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Ventilatory roll off during sustained hypercapnia is gender specific in pekin ducks

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Abstract

The objective of the present study was to examine the relative roles of peripheral *versus* central mechanisms in producing ventilatory adjustments in pekin ducks during prolonged (5 h) hypercapnia (5% inspired CO_2), and to determine whether these adjustments differed between male and female ducks. After 20 min of CO_2 exposure, intact ducks increased total ventilation ($\dot{V}_E$) 2.5–3-fold above control values, due to large increases (~200%) in tidal volume (V_T) and slightly smaller increases (~140%) in breathing frequency (f_R). This response was accompanied by respiratory acidosis (pHa fell from ~7.46 to ~7.41) and hypercapnia (Pa_{CO_2} increased from ~35 to ~40 Torr). In males, $\dot{V}_E$ fell progressively thereafter due exclusively to a fall in f_R, in parallel with a rapid partial recovery of pH (to 7.44) while Pa_{CO_2} continued to climb (to ~42 Torr). In females, $\dot{V}_E$ remained elevated during hypercapnia, and no pH recovery occurred. This suggests that a respiratory decline resulting from acid–base compensation (probably due to HCO_3^- mobilization) occurred in males but not in females. Bicarbonate mobilization, and thus pH compensation, may have been reduced in females due to the $CaCO_3$ requirements of eggshell formation. In males, the acute ventilatory response was reduced slightly by denervation of the carotid bodies or intrapulmonary chemoreceptors, but there was no effect of denervation of either receptor group on the responses to prolonged CO_2. We conclude that pH compensation triggered by constant or increasing Pa_{CO_2}, acting at central chemoreceptors, likely mediates the respiratory adjustments seen in male pekin ducks during hypercapnia. Furthermore, we suggest that this ventilatory response be considered a gender-specific hypercapnic ventilatory roll off, in the context of the various time domains of the hypercapnic ventilatory response.
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1. Introduction

Carbon dioxide is a powerful respiratory stimulant in most air-breathing vertebrates. The coordinated response of central and peripheral chemoreceptors to hypercapnia, which increases arterial P_{CO_2} (Pa_{CO_2}) and decreases arterial pH (pHa), generally increases ventilation (Milsom, 2002). The magnitude of the ventilatory response to hypercapnia often decreases with time, which may reflect independent effects of Pa_{CO_2} and pHa on breathing (Dempsey and Forster, 1982). For example, in male pekin ducks breathing 5% CO_2, the immediate increase in total ventilation diminishes over time, which occurs in conjunction with a partial recovery of pHa (Dodd and Milsom, 1987). These ventilatory changes are entirely due to a decrease in breathing frequency, and occur even though Pa_{CO_2} remains constant.

Multiple CO_2-sensitive chemoreceptors exist in air-breathing vertebrates, all of which could influence ventilation during prolonged hypercapnia (Milsom, 2002). Birds in particular are thought to possess at least three dominant chemoreceptor groups. Stimulation of central chemoreceptors appears to be the most effective at increasing ventilation in response to hypercapnia, while the carotid body chemoreceptors also contribute substantially to ventilatory drive during hypercapnia (Milsom et al., 1981). Intrapulmonary chemoreceptors (IPCs), which are unique amongst birds and diapsid reptiles, are also CO_2 sensitive (Hempleman and Posner, 2004; Milsom et al., 2004), but the role of this chemoreceptor group is unclear: IPCs are definitely known to regulate breathing pattern, and may also influence total ventilation. In addition to the contributions of central and peripheral chemoreceptors, the ventilatory response to hypercapnia can be modulated by mechanisms of central integration,

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depending on the pattern and time course of the CO_2 exposure (Baker et al., 2001).

The ventilatory response to hypercapnia has multiple time domains, similar to the hypoxic ventilatory response (Powell et al., 1998). Initiation of chronic hypoxia causes an acute rise in ventilation, followed by a decrease in ventilation over several minutes to hours of continued hypoxic exposure. This decrease can be due to hyperventilation-associated hypocapnia/alkalosis in the case of poikilocapnic hypoxia ("roll off"), as well as hypoxic ventilatory decline, which also occurs during isocapnic hypoxia. Whereas the former can be explained by changes in arterial P_{CO_2}/pH, the latter may be caused by changes in chemosensitivity or central integration (Powell et al., 1998). Prolonged hypercapnia results in analogous ventilatory adjustments, but the mechanisms underlying this acclimation are unknown.

The present study was undertaken to examine the chemoreceptor control of the ventilatory response during prolonged (5 h) hypercapnia (5% inspired CO_2) in pekin ducks. The primary objective of this study was to examine the relative roles of peripheral *versus* central mechanisms in producing ventilatory acclimation to hypercapnia. Because our previous study only characterized ventilatory acclimation in male ducks (Dodd and Milsom, 1987), an additional objective of this study was to determine if there were differences between male and female ducks in the magnitude and time course of this response.

2. Materials and methods

2.1. Experimental animals

Experiments were performed on adult and juvenile (6–24-week old) pekin ducks (*Anas platyrhynchos*), which were housed outdoors at the Animal Care Facility of the University of British Columbia. Adult animals ranged in body mass from 2.2 to 3.3 kg, whereas juveniles were somewhat smaller, ranging between 1.5 and 3.0 kg in mass. Two days prior to any experimentation, the animals were brought indoors, maintained in individual cages (60 cm × 63 cm × 91 cm) with free access to food (Buckerfields Goose and Duck Grow Pellets) and water, and allowed to adjust to room temperature (21–22 °C). All animal care and experimentation was approved by the UBC Animal Care Committee.

The experiments performed were grouped into four separate series as follows:

(i) Series A—Experiments were conducted on adult ($n = 18$) and juvenile ($n = 12$) male ducks that had fully intact respiratory chemoreceptor groups to determine the effects of long term (5 h) exposure to hypercapnia (5% inspired CO_2) on ventilation, arterial P_{CO_2} and pH. Plasma ion levels were also measured in a subgroup of adult males ($n = 8$).

(ii) Series B—Experiments that were identical to those in Series A were conducted on adult female birds, again with all chemoreceptor groups intact ($n = 6$), to determine whether the responses measured in Series A differed between the sexes.

(iii) Series C—Experiments were conducted on adult males whose carotid bodies were denervated ($n = 6$) to examine the role of this chemoreceptor group in the responses observed in Series A.

(iv) Series D—Experiments were conducted on juvenile male birds whose pulmonary afferent nerves were denervated ($n = 5$) to examine the role of CO_2-sensitive IPCs in the responses observed in Series A. We could only perform the surgery for this series on juvenile birds, which is why both adults and juveniles were used in Series A.

2.2. Surgical procedures

All birds undergoing experimentation had a flexible polyethylene cannula (PE-90; Clayton Adams) implanted in their right brachial artery for both blood sampling and for monitoring arterial blood pressure. In a subset of animals (Series C and D, below), the right brachial vein was also cannulated for injecting general anaesthetic. The surgery required to implant the cannulae was minor and was conducted under local anaesthesia (20 mg/ml Xylocaine, administered subcutaneously). Each animal was lightly restrained in dorsal recumbency, and the cannulae (filled with 1000 IU/ml heparinized saline) were inserted lateral to the humerus and slowly advanced approximately 7 cm. In Series A and B, each animal was allowed to recover from cannulation for 24 h before experimentation. In Series C and D, additional surgery was required as described below.

2.2.1. Carotid body denervation

In Series C, adult male birds underwent additional surgery to denervate the carotid bodies. Bilateral denervation was performed under intravenously administered general anaesthesia: an initial dose of 30 mg/kg sodium pentabarbitol (MTC Pharmaceuticals) achieved a surgical plane of anaesthesia, after which smaller supplemental doses (6.5 mg/kg) were used as necessary. With each animal in dorsal recumbency, feathers were removed from a region of the chest, a midsagittal incision approximately 7 cm in length was made, and the skin and subcutaneous fat were reflected to expose the clavicular airsac. The airsac was carefully opened and reflected. The animal was then intubated with an endotracheal tube (4.0 mm inner diameter, cuffed; Mallinckrodt) and unidirectionally ventilated with a hyperoxic gas mixture (30% O_2), by passing gas in through the trachea and out *via* the ruptured inter-clavicular airsac. Intramuscular injection of atropine sulphate (2.1 μg/kg) was used to prevent the buildup of mucous in the trachea and bronchi, which is a known side-effect of the pentobarbitol anaesthesia.

The carotid body chemoreceptors are located at the bifurcations of the left and right common carotid arteries (Jones and Purves, 1970). They are each innervated by a carotid sinus nerve, which is a direct branch of the ipsilateral vagus nerve arising from the nodose ganglion. The left and right vagus nerves were therefore identified on the dorsal surface of the cervical airsac and were traced posteriorly to the left and right thyroid glands. On each side, the thyroid gland was carefully reflected to reveal the underlying nodose ganglion. To ensure complete

section of the carotid sinus nerves, the 2 or 3 nerve fibers branching from each vagal nerve trunk in the region of the ganglion (1 cm both posterior and anterior) were sectioned. This was done on both the left and right side to accomplish the desired bilateral denervation of the carotid bodies. Once completed, the plane of anaesthesia was reduced, and when spontaneous breathing movements became apparent, the endotracheal tube was removed and the clavicular airsac was tightly sutured to prevent air leakage. The overlying muscle and skin were then sutured independently and the animal was given analgesics (Demerol) and a prophylactic intramuscular injection of ampicillin (Ayerst Laboratories), and was then allowed to recover for several days.

The effectiveness of the denervation was determined several days after the surgery by intravenous injection of sodium cyanide. At small sublethal doses, NaCN creates histotoxic hypoxia in the highly O_2-sensitive tissues of the carotid body chemoreceptors. This increases carotid body activity, which increases overall ventilation in animals with intact afferent signalling from these chemoreceptors. If ventilation did not increase after cyanide injection, denervation of the carotid bodies was considered successful.

2.2.2. *Pulmonary afferent denervation*

In Series D, juvenile male birds underwent additional surgery to denervate the intrapulmonary chemoreceptors. These chemoreceptors are located throughout each parabronchial lung, and send afferent input to respiratory centres in the brain *via* the vagus nerves. Several nerve fibres arise from IPCs, and these fibres converge on the vagus nerve at several points along the length of the lung. Bilateral section of both vagi between the lungs and the nodose ganglion will therefore eliminate all afferent input from the IPCs, but also denervates all visceral branches of the vagus, which is known to be fatal (Fedde and Burger, 1963). Denervation of the IPCs was therefore accomplished by completely sectioning the left vagus (unilateral vagotomy is well tolerated) and sectioning all pulmonary branches of the right vagus. In this way, pulmonary denervation was complete while right unilateral visceral innervation was maintained. It was not possible to discern between IPC-specific nerve fibres and those travelling in the vagi that arose from other lung receptors (e.g., mechanoreceptors), so all vagal afferent branches from each lung were sectioned.

The surgery required to remove these receptors was extensive and required opening the thoracic cavity to expose the lungs. Juvenile birds were therefore used in this series of experiments because their sternums are incompletely ossified, assisting the necessary bisection of the sternum. The surgery was performed under the same general anaesthetic regime and atropine treatment described above. With each animal in dorsal recumbency, it was fully anaesthetized and unidirectionally ventilated as described above. At this point, the right internal thoracic artery was ligated at a point immediately ventral to the right thyroid gland. A sagittal incision extending along the entire length of the sternum was made in the skin immediately right of the midline, thus exposing the large pectoralis major muscle. Using an electro-surgical unit (Electrosectilis, model 770; Birtcher), a sagittal incision was made through the pectoralis major extending the full length of the sternum as close to the midline as possible. A second incision was then made through the smaller underlying pectoralis minor muscle. Excessive bleeding was stopped either by clamping the tissue with hemostatic forceps, or by electrocoagulation (Hyfrecator, model X-712; Birtcher). Both muscle layers were then laterally reflected to expose the underlying sternum. Because of its predominantly cartilaginous composition, the sternum was quite easily cut with scissors, in a posterior–anterior direction approximately 1 cm to the right of the carina. Extreme care was taken to keep the incision through the sternum shallow to avoid rupturing the underlying pericardium. Once bisected, the sternum was carefully pried apart with a retractor.

The pulmonary branches of the right vagus were denervated first. The right vagus nerve was identified posterior to the right thyroid gland, in an area bordered by the right bronchi and the right bracheocephalic vein, and was then traced over the entire length of the right lung. All nerve fibers that branched from the vagus in the region of the lung were carefully traced. Those that projected dorsally towards the lung were sectioned. Each animal was then sutured closed in a layered fashion. The sternum was laced together with surgical silk, through holes bored on either side of the incision, and the pectoralis muscles were individually sewn to the carina of the sternum. The left vagus was then prepared for sectioning, while the animal was still under general anaesthesia. A small region of the skin on the ventral surface of the neck (approximately 8 cm posterior to the base of the bill) was exposed, and a sagittal incision (3 cm) exposed the trachea. Gentle retraction of the trachea and surrounding tissue exposed the left carotid sheath, a fascial compartment that enclosed the left external carotid artery, left jugular vein, and left vagus nerve. Without damaging the pulsatile carotid artery, the vagus nerve was gently teased from this fascia and a 1 cm segment of it was isolated and wrapped with latex sheeting to prevent tissue regrowth and allow easy relocation of the nerve at a later time. Once this was completed and the neck incision closed, the plane of anaesthesia was reduced and the endotracheal tube removed once spontaneous breathing movements became apparent. The clavicular airsac and overlying skin were finally sutured closed. Ampicillin (25 mg/kg) and Demerol (meperidine hydrochloride, 5 mg/kg; Winthrop Laboratories) were administered intramuscularly every 4–6 h for 2 days.

After a 5–6 day recovery period, the left vagus was denervated under local anaesthesia by relocating and sectioning the previously isolated left vagus nerve. Although the completeness of pulmonary denervation was immediately apparent from the marked change in breathing pattern (breathing became slower and deeper), all surgical denervations were confirmed postmortem. Successfully denervated birds in this series were devoid of input from all pulmonary receptors, including intrapulmonary chemoreceptors, and from one carotid body chemoreceptor. This surgical protocol therefore maintained input from one carotid body, as well as the influence of the parasympathetic nervous system upon cardiovascular, excretory, and digestive organ systems, avoiding the problems associated with bilateral cervical vagotomy (Fedde and Burger, 1963).

2.3. Measurements

Body plethysmography was used to measure ventilation in all experimental series, as previously described (Dodd and Milsom, 1987). Briefly, the plethysmograph consisted of two parts, a body compartment and a head compartment, separated from each other by a flexible latex collar (Dental Dam; Hygenic Corp.). The body compartment was water jacketed, which allowed the chamber to be maintained at normal avian body temperature ($41 \pm 1\,^{\circ}C$). The head compartment was used to administer specific gas mixtures for the animals to breath, which entered and exited the head compartment on opposite sides of the compartment. The composition of gas flowing into the head compartment was controlled by mixing pure gases (CO_2 and O_2) with compressed air through a series of calibrated flow meters, and was monitored with Beckman oxygen (OM-11) and carbon dioxide (LB-2) analyzers. The flow rate of gas entering the head chamber was never less than 20 l/min to prevent CO_2 accumulation. Gas was bubbled through a humidifier before entering the head compartment.

Changes in body volume (due to ventilatory movements) in the body compartment resulted in changes in air flowing through a pneumotachograph (Fleisch) connected to a single port in the body chamber, which was measured as a change in differential pressure (Validyne). This flow measurement was integrated to yield tidal volume. Mean arterial blood pressure (MAP) was continuously monitored using a physiological pressure transducer (Narco Scientific) connected to the brachial artery cannula. MAP was maintained relatively constant throughout the experiment by replacing any blood volume lost through sampling with intravenous infusion of saline. Tidal volume, MAP, airflow, and inspired CO_2 levels were continuously recorded on a Gould multi-channel pen recorder.

Arterial blood samples (0.5 ml) were drawn aerobically and immediately placed on ice to arrest erythrocyte metabolism (Scheid and Kawashiro, 1975). Within 5 min of sampling, Pa_{CO_2} and pHa were determined using a Radiometer blood gas/pH analyzer maintained at avian core body temperature ($41 \pm 1\,^{\circ}C$). The analyzer was calibrated before each sample using saturated gases and commercially prepared pH buffers (Radiometer/Bach-Simpson). For birds in Series A, CO_2 content was also determined using the method of Cameron (1971).

At select times in the experimental protocol (described below) a second arterial blood sample (2.3 ml) was taken for determining the concentrations of several ions and lactate. A 2.0 ml aliquot of this sample was allowed to clot, after which approximately 0.8 ml of serum was separated from this aliquot by centrifugation (4000 rpm). Ion concentrations were determined for this serum within 2 days of sampling using a sequential multiple analyzer computer (SMAC) system. The remaining 0.3 ml of sampled blood was immediately centrifuged at 4000 rpm to isolate the plasma, and the lactate concentrations of these samples were determined using a commercially available assay kit (Sigma) following manufacturer instructions. A small amount of blood was also taken at the beginning and end of each experiment to make sure that haematocrit was at normal levels.

2.4. Experimental protocol

The overall experimental protocol was the same for each of the four series of experiments in this study. In all cases, birds were allowed sufficient recovery time before undergoing any experimentation. Ducks were placed in the experimental apparatus and allowed 45–60 min to adjust to their surroundings. To minimize the contribution of oxygen to overall respiratory drive, hyperoxic gas (50% O_2) was used as the control condition in all experiments, and all CO_2 gas mixtures contained this same hyperoxic background. After the initial 45–60 min of breathing control hyperoxic gas, ventilatory, blood pressure, Pa_{CO_2}, and pHa measurements were taken. The level of inspired CO_2 was then quickly increased from 0 to 5% in a single step. Subsequent measurements were taken 20, 60, 180, and 300 min later. Serum ion and plasma lactate concentrations were determined in a subset of adult birds from Series A at 0, 60, and 300 min. The effects of carotid body and pulmonary afferent denervation were assessed by comparing animals in Series C and D with intact adult males and intact juvenile males, respectively.

2.5. Calculations and statistical analyses

Total ventilation ($\dot{V}_E$) was calculated as the product of the expired tidal volume (V_T) and breathing frequency (f_R). The bicarbonate ion concentration of arterial blood was calculated in all series using the Henderson-Hasselbach equation, and in Series A was also calculated from total CO_2 content (C_{CO_2}) and CO_2 solubility (α):

$$[HCO_3^-] = C_{CO_2} - \alpha \cdot P_{CO_2} \quad (1)$$

The strong ion difference (SID) was calculated as the difference between the total concentration of strongly dissociated cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) and strongly dissociated anions (Cl^-, lactate$^-$). [Ca^{2+}] was calculated by assuming that 47.5% of total serum calcium was ionic calcium (Bianchi, 1968).

Data are reported as means ± S.E. Depending on the data sets being considered, statistical analyses were carried out using either Student's *t*-tests or ANOVA with Tukey post hoc comparison tests. Regression analyses and analyses of covariance (ANCOVA) were used to compare slopes between different groups. A significance level of 0.05 was used throughout.

3. Results

3.1. Responses of intact male ducks to prolonged hypercapnia

Hypercapnia (5% inspired CO_2) increased ventilation substantially in both adult and juvenile male ducks after 20 min of exposure (Table 1; Fig. 1). The three-fold rise in total ventilation ($\dot{V}_E$; Fig. 1A) resulted from an increase in both tidal volume (V_T, two-fold; Fig. 1B) and breathing frequency (f_R, 1.5-fold; Fig. 1C). After this initial response to hypercapnia, $\dot{V}_E$ decreased continuously during prolonged CO_2 exposure in both adult and juvenile males, such that after 300 min total ventila-

Table 1
Ventilatory and acid–base characteristics in ducks during prolonged hypercapnia (5% inspired CO_2)

Time (min)	Intact adult male	Intact juvenile male	Intact adult female	CBX adult male	PAX juvenile male
$\dot{V}_E$ (ml BTPS/min/kg)					
0	315 ± 21	229 ± 14†	256 ± 26	269 ± 16	293 ± 37
20	936 ± 56	668 ± 47†	612 ± 65†	707 ± 100	625 ± 88
60	848 ± 44	692 ± 62	595 ± 58†	638 ± 59†	655 ± 67
180	671 ± 44*	576 ± 49*	576 ± 57	507 ± 56	598 ± 63
300	635 ± 40*	530 ± 43*	547 ± 52	446 ± 49*†	512 ± 76*
V_T (ml BTPS/kg)					
0	29.1 ± 2.6	19.7 ± 1.4†	21.5 ± 1.6	29.0 ± 3.4	35.7 ± 6.0†
20	59.2 ± 3.8	41.3 ± 3.0†	38.5 ± 2.5†	57.6 ± 6.4	64.9 ± 3.5†
60	60.3 ± 4.0	42.4 ± 3.0†	37.5 ± 2.0†	58.8 ± 6.6	66.2 ± 2.5†
180	55.1 ± 5.3	42.3 ± 3.0†	37.2 ± 2.1†	55.7 ± 6.3	62.2 ± 2.2†
300	61.1 ± 4.3	41.0 ± 2.7†	35.1 ± 3.0†	56.9 ± 5.7	63.5 ± 0.8†
f_R (min^{-1})					
0	11.2 ± 1.0	12.3 ± 1.3	12.3 ± 1.2	10.0 ± 1.3	8.5 ± 2.0
20	16.1 ± 1.1	16.5 ± 1.0	15.9 ± 1.4	12.5 ± 1.5	9.7 ± 1.5†
60	14.3 ± 1.0	16.3 ± 0.9	15.9 ± 1.6	11.3 ± 1.1	9.9 ± 1.0†
180	11.8 ± 1.0*	13.7 ± 0.7*	16.3 ± 1.8	9.3 ± 1.0	9.7 ± 1.1†
300	10.5 ± 0.4*	13.0 ± 0.7*	16.1 ± 2.0†	8.0 ± 0.8†	8.1 ± 1.3†
pHa					
0	7.46 ± 0.005	7.46 ± 0.009	7.45 ± 0.015	7.45 ± 0.005	7.46 ± 0.014
20	7.41 ± 0.006	7.42 ± 0.010	7.40 ± 0.220	7.41 ± 0.006	7.38 ± 0.007
60	7.42 ± 0.007	7.42 ± 0.010	7.41 ± 0.019	7.42 ± 0.010	7.39 ± 0.006
180	7.43 ± 0.007	7.43 ± 0.009*	7.41 ± 0.015	7.43 ± 0.007	7.41 ± 0.010*
300	7.44 ± 0.005*	7.44 ± 0.009*	7.41 ± 0.015	7.44 ± 0.005	7.42 ± 0.009*
Pa_{CO_2} (Torr)					
0	34.5 ± 0.8	32.6 ± 1.4	36.6 ± 0.6	35.6 ± 1.2	30.5 ± 1.2
20	39.5 ± 0.7	37.9 ± 0.9	42.3 ± 1.0	39.7 ± 1.3	41.2 ± 2.3†
60	40.1 ± 0.7	38.5 ± 0.7	42.1 ± 1.2	41.4 ± 1.0	41.4 ± 1.0
180	42.0 ± 0.9	39.9 ± 1.0	43.1 ± 1.1	44.0 ± 1.5	42.8 ± 1.6
300	42.5 ± 0.8*	41.5 ± 0.7*	43.8 ± 0.9	46.2 ± 1.2*†	44.6 ± 1.9
$[HCO_3^-]$ (mM)					
0	22.1 ± 0.5	20.9 ± 0.8	23.3 ± 0.8	22.6 ± 0.9	19.8 ± 0.7
20	22.9 ± 0.5	22.3 ± 0.4	23.9 ± 1.0	23.0 ± 0.8	21.9 ± 0.9
60	23.6 ± 0.4	22.6 ± 0.5	24.2 ± 0.7	24.3 ± 0.7	22.6 ± 0.5
180	25.1 ± 0.5*	24.3 ± 0.5	24.7 ± 0.8	26.4 ± 0.8	24.9 ± 1.2
300	26.0 ± 0.5*	26.0 ± 0.4*	25.4 ± 0.8	28.2 ± 0.8*†	26.4 ± 1.0

CBX, carotid body denervated; PAX, pulmonary afferent denervated; $\dot{V}_E$, total ventilation; V_T, tidal volume; f_R, breathing frequency; pHa, arterial pH; Pa_{CO_2}, arterial CO_2 tension. Data are means ± S.E. *Significant difference from value at 20 min. †Significant difference from intact adult male group (for intact juvenile male, intact adult female, and CBX adult male groups) or intact juvenile male group (for PAX juvenile male group) ($p < 0.05$).

tion was only elevated 2–2.5-fold (~30% reduction compared to 20 min values). This ventilatory decline was primarily due to reductions in f_R, which decreased continuously over time in both adult and juvenile males, while V_T remained relatively constant. Although the ventilatory responses of adult and juvenile males to hypercapnia were similar when considered relative to control conditions (0% CO_2), absolute $\dot{V}_E$ and V_T were generally lower in juveniles, even when corrected for body mass (Table 1). In two adult male birds that were not exposed to CO_2, $\dot{V}_E$, V_T, and f_R remained at control levels throughout the experimental duration (300 min; data not shown).

The initial increase in ventilation in adult and juvenile male ducks was accompanied by an increase in arterial CO_2 tension (~5 Torr) and a decrease in arterial pH (~0.05 units) (Table 1; Fig. 2). The nature of the blood acid–base disturbance by hypercapnia was comparable in adult and juvenile male ducks, indicated by a similar inherent plasma buffering capacity (i.e., the slope of the line joining the first, 0 min, and second, 20 min, points in Fig. 2). After the initial acidosis, arterial pH recovered over time, even though Pa_{CO_2} continued to increase for the duration of CO_2 exposure (~8 Torr above control levels after 300 min of 5% CO_2). Despite these substantial changes in acid–base variables, there was no change in the plasma concentration of any strong cation (Na^+, K^+, Ca^{2+}) or anion (Cl^-, $lactate^-$) in adult male ducks; therefore, prolonged hypercapnia had no effect on strong ion difference (Table 2). The pH recovery was likely due to 4–5 mM increases in blood bicarbonate levels.

The respiratory data are re-plotted in Fig. 3 as a function of Pa_{CO_2} and pHa. There is a tight correlation of changes in $\dot{V}_E$ ($R \geq 0.98$) or f_R ($R \geq 0.84$) to pHa, regardless of the levels of Pa_{CO_2} (Fig. 3B, F *versus* A, E). Tidal volume, in contrast, reached peak values quickly and remained there, despite the slow recovery in pHa and slowly increasing Pa_{CO_2} (Fig. 3C and D).

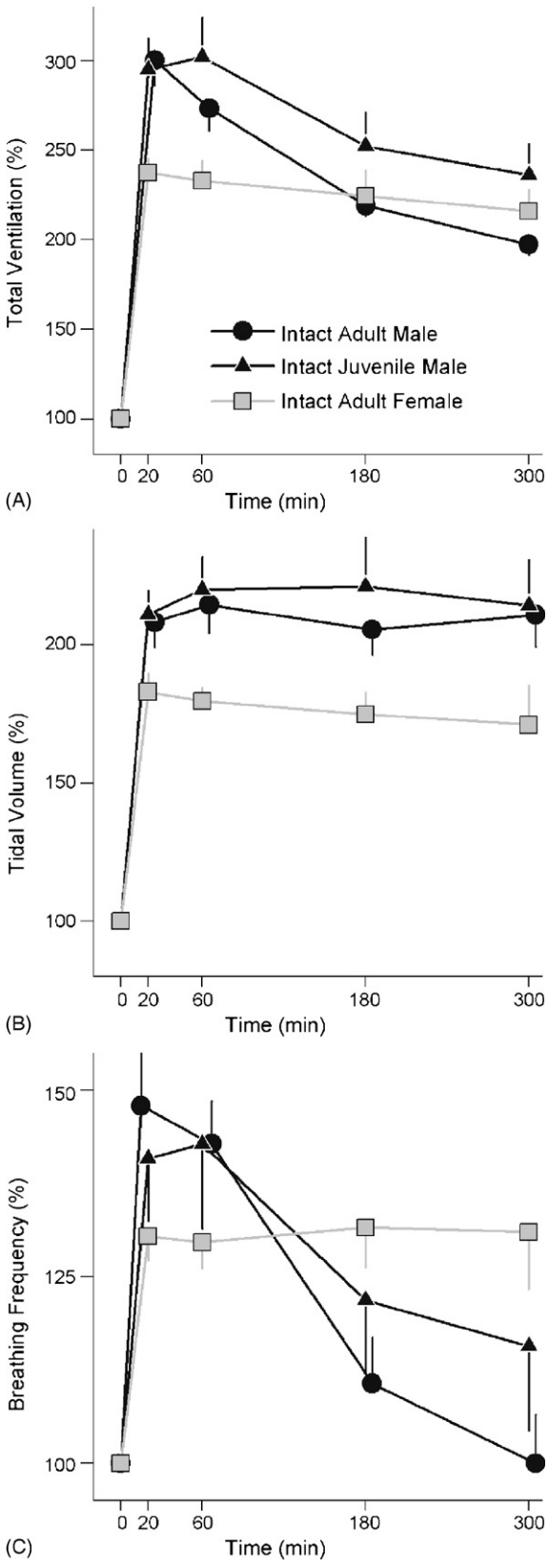

Fig. 1. The relative changes in total ventilation (A), tidal volume (B), and breathing frequency (C) in intact adult male (black circles), intact juvenile male (black triangles), and intact adult female (grey squares) pekin ducks before (0 min) and after a step increase in inspired CO_2 from 0 to 5%. Data are expressed relative (%) to levels before CO_2 administration as mean ± S.E. In some cases, data are offset for clarity. Absolute data are shown in Table 1.

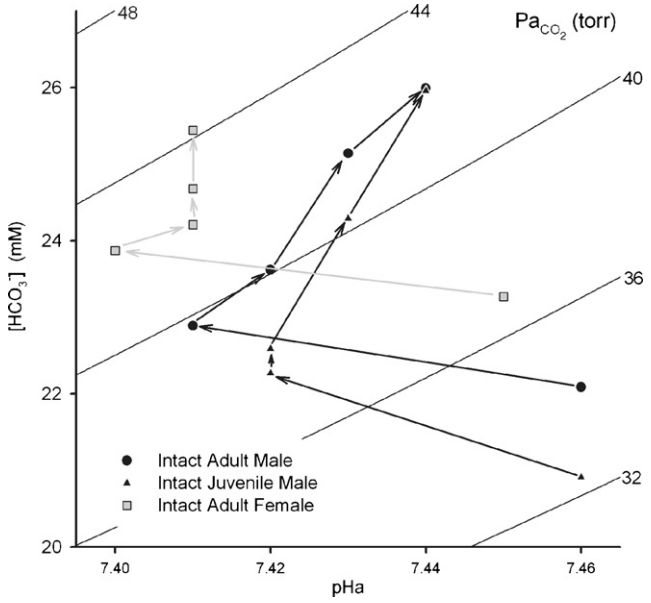

Fig. 2. Davenport diagram showing the effect of a step increase in inspired CO_2 (from 0 to 5%) on arterial pH, P_{CO_2}, and [HCO_3^-] in intact adult male (black circles), intact juvenile male (black triangles), and intact adult female (grey squares) pekin ducks. Data means ± S.E. are shown in Table 1.

3.2. Responses of intact adult female ducks to prolonged hypercapnia

Like adult and juvenile male ducks, adult female ducks increased ventilation immediately upon 5% CO_2 exposure. The relative magnitude of this increase was somewhat lower, however, as total ventilation increased less than 2.5-fold (Fig. 1A), due to an approximate 1.9-fold increase in tidal volume (Fig. 1B) and 1.3-fold increase in breathing frequency (Fig. 1C) (see also Table 1). After this initial response to hypercapnia, females differed substantially from males, as $\dot{V}_E$, V_T, and f_R remained constant over the entire duration of CO_2 exposure.

Table 2
Plasma strong ion levels in intact adult male ducks during prolonged hypercapnia (5% inspired CO_2)

	Time (min)		
	0	60	300
Cations (mM)			
Na^+	145.5 ± 1.68	142.75 ± 1.16	142.00 ± 1.41
Ca^{2+}	3.83 ± 0.41	3.71 ± 0.46	3.64 ± 0.45
K^+	2.56 ± 0.11	2.64 ± 0.1	2.79 ± 0.13
Total cations	151.9 ± 1.66	149.1 ± 1.24	148.81 ± 1.52
Anions (mM)			
Cl^-	110.13 ± 1.22	107.88 ± 0.91	107.38 ± 1.5
Lactate$^-$	1.61 ± 0.17	1.55 ± 0.11	1.40 ± 0.08
Total anions	111.74 ± 1.31	109.42 ± 0.93	108.78 ± 1.44
SID (mM)	40.16 ± 1.08	39.67 ± 0.94	40.02 ± 0.82

SID, Strong ion difference. Data are means ± S.E. No significant changes in the plasma concentration of any strong ion occurred following 60 and 300 min of hypercapnia, neither was there any change in strong ion difference ($p < 0.05$).

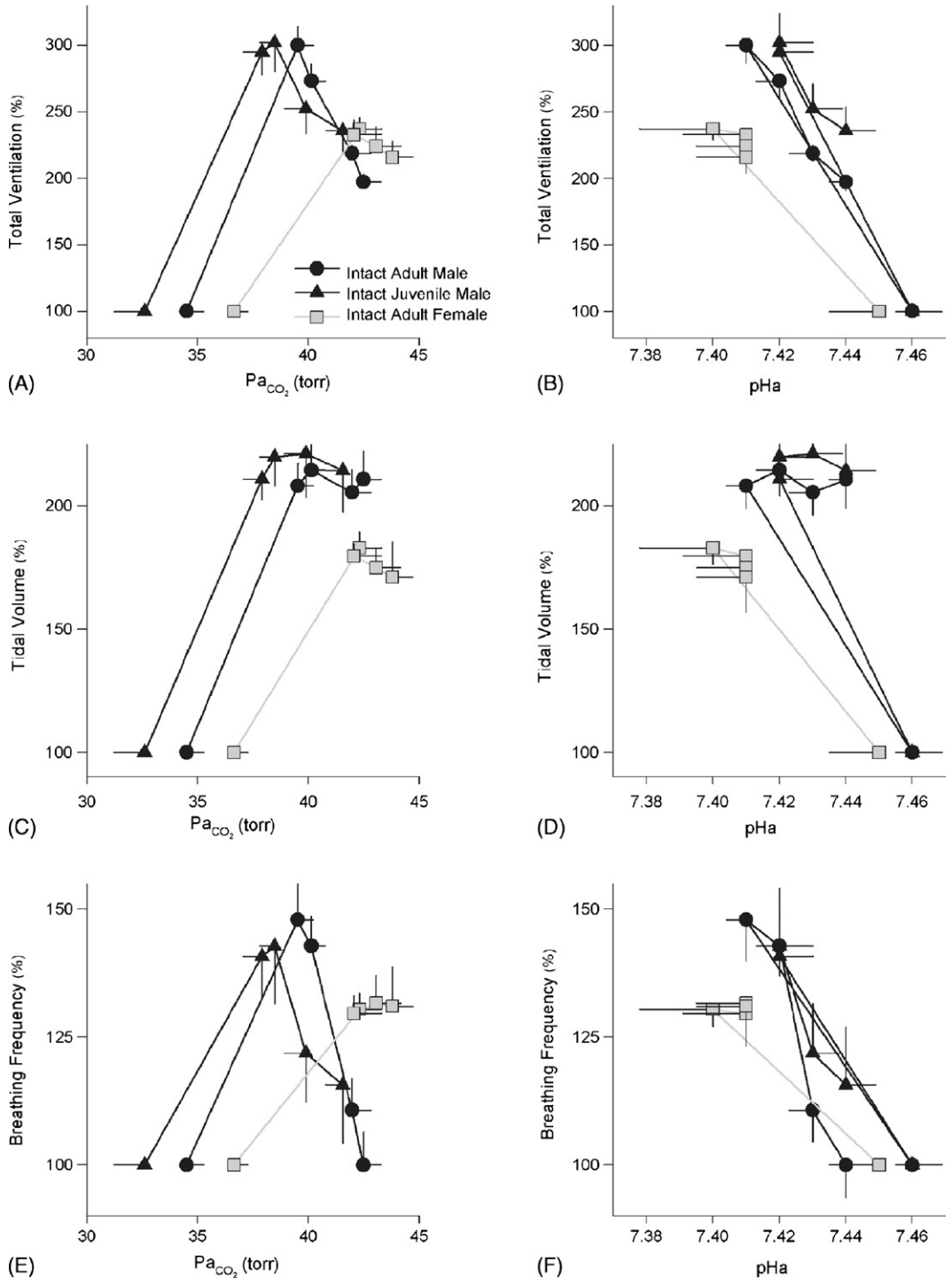

Fig. 3. Total ventilation (A and B), tidal volume (C and D), and breathing frequency (E and F) as a function of arterial P_{CO_2} (A, C, E) or arterial pH (B, D, F) in intact adult male (black circles), intact juvenile male (black triangles), and intact adult female (grey squares) pekin ducks. Data are means ± S.E. Breathing data are expressed relative (%) to levels before CO_2 administration, and the absolute data are shown in Table 1.

Females were also unlike males in the response of acid–base variables to prolonged hypercapnia. Whereas the initial magnitude of the hypercapnia-induced acidosis in female ducks was similar to that in male ducks (~6 Torr decrease in Pa_{CO_2} and ~0.05 unit decrease in pH), as was the inherent plasma buffering capacity (i.e., the slope of the line joining the first, 0 min, and second, 20 min, points in Fig. 2), pHa did not recover in females as it did in males (Table 1; Fig. 2). Furthermore, the calculated increase of blood $[HCO_3^-]$ in females was only half (~2 mM) of that observed in males. It appears that this small mobilization of bicarbonate was sufficient to prevent continued acidosis during prolonged hypercapnia, but was not able to restore pHa towards control levels. When the data for female ducks were plotted as a function of Pa_{CO_2} and pHa, tight correlations were observed between all variables and pHa, but not Pa_{CO_2} (Fig. 3).

3.3. Effects of carotid body denervation on the responses to prolonged hypercapnia

Carotid body denervation (CBX) did not appear to affect the response to prolonged hypercapnia in adult male ducks. Total ventilation in CBX adult male ducks before hypercapnia was ~15% lower than intact adult male ducks, which was due to a reduced breathing frequency, but the difference was not significant (Table 1). After the first 20 min of hypercapnia, CBX ducks increased $\dot{V}_E$ 2.5-fold (Fig. 4A), by increasing V_T (two-fold; Fig. 4B) and f_R (1.25-fold; Fig. 4C). The initial response to hypercapnia therefore appears to be slightly reduced by carotid body denervation, primarily due to a reduced increase in breathing frequency; however, the ventilatory adjustments that occurred during prolonged hypercapnia in CBX ducks appeared identical to those in the intact animals. After 300 min of hypercapnia, total ventilation was reduced by 30% in both CBX ducks (only 1.7-fold above control value) and intact ducks. Like the intact ducks, this decline in $\dot{V}_E$ was due to reductions in f_R; V_T remained constant.

Acid–base variables after prolonged hypercapnia in carotid body denervated ducks were similar to those in intact ducks. The initial changes in pHa, Pa_{CO_2}, and $[HCO_3^-]$ after 20 min of 5% CO_2 were similar between CBX and intact ducks, as was the degree of pH recovery (Fig. 5). In fact, pHa in CBX ducks recovered to the same value as intact ducks after 300 min of hypercapnia despite a nearly 4 Torr higher arterial CO_2 tension (Table 1; Fig. 5). CBX ducks therefore mobilized nearly 50% more bicarbonate than intact ducks. Although the magnitude of the responses were different, the qualitatively similar responses of CBX and intact ducks to chronic CO_2 exposure suggest that changes in acid–base variables acting at the carotid body do not contribute to ventilatory acclimation to prolonged hypercapnia.

3.4. Effects of pulmonary denervation on the responses to prolonged hypercapnia

In the control condition before hypercapnia, the breathing pattern in PAX juvenile male ducks appeared to be altered compared to intact juvenile male ducks. PAX ducks had a higher tidal volume than intact ducks (1.8-fold), and a lower breathing frequency (~25%). These respiratory variables may not have been equally offset, however, as there was a trend towards higher total ventilation in PAX ducks (though not significant; Table 1). Furthermore, the initial response to hypercapnia was reduced in PAX ducks. After the first 20 min of hyerpcapnia, PAX ducks increased $\dot{V}_E$ only two-fold (Fig. 6A), primarily by increasing V_T (two-fold; Fig. 6B). The slight increase in f_R was statistically insignificant (Table 1 and Fig. 6C). Compared to intact ducks, the initial *relative* response to hypercapnia was therefore reduced by approximately 50% by pulmonary afferent denervation, and this was primarily because the increase in breathing frequency was reduced. Similar to carotid body denervation, however, pulmonary afferent denervation (PAX) did not appear to effect the response to prolonged hypercapnia: after 300 min of hypercapnia, $\dot{V}_E$ was reduced by 20% in both PAX juvenile male ducks and intact juvenile male ducks. The effect appeared to be due to a

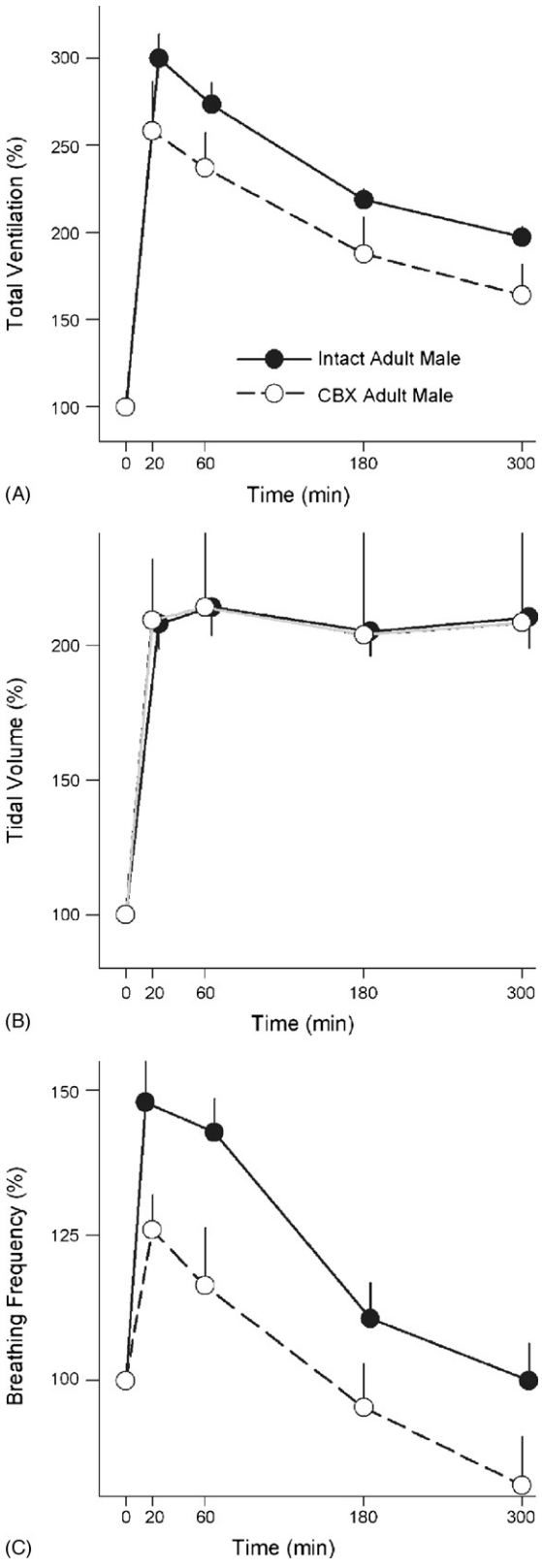

Fig. 4. The relative changes in total ventilation (A), tidal volume (B), and breathing frequency (C) in intact adult male (black circles) and carotid body denervated (CBX) adult male (open circles) pekin ducks before (0 min) and after a step increase in inspired CO_2 from 0 to 5%. Data are expressed relative (%) to levels before CO_2 administration as mean ± S.E. In some cases, data are offset for clarity. Absolute data are shown in Table 1.

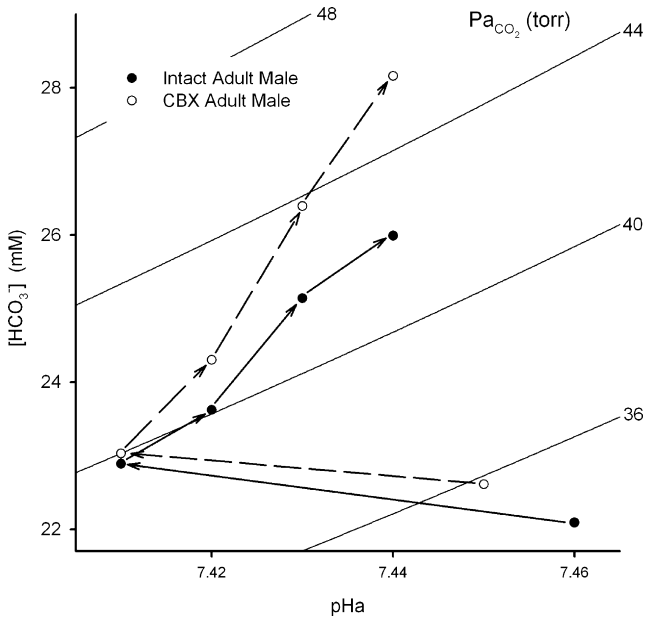

Fig. 5. Davenport diagram showing the effect of a step increase in inspired CO_2 (from 0 to 5%) on arterial pH, P_{CO_2}, and $[HCO_3^-]$ in intact adult male (black circles) and carotid body denervated (CBX) adult male (open circles) pekin ducks. Data means ± S.E. are shown in Table 1.

reduction in f_R in both groups, but this suggestion must be taken with some caution. In PAX ducks, there was a trend for mean f_R to decrease over time during hypercapnia; however, because the initial increase in f_R was reduced, there was very little scope for f_R to decrease throughout hypercapnia exposure.

Whereas the changes in acid–base variables after prolonged hypercapnia appeared to be of greater magnitude in pulmonary afferent denervated ducks than in intact ducks, the pattern of these changes were similar in each group. The initial decrease in pHa (0.08 units) and increase in Pa_{CO_2} (11 Torr) in PAX ducks was greater than in intact ducks (0.04 units and 5 Torr, respectively) (Table 1; Fig. 7). PAX ducks also had a greater pH recovery between 20 and 300 min (0.04 units, compared to 0.02 units in intact ducks), however, despite a final Pa_{CO_2} that was 14 Torr higher than control levels. Plasma $[HCO_3^-]$ also increased in PAX ducks after prolonged 5% CO_2 exposure, to an extent slightly greater than in intact ducks. As was the case for carotid body denervation, the similar responses of PAX and intact juvenile ducks to prolonged hypercapnia suggest that changes in acid–base variables acting at the intrapulmonary chemoreceptors do not contribute to ventilatory acclimation to prolonged hypercapnia.

4. Discussion

The mechanisms through which breathing is controlled in birds and mammals are generally believed to be similar (Bouverot, 1978). The data in this study, however, support our earlier work (Dodd and Milsom, 1987) suggesting that birds and mammals differ in both the speed and magnitude of their ventilatory and acid–base responses to prolonged hypercapnia. In fact, the responses exhibited by pekin ducks appear to be unequalled by any other vertebrate species studied to date.

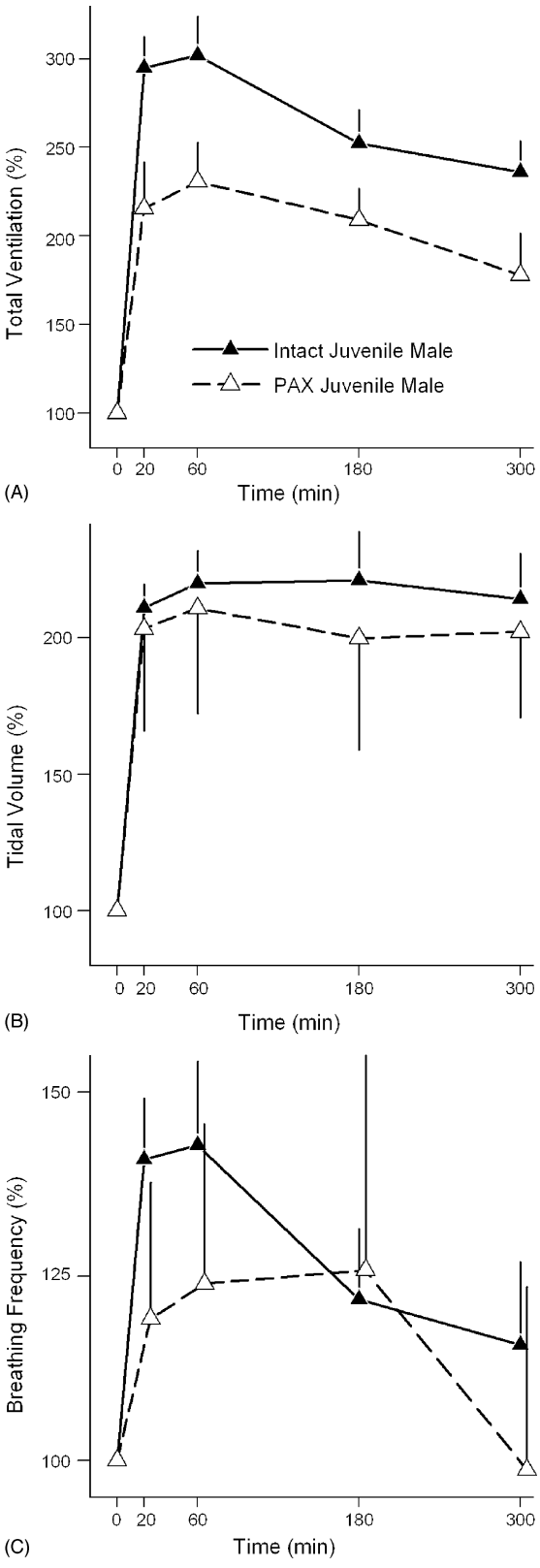

Fig. 6. The relative changes in total ventilation (A), tidal volume (B), and breathing frequency (C) in intact juvenile male (black triangles) and pulmonary afferent denervated (PAX) juvenile male (open triangles) pekin ducks before (0 min) and after a step increase in inspired CO_2 from 0 to 5%. Data are expressed relative (%) to levels before CO_2 administration as mean ± S.E. In some cases, data are offset for clarity. Absolute data are shown in Table 1.

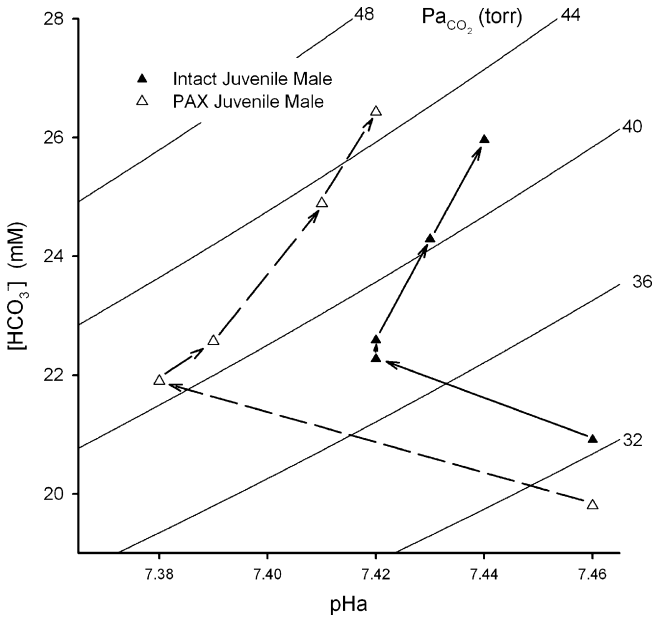

Fig. 7. Davenport diagram showing the effect of a step increase in inspired CO_2 (from 0 to 5%) on arterial pH, P_{CO_2}, and [HCO_3^-] in intact adult male (black circles) and pulmonary afferent denervated (PAX) juvenile male (open triangles) pekin ducks. Data means ± S.E. are shown in Table 1.

4.1. Ventilatory adjustments during prolonged hypercapnia in intact ducks

Hypercapnia (5% inspired CO_2) resulted in an immediate increase in all respiratory variables ($\dot{V}_E$, V_T, and f_R), regardless of age or gender. Similar ventilatory adjustments to acute CO_2 have been observed qualitatively numerous times in birds (e.g., Jones and Purves, 1970; Bouverot et al., 1974; Powell et al., 1978; Brackenbury et al., 1982), and are a response shared by mammals and many (but not all) reptiles (Milsom, 1998). Furthermore, the acute change in ventilation of ducks in this study upon exposure to hypercapnia was largely due to an increase in tidal volume (~2-fold), and to a lesser extent due to an increase in breathing frequency (~1.4-fold), as has been observed several times previously (e.g., Bouverot et al., 1974; Powell et al., 1978; Milsom et al., 1981; Brackenbury et al., 1982; Dodd and Milsom, 1987). Breathing frequency has actually been observed to decrease during hypercapnia in some situations (Jones and Purves, 1970; Bouverot and Leitner, 1972; Osborne et al., 1977; Colby et al., 1987), possibly due to the effects of CO_2 on intrapulmonary chemoreceptors (Milsom et al., 1981).

Whereas the acute responses of adult males, juvenile males, and adult females were qualitatively similar, some notable quantitative differences existed. In particular, the relative magnitude of the acute response of adult females was lower than that of adult and juvenile males, due to both smaller increases in V_T and f_R. Although the reasons for this are unclear, the mechanisms controlling ventilation and acid–base status in male and female ducks may be quite different (discussed further below).

After the initial acute increase in respiratory variables, continued exposure to 5% CO_2 resulted in a progressive decline of $\dot{V}_E$ in both adult male and juvenile male ducks. This reduction was entirely due to a decline in breathing frequency, such that over the relatively short duration of this experiment (300 min) f_R returned to pre-hypercapnic levels. These results support previous findings in adult male ducks (Dodd and Milsom, 1987), which demonstrated the remarkably rapid respiratory acclimation of male ducks during prolonged hypercapnia. In contrast, respiratory acclimation in humans and other mammals occurs over a period of days (Schaefer et al., 1963; Lai et al., 1981) if it happens at all (Kondo et al., 2000; Crosby et al., 2003). Interestingly, prolonged hypercapnia in adult female ducks did not result in a decline in respiration, at least over the time course of this study. The possible mechanistic basis for these differences is discussed in the next section.

4.2. Regulation of ventilation by P_{CO_2} and pH

As well as there being an immediate increase in all respiratory variables in response to hypercapnia, a significant respiratory acidosis occurred in all ducks after 20 min of breathing 5% CO_2, as previously observed by others (e.g., Jones and Purves, 1970; Bouverot et al., 1974; Milsom et al., 1981; Dodd and Milsom, 1987). This is a common occurrence during hypercapnia, and is observed in other birds (Kilgore et al., 1994), humans (Crosby et al., 2003), and other mammals (Lai et al., 1981), reptiles (Silver and Jackson, 1985; 1986), and amphibians (Kinkead and Milsom, 1994). In most air-breathers, hypercapnia and/or acidosis of the blood is the primary cause of the hyperventilatory response to acute hypercarbia, although in birds and some reptiles airway CO_2 also influences this response.

After the initial hypercapnic-acidosis, there was a substantial pH recovery in adult and juvenile male birds, despite there being a continued and progressively increasing hypercapnia. A likely mechanism for this recovery was an increase in bicarbonate concentration, which increased progressively for the duration of hypercapnia (see also Dodd and Milsom, 1987; Bebout and Hempleman, 1999). The source of HCO_3^- is unclear, as there were no detectable changes in the strong ions in the blood (at least in adult males). However, because the calculated changes in bicarbonate levels were small (3–5 mM), equivalent changes in strong ion difference could have occurred that were small enough to go undetected. This possibility, as well as previous theoretical concerns about the role of strong ion difference in acid–base regulation (Cameron, 1989), make this aspect of our results difficult to justify. Regardless, our data contrast previous work in turtles, which increase blood [Ca^{2+}] and [Mg^{2+}] as well as [HCO_3^-] during hypercapnia, presumably because the source of bicarbonate is bone and/or shell (Silver and Jackson, 1986). In addition to bone, adult and juvenile male ducks may attempt to restore acid–base balance through renal and extra-renal mechanisms, as occurs in mammals (Tannen and Hamid, 1985) and reptiles (Schilb and Brodsky, 1966). Regardless the mechanisms involved, the speed of pH compensation in male pekin ducks is impressive, and exceeds that of many mammals (Lai et al., 1981; Nattie and Edwards, 1981), reptiles (Silver and Jackson, 1985; Glass and Heisler, 1986; Silver and Jackson, 1986), and amphibians (Boutilier et al., 1979; Boutilier and Heisler, 1988). Interestingly, pH recovery during chronic hypercapnia appears

to be faster in the cerebrospinal fluid than in the blood of dogs (Nattie and Edwards, 1981); it is unknown whether this is also the case in birds.

As discussed above, respiratory acclimation occurred during prolonged hypercapnia in adult and juvenile male ducks. This decrease in ventilation during continued hypercapnia could be the result of at least three different mechanisms (Lai et al., 1981; Dodd and Milsom, 1987): (i) a change in the sensitivity of chemoreceptors to CO_2/H^+; (ii) a change in the setpoint for P_{CO_2}/pH regulation by the chemoreceptors; or (iii) a decrease in stimulation of chemoreceptors by H^+ due to acid–base compensation. The first possibility is unlikely, because the *slope* of the $\dot{V}_E$ response curve to Pa_{CO_2} or pHa is unaltered when sequential step changes in inspired CO_2 are repeated several times (Dodd and Milsom, 1987) (CO_2 sensitivity is frequently assessed by the slope of the ventilation–P_{CO_2} curve; e.g., Woodin and Stephenson, 1998). Although the second possibility cannot be excluded entirely, what seems most likely is that the respiratory acclimation was caused by pH recovery. This is supported by the close relationship between the decrease in ventilation and the degree of acid–base compensation (Fig. 3B and F). Indeed, the pH recovery and respiratory adjustments (or lack thereof) are well correlated in the birds of this study and in other studies of birds (Dodd and Milsom, 1987; Bebout and Hempleman, 1999), mammals (Lai et al., 1981; Jennings and Davidson, 1984), and reptiles (Silver and Jackson, 1985). Interestingly, respiratory adjustments in the male ducks of this study occurred exclusively *via* reductions in breathing frequency, whereas in rats this may also involve reductions in tidal volume (Lai et al., 1981).

Further support for the role of acid–base compensation in respiratory acclimation during prolonged hypercapnia comes from the results from the adult female pekin ducks. Unlike their male counterparts, females did not experience respiratory acclimation and did not restore pH to nearly the same degree. It seems much more likely that females experience no respiratory adjustments because of an inability to raise pH levels than the alternative possibility, that females are *both* less effective than males at pH compensation and lack the ability to change the P_{CO_2} setpoint. Reasons for the observed gender differences are nevertheless curious. Calcium carbonate in egg shells is known to come from stores of calcium and bicarbonate in the blood and/or skeleton (Hunt and Simkiss, 1967; Mongin, 1968), so shell formation results in a temporary metabolic acidosis, as well as elevated H^+ secretion and HCO_3^- reabsorption at the kidneys (Mongin, 1968; Hodges, 1970; Prashad and Edwards, 1973). Females in the laying cycle may therefore be less able to regulate pH during hypercapnia because HCO_3^- stores have already been mobilized to make $CaCO_3$ for eggshells, and because acid and base transport by the kidneys is already elevated. Pekin ducks are not seasonally laying birds, but instead lay eggs year round, and unfortunately no attempt was made to establish laying cycles for the birds used in this experiment.

It is interesting to note that while the changes in f_R were tightly linked to changes in pHa (independent of changes in Pa_{CO_2}), the changes in V_T appeared to arise due to changes in both pHa and Pa_{CO_2}. During prolonged hypercarbia, when Pa_{CO_2} was progressively rising while changes in pHa were slowly being buffered, V_T remained constant.

4.3. Carotid body chemoreceptor control of ventilatory adjustments

Peripheral arterial (e.g., carotid body) chemoreceptors appear to be present in all terrestrial vertebrates (Milsom, 2002), and are believed to be sensitive to changes in both arterial P_{CO_2} and arterial pH (Summers et al., 2002). This receptor group is typically considered to be important for the immediate ventilatory responses to changes in ambient or blood gas levels, but may also be important for regulating breathing at rest (Smatresk, 1990; Milsom, 1998). In this study, denervation of the carotid bodies appeared to reduce total ventilation by 15% when breathing control gas (0% CO_2), as observed previously (Bouverot et al., 1974). The carotid bodies are known to discharge even under normoxic-normocapnic conditions, which also supports a role for this chemoreceptor group in resting ventilation.

Carotid body denervation did not appear to significantly alter the acute ventilatory response to CO_2 exposure (20 min), and had remarkably little effect on the changes in blood acid–base values. Similar results in ducks were observed by Jones and Purves (1970), who noted that carotid body denervation only influenced the rate of the initial ventilatory response, on a scale of seconds after the onset of hypercapnia. In contrast, Bouverot et al. (1974) observed a substantial reduction in the ventilatory response of ducks to hypercapnia after denervating the carotid bodies. In the present study, the relative increase in total ventilation after 20 min of hypercapnia was reduced (though not statistically significant) approximately 15% by carotid body denervation, which was caused entirely by a reduction in breathing frequency. Similar variability among studies on the effects of carotid body denervation in birds also exists among studies in mammals (Berkenbosch et al., 1979; Heeringa et al., 1979; O'Regan and Majcherczyk, 1982).

Carotid body denervation also had no influence on the respiratory adjustments or pH recovery during prolonged hypercapnia. From 20 to 300 min after the onset of CO_2 exposure, all respiratory variables ($\dot{V}_E$, V_T, and f_R) responded in the same manner, regardless of whether or not the carotid bodies were intact. Furthermore, pH recovered to the same level in intact and denervated ducks, and CBX ducks increased blood bicarbonate levels to a greater extent than intact ducks. A decreased stimulation of carotid body chemoreceptors, as pH recovers throughout hypercapnia, therefore does not contribute to the respiratory acclimation to CO_2 in birds.

4.4. Intrapulmonary chemoreceptor control of ventilatory adjustments

The IPC are believed to have an analogous function in birds to that of the mammalian pulmonary stretch receptors, which are involved in terminating the inspiratory cycle (Hempleman and Posner, 2004; Milsom et al., 2004). IPC firing rate is inversely proportional to inspired P_{CO_2}, unlike most CO_2-sensitive chemoreceptors, and activity by IPC afferents is known

to inhibit breathing (Peterson and Fedde, 1968; Scheid et al., 1978). IPCs are therefore involved in the breath-by-breath regulation of CO_2 exchange under both resting conditions and during hypercapnia. Denervating the IPCs in this study appeared to increase overall total ventilation by 25%, probably because tonic inhibitory afferent activity from these receptors was eliminated. An additional mechanism through which the IPCs regulate CO_2 exchange is likely through the control of breathing pattern (Hempleman and Posner, 2004; Milsom et al., 2004); indeed, in this study IPC denervation also altered breathing pattern, such that breaths became deeper and slower.

Denervation of afferents from intrapulmonary chemoreceptors reduced the relative increase in ventilation in response to hypercapnia by 30%, and resulted in a more substantial hypercapnia and acidosis of the blood. Intrapulmonary chemoreceptors have been suggested to play a role in the respiratory response to elevated inspired CO_2 (Powell et al., 1978; Scheid et al., 1978). Therefore, in intact ducks the acute respiratory response to hypercapnia would be mediated by an increased stimulation from central and arterial chemoreceptors, and a decrease in the inhibitory influence from IPCs. In pulmonary afferent denervated ducks, however, chemoreceptor drive to breath would likely be the same in hypercapnia as in intact ducks: each would receive stimulation from central and arterial chemoreceptors, and neither would receive inhibition from IPCs. In this study, absolute $\dot{V}_E$ was the same during hypercapnia in intact and IPC denervated ducks. The reduction caused by IPC denervation in the relative increase in ventilation immediately after the onset of hypercapnia was therefore largely due to the effect of denervation on resting ventilation. This conclusion must be taken with slight caution though, because our IPC denervation procedure required the removal of afferent activity from one carotid body (see Section 2). Considering the small effect that bilateral carotid body denervation had on the ventilatory response to CO_2, however, the effects of this were likely minimal.

As was the case for carotid body denervation, IPC denervation did not appear to influence respiratory adjustments or pH recovery during prolonged hypercapnia. $\dot{V}_E$ and V_T responded in the same manner between 20 and 300 min of CO_2 exposure in intact and IPC-denervated ducks. Reductions in f_R likely caused the decline of $\dot{V}_E$ over time in both groups, but this suggestion must be taken with some caution (see Section 3). Although pH did not recover to the same level in the denervated ducks as in intact ducks, the increase in pH and blood bicarbonate levels between 20 and 300 min was greater in denervated ducks than intact ducks. Reduced stimulation of intrapulmonary chemoreceptors therefore does not appear to contribute to the respiratory adjustments to prolonged CO_2 exposure.

4.5. Central chemoreceptor control of ventilatory adjustments

The results of the present study are consistent with the hypothesis that central chemoreceptors provide the majority of ventilatory drive during hypercapnia, and mediate the respiratory adjustments to prolonged CO_2 exposure (due to a decrease in stimulation as pH recovery occurs). Unfortunately, because both receptor groups could not be successfully removed together, this conclusion depends on three assumptions: (i) the carotid body and intrapulmonary chemoreceptors contribute to the control of ventilation in an additive rather than an interactive fashion; (ii) there is little or no redundancy between carotid body and pulmonary receptors in their effects on ventilatory control, so that removal of one receptor group did not simply shift its contribution to another group; and (iii) that no other peripheral chemoreceptors exist in pekin ducks other than the carotid body and intrapulmonary chemoreceptors.

Central chemoreceptors have previously been suggested to contribute the majority (60–80%) of the increased ventilatory drive during hypercapnia (Milsom et al., 1981). The exact location of these receptors remain unknown in birds, but because of similarities in the chemical control of respiration between birds and mammals, central chemoreceptors in birds are thought to be in a similar location to those in mammals (Bouverot, 1978). Chemoreceptive neurons are spread among numerous brain stem regions (Nattie, 1999; Lahiri and Forster, 2003; Putnam et al., 2004). The arterial pH recovery observed in our study probably influenced signalling by chemosensitive neurons by altering cerebrospinal fluid (CSF) pH and intracellular pH of the neurons, but the relationship may not be direct as CSF and arterial pH are regulated differently (e.g., Nattie and Edwards, 1981).

4.6. Time domains of the hypercapnic ventilatory response

In contrast to the hypoxic ventilatory response (HxVR) (Powell et al., 1998), the time domains of the hypercapnic ventilatory response (HcVR) are relatively poorly understood. Hypercapnia causes a post-hypercapnic frequency decline (PHcFD), which may be mediated by both the carotid body and central mechanisms (Coles et al., 2002; Day and Wilson, 2005). If PHcFD is analogous to post-hypoxic frequency decline (PHxFD), then PHcFD would be equivalent to short term depression (STD). Whether other time domains exist during hypercapnia is unclear; it has been argued that prolonged hypercapnia does not induce changes in carotid body chemosensitivity, in contrast to long-term hypoxia, which increases carotid body O_2-sensitivity (Bisgard et al., 1986), but little is known of its mechanism. The time domains after CO_2 exposure ends are better understood. For example, continuous and intermittent hypercapnia have been shown to induce long-term depression of respiratory activity after CO_2 exposure ends, regulated primarily by noradrenergic influences (Bach and Mitchell, 1998; Baker et al., 2001; Kinkead et al., 2001). PHcFD also occurs after CO_2 exposure, as described above. Regardless, several aspects of the HcVR are still unexplored, so a more complete analysis of all the time domains of this response is unavailable.

If we apply the same logic to an analysis of the time domains of the HcVR as applied to the HxVR (Powell et al., 1998), the ventilatory adjustments during prolonged hypercapnia in ducks are likely caused by several different mechanisms. It is possible that the ventilatory adjustments in this study are a form of STD. STD, however, is generally considered to occur within only a few minutes of the onset of CO_2 exposure, which is inconsistent with the time course of the ventilatory adjustments in

this study. Our observations could instead be considered hypercapnic ventilatory decline (HcVD), which occurs after several minutes to hours. Unlike STD, however, hypoxic ventilatory decline (HxVD) reduces tidal volume and not breathing frequency. Both hypoxic STD and HxVD are mediated centrally, consistent with our findings on hypercapnic ventilatory acclimation; nonetheless, STD and HxVD generally occur without secondary changes in CO_2/pH (Powell et al., 1998), so it may be incorrect to consider our observations to be the CO_2 equivalent of either of these phenomena. In this regard, the ventilatory response to CO_2 may decline over time in the absence of pH compensation (Makeham et al., 2004) and this situation may be more accurately termed STD or HcVD.

Because the ventilatory adjustments in this study appeared to be entirely due to pH compensation, we feel they are more appropriately considered hypercapnic ventilatory roll off. Similar ventilatory adjustments occur in birds, mammals, and reptiles during prolonged hypercapnia, which are well correlated with the magnitude of pH recovery (Lai et al., 1981; Jennings and Davidson, 1984; Silver and Jackson, 1985; Dodd and Milsom, 1987). That ventilation in this study and others (Dodd and Milsom, 1987) are so well correlated to pH, and that pH sensitivity does not change over time (e.g., Fig. 3), further suggests that the mechanism of hypercapnic ventilatory roll off is pH recovery (and not plasticity or modulation of central respiratory control). The present study advances our knowledge of this phenomenon, suggesting that central chemoreceptors are the site where the pH compensation is detected, and consequently that central mechanisms are responsible for hypercapnic ventilatory roll off.

4.7. Conclusions

The data in this study support our earlier work (Dodd and Milsom, 1987) suggesting that ventilatory and acid–base responses to prolonged hypercapnia are extremely rapid in male pekin ducks. After an immediate rise in ventilation after the onset of CO_2 exposure, ventilation falls progressively, in parallel with a rapid pH recovery. Although females differ from males in this regard, the responses exhibited by males may be unequalled by other vertebrates. The likely cause of the respiratory adjustments during prolonged hypercapnia is an effect of pH recovery on central chemoreceptors, because carotid body or intrapulmonary chemoreceptor denervation had no effect on this response. By analogy to the time domains of the hypoxic ventilatory response, we suggest that the ventilatory adjustments during prolonged CO_2 in this study be considered hypercapnic ventilatory roll off.

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