BT-70 pressure transducer and breathing was monitored by a pneumotachograph attached to the tracheal cannula. 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 350-3700A integrating preamplifier to give tidal volume. Heart rate was obtained from an electrocardiogram (ECG) recorded with bipolar copper wire electrodes and fed into an instantaneous heart rate meter to give pulse frequency. Blood flow in the left pulmonary artery was recorded with a Biotronix BL 610 pulsed-logic electromagnetic flowmeter and this flow was doubled to give cardiac output. Zero flow was assumed to occur when a constant flowmeter output was obtained in diastole, which was confirmed during elicitation of diving bradycardia. Each flowmeter was calibrated at the end of an experiment after the duck was killed by an overdose of Nembutal. The pulmonary artery was cannulated above and below the flow probe and saline was passed through the vessel from a reservoir. The fluid passing through the transducer was collected in a graduated cylinder and the period required for the collection of 100 mL was timed with a stopwatch. We have already established that for our flowmeter system, saline and blood give similar calibrations when the same probe is tested with both (Langille and Jones 1975). All signals were amplified by conventional means and the blood flow and blood pressure, tracheal airflow, tidal volume, ECG, and heart rate were displayed on a Technirite eight-channel thermal pen recorder writing on rectilinear coordinates and stored on an eight-channel frequency-modulated tape system.

Arterial and venous blood samples were taken immediately before the measurement of all respiratory variables in each experimental run and analyzed using an Instrumentation Laboratories IL micro-13 blood gas analyzer maintained at 41 ± 1°C. The arterial and venous blood samples were also analyzed for CO_2 content using the method described by Cameron (1971) for small blood samples. Venous and arterial CO_2 loads were then calculated by multiplying blood content values by cardiac output and the CO_2 flux across the lungs ($\dot{V}CO_2$) may be obtained from the difference between the venous CO_2 load arriving at the lung and the arterial CO_2 load leaving the lung.

Protocol

In all experiments, the animals were unanesthetized but lightly restrained, ventral side down, on operating tables. This type of restraint had no noticeable effect on the breathing pattern of the birds. Before the start of gut ventilation, the head of each animal was submerged for up to 60 s (Jones and Purves 1970). The achieve this, the head was ventriflexed into the mouth of a large plastic funnel and clamped in position. The funnel was filled with tap water (10−14°C) from a beaker for submergence, while emersion was brought about by draining the funnel through the spout. The long diastolic intervals during diving bradycardia were used to confirm our estimation of zero flow on the flowmeter trace. The body temperature of all birds used was continuously monitored during experiments using a thermistor probe (1 mm diameter) inserted into the abdominal cavity. Body temperature was maintained at 41 ± 1.0°C by infrared lamps mounted above the birds.

Mixed venous P_{CO_2} ($P\bar{v}_{CO_2}$) was altered by perfusing the gut with warm humidified gas. This procedure resulted in CO_2 loading of the venous blood via diffusion into the gastrointestinal circulation. All variables were then recorded while animals were not gut ventilated and while animals were gut ventilated with (*i*) 2 L air·min^{-1}, (*ii*) 4 L air·min^{-1}, (*iii*) 2 L CO_2·min^{-1}, and (*iv*) 4 L CO_2·min^{-1}. Sufficient time was allowed following each change in the gut-ventilation regime for blood gas values to stabilize. Increasing flow from 2 to 4 L·min^{-1} did not enhance gas exchange across the gut and thus data from the two air-loading and two CO_2-loading regimes have been combined in the data analysis. The various gut-ventilation regimes were administered in a random sequence, but care was taken to ensure that animals were returned to control conditions (no gut ventilation) between all gut-ventilation runs.

Data analysis

All measurements are given as means (±SD of the mean) of *n* observations on *N* animals. The statistical significance of differences between means was assessed by Scheffe's method after first performing an analysis of variance with a specially prepared computer program for balanced experimental designs (Anvart, University of British Columbia). Critical values of the *F* statistic were obtained from tables (Schefler 1980).

Results

Gut ventilation with air had no effect on any respiratory or cardiovascular variables compared with the values obtained from ducks which were not gut ventilated (Table 1). Gut ventilation with 100% CO_2, however, significantly changed all measured variables, except blood pressure, from those obtained during gut ventilation with air (Table 1, Fig. 1). Mixed venous CO_2 content was elevated by 1.8 mM·L^{-1} and $P\bar{v}_{CO_2}$ rose by 5.7 mmHg (1 mmHg = 133.322 Pa). In spite of an increase in $\dot{V}_E$ of 54%, P_{aCO_2} rose by 3.9 mmHg. Increases in both V_T and f contributed to the rise in $\dot{V}_E$. Cardiac output increased, but only by 22%, so there was a marked rise in the $\dot{V}_E$:$\dot{Q}$ ratio (Table 1). Ventilatory sensitivity ($\Delta\dot{V}_E/\Delta P_{aCO_2}$) calculated as a mean from values for each individual animal was 137 ± 55 mL·min^{-1}·mmHg^{-1} when CO_2-loaded ducks were compared with those on gut ventilation with air and 142 ± 21 mL·min^{-1}·mmHg^{-1} when CO_2-loaded ducks were compared with those not on gut ventilation. Normalizing these values to those from animals not on CO_2 loads gave sensitivities of 15%·mmHg^{-1} for both comparison groups.

Discussion

This study has shown the feasibility of venous CO_2 loading in birds by means of ventilating the gut with 100% CO_2. Furthermore, gut ventilation with air had no effect on breathing and did not appear to cause any distress to the bird, even at gut

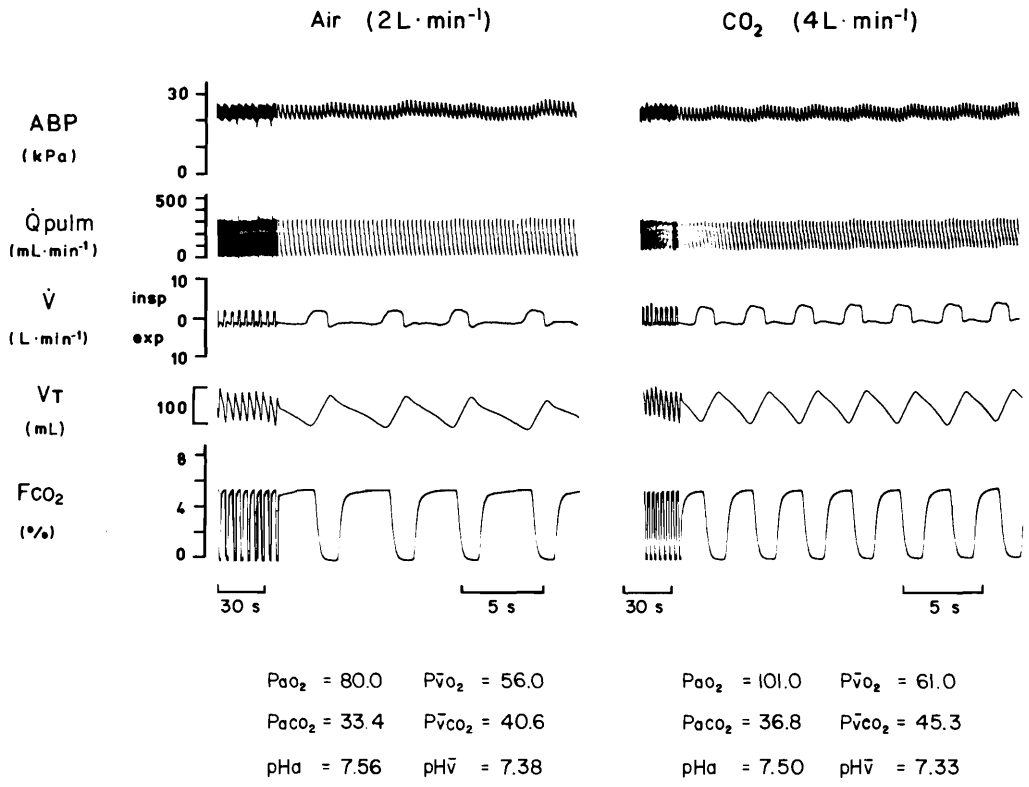

FIG. 1. The effects of gut ventilation with air and CO_2 on arterial blood pressure (ABP), blood flow through one pulmonary artery ($\dot{Q}$pulm), ventilatory air flow ($\dot{V}$; insp, inspiration; exp, expiration), tidal volume (V_T), and the CO_2 fraction of tracheal air (F_{CO_2}) in one animal. The partial pressures of O_2 (P_{O_2}), CO_2 (P_{CO_2}), and the pH of arterial (a) and mixed venous ($\bar{v}$) blood at the time of each recording are listed under each set of traces.

TABLE 1. Average ventilatory and cardiovascular values (±SD) under steady-state conditions in five white Pekin ducks at rest and during gut ventilation

Variable	No gut ventilation	Air gut ventilation	CO_2 gut ventilation
V_T (mL)	64.6±18.8	64.4±12.6	78.6±20.1*
f (min^{-1})	15.8±5.8	15.0±3.9	19.5±6.5*
$\dot{V}_E$ (mL·min^{-1})	931±111	929±120	1431±211*
$\dot{Q}$ (mL·min^{-1})	598±106	575±112	700±109*
$\dot{V}/\dot{Q}$	1.63±0.36	1.65±0.26	2.03±0.2*
Pa_{O_2} (mmHg)	79.3±5	78.6±7.0	88.3±5*
Pa_{CO_2} (mmHg)	28.1±2.7	27.6±2.2	31.5±2.0*
Arterial pH (pHa)	7.6±0.04	7.6±0.04	7.57±0.03*
Ca_{CO_2} (mM·L^{-1})	20.8±1.6	20.6±2.5	22.2±2.5*
$P\bar{v}_{O_2}$ (mmHg)	46.8±8.5	46±8	49.8±7.6*
$P\bar{v}_{CO_2}$ (mmHg)	31.2±2.1	30.8±2.6	36.5±1.8*
Mixed venous pH (pH$\bar{v}$)	7.57±0.03	7.56±0.03	7.5±0.04*
$C\bar{v}_{CO_2}$ (mM·L^{-1})	22.2±1.5	22.3±2.2	24.1±2.9*
Arterial CO_2 load (mM·L^{-1}·min^{-1})	12.35±1.9	11.97±3.3	15.8±3.2*
Venous CO_2 load (mM·L^{-1}·min^{-1})	13.2±2.4	13.4±3.4	17.2±4*
n	10	10	10

*Significantly different ($P < 0.05$) from air gut-ventilation values.

gas flows of 4 L·min^{-1}. Unfortunately, this method of venous CO_2 loading is not without its drawbacks in birds. The short length of the intestine, coupled with the effectiveness of its wall as a diffusion barrier, severely limits the amount of CO_2 that can be loaded and may completely prevent the unloading of significant amounts of CO_2 from venous blood. Judging from acid–base criteria, however, our ducks were hyperventilating even without gut ventilation, so that Pa_{CO_2} was already considerably lower than normal (Table 1). This may explain why attempts to unload CO_2 were unsuccessful; therefore it may be

that some CO_2 can be unloaded when P_{aCO_2}, before gut ventilation, is in the normal range of 35–38 mmHg (Kawashiro and Scheid 1975).

The present study has also shown that changes in ventilation in response to venous CO_2 loading via the gut are associated with increases in P_{aCO_2} in ducks. Ventilatory sensitivities to changes in P_{aCO_2} ($\Delta\dot{V}E/\Delta P_{aCO_2}$) expressed with the values of $\dot{V}E$, obtained during gut ventilation with CO_2, normalized to minute ventilation without gut ventilation, or during air ventilation of the gut, were similar at about $15\% \cdot mmHg^{-1}$. This value is somewhat lower than the $22-29\% \cdot mmHg^{-1}$ for ventilatory sensitivity of decerebrate Pekin ducks obtained from studies of CO_2 inhalation and venous CO_2 loading using an extracoporeal circuit by Tallman and Grodins (1982*a*). In the present experiments, P_{aO_2} rose significantly during CO_2-induced hyperventilation which could have reduced ventilatory drive. On the other hand, our previous estimates for ventilatory sensitivity of unanesthetised ducks ($10-11\% \cdot mmHg^{-1}$), obtained by inhalation of low levels of CO_2 and by arterial CO_2 loading, are somewhat lower than the present estimate (Milsom *et al.* 1981). Nevertheless, the similarities between the present and other data are sufficient for us to argue against any non-specific effects on breathing of CO_2 gut ventilation, which might have been caused by gut afferents stimulated by, for instance, large changes in pH of the cells of the gut wall.

In contrast to the studies mentioned above, there are a number of reports of isocapnic hyperpnea accompanying CO_2 loading in both birds and mammals giving an infinite ventilatory sensitivity to CO_2 (Wasserman *et al.* 1975; Osborne and Mitchell 1977; Powell *et al.* 1978; Scheid *et al.* 1978; Stremel *et al.* 1978; Mitchell and Osborne 1979; Phillipson *et al.* 1982). It has been suggested that intrapulmonary chemoreceptors in birds could elicit such a response (Fedde *et al.* 1982); however, in mammals, which lack this chemoreceptor group, extremely high CO_2 sensitivity has been claimed to be a reflex response to either carotid body stimulation (Phillipson *et al.* 1981) or to an elevation in right ventricular pressure caused by increased venous return (Ponte and Purves 1978; Jones *et al.* 1982). Cardiac receptors have been demonstrated in the avian heart (Jones 1969; Estavillo and Burger 1973), which could function as the afferent arm of a cardiogenic–respiratory reflex similar to that which may exist in mammals. The carotid bodies in birds seem to be essentially similar to those in mammals in terms of their reflex effects on ventilation (Bouverot *et al.* 1974; Bouverot 1978); therefore, any role they play in generating isocapnic hyperpnea, if and when it occurs, must also remain open. The present gut-loading experiments did not cause isocapnic hyperpnea, however, so a group of receptors located between the venous and arterial sides of the circulation, making a significant contribution to $\dot{V}E$, is not indicated. Nevertheless, we hope that this technique will allow us to resolve the relative contribution of all CO_2-sensitive receptor groups to increasing $\dot{V}E$ in response to CO_2 loads in a series of future experiments.

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