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Original Communication

Effects of hypoxia and hypercapnia on neopulmonic and paleomonic pulmonary blood flow in *Gallus domesticus*

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ABSTRACT

Changes in the O_2 and CO_2 content of respired gases can alter regional pulmonary blood flow distribution in the mammalian lung. It is known that alterations in respiratory gases can cause a non-homogenous redistribution of pulmonary blood flow between the paleopulmo (PALEO) and neopulmo (NEO) of the avian lung; however the effect of alterations in respired gas content on the distribution of pulmonary blood flow in birds, such as the chicken, which possess a highly developed NEO is not known. This study utilized a colorimetric microsphere method to determine the effects of hypoxia and hypercapnia on the relative distribution of pulmonary blood flow in anesthetized chickens (*Gallus domesticus*) during control (normoxic) and experimental (hypoxic or hypercapnic) conditions where the relative regional distribution of blood flow in the lung is expressed as the ratio NEO/ PALEO. Administration of a hypoxic gas mixture (16.0% O_2) produced a 13.4% increase in NEO/ PALEO, and, administration of a hypercapnic gas mixture (5.0% CO_2) resulted in a 27.8% increase in NEO/PALEO. NEO/PALEO was not changed in normoxic sham controls. Our results are consistent with a mechanism in which the regional redistribution of pulmonary blood flow is mediated by local intrapulmonary factors.

KEYWORDS: avian pulmonary circulation, lung ventilation perfusion, avian lung, pulmonary blood flow, microspheres

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INTRODUCTION

The avian lung is the most efficient gas-exchanger among air-breathing vertebrates and many structural and physiological features of the avian pulmonary system have been described which facilitate this phenomenon. The anatomy of the avian respiratory system has been extensively reviewed [1, 2, 3]. Ventilation in the avian respiratory system occurs via of a series of bellows-like air sacs which are connected to large bronchi which in turn give rise to smaller parabronchi which form the gas-exchanging units of the avian lung. It is well accepted that ventilatory gas flow is unidirectional during both phases of respiration in that portion of the avian lung in which the parabronchi are arranged in parallel, the *paleopulmo*. In most avian species the paleopulmo has been supplemented by the *neopulmo* [2], an additional network of anastomosed parabronchi which arises from the caudal primary bronchial system and through which ventilatory gas flow is bidirectional with air flowing through the neopulmonic parabrochi into the caudal air sacs during inspiration and in the reverse direction during expiration. The neopulmo is most extensively developed in song birds and chickens.

It is well established in mammals that respired gases, particularly regional hypoxia, can alter regional pulmonary blood flow [4, 5]. Studies in the duck (*Cairina moschata*) and ostrich (*Struthio camelus*) have shown that regional pulmonary blood flow distribution not only can be changed by alterations in local blood gas content, but that redistribution of pulmonary blood flow is nonhomogenous with respect to the paleopulmo

and neopulmo [6, 7]. The effect of alterations in respiratory gases on the regional distribution of pulmonary blood flow in birds possessing a highly developed neopulmo, such as the chicken, is not known. In the present study we utilized an established colorimetric microsphere technique to examine the effects of hypoxia and hypercapnia on the distribution of pulmonary blood flow in the paleopulmo and neopulmo of the lung of the chicken (*Gallus domesticus*).

MATERIALS AND METHODS

General experimental procedures

All studies utilized White Leghorn cocks (n = 32) with an average body weight of 2.4 kg (range 1.85-3.30 kg). All experiments were conducted under anesthesia according to an approved protocol of the University of California, Davis (Protocol for Animal Use and Care no. 15524) and were terminal. Animals were manually restrained and anesthetized with sodium pentobarbital (30 mg/kg, i.v.) through a brachial vein cannulated under local anesthesia (Lidocaine 1%). A brachial artery was cannulated for the measurement of systemic arterial blood pressure (Psa, Bentley Trantec). Arterial blood samples (0.3 ml) were drawn from this catheter 3 min prior the injection of control and experimental microspheres for the determination of PaO_2 and $PaCO_2$ (Radiometer BMSlll Mk2). Body temperature was monitored and maintained at 41ºC with an infrared heat lamp and a heated pad. During preparatory surgery animals were kept in a supine position. Animals breathed spontaneously through a cuffed endotracheal tube fitted with a one- way expiratory valve and were ventilated with appropriate gas mixtures delivered by a gas-mixing flow meter (Cameron Instruments GF- 4).

Experimental protocol

Regional distribution of pulmonary blood flow in both left and right lungs was determined with a non-radioactive, colored microsphere technique according to established procedures [8, 9]. Animals in each of three groups were initially ventilated with a normoxic gas mixture of 20.9% O_2 and 79.1% N_2 during a 10 min baseline period which was followed immediately by the i.v. injection of a sonicated 0.3 ml suspension containing approximately 300,000 control (yellow) microspheres (Triton Dye-Trak, 15 µm diameter) in avian ringer solution. The injection catheter was flushed with 1.0 ml of avian ringer. After a 20 min equilibration period, Group l animals (*Hypoxic*, n =14) were switched to an inspired gas mixture of 16.0% O_2 and 84.0% N_2; Group II animals (*Hypercapnic*, n = 10) were switched to an inspired gas mixture of 20.9% O_2, 5.0% CO_2, and 74.1% N_2, and Group III animals (*Sham control*, n = 8) remained on the initial normoxic inspiratory gas mixture. Immediately following a 10 min baseline period, a 0.3 ml suspension of approximately 300,000 experimental (red, eosin) microspheres was administered as described above. After a 20 min equilibration period, the animals were euthanized via anesthetic overdose, the chest was immediately opened via median sternotomy, and both the right and left lungs were carefully removed from the thorax for subsequent tissue processing.

Tissue processing and microsphere recovery

Using the first mediodorsal secondary bronchus and the caudal half of the primary bronchus as markers, the neopulmo was carefully dissected ventrolaterally from the paleopulmo. Neopulmonic and paleopulmonic tissues were processed separately for microsphere recovery, quantification of dye content, and determination of relative regional pulmonary blood flow utilizing a procedure adapted from Hakkinen *et al.* 1995. Briefly, the right and left neoplumo and paleopulmo were cut into 2 to 4 sections and from these smaller tissue samples (0.5 g to 1.5 g) were randomly obtained and processed in duplicate. Each small tissue sample was placed in a tared 15 ml polypropylene centrifuge tube and the wet tissue weight was determined. To each tube 6 ml of a 1.0 M KOH alkaline digestion reagent was added and the tissues were digested in the capped tubes overnight in a 60ºC oven. The tubes were vented and vortexed for 30 sec and returned to the oven for 60 min periods as necessary to thoroughly homogenize the tissue. The tubes were removed from the oven, 50ºC distilled H_2O was added, and after centrifugation for 15 min at 2500 rpm, the supernatant was aspirated from the precipitated pellet. The tissue pellet was first suspended in 10% Triton X-100 reagent (10 ml, 50ºC), sonicated, centrifuged for 5 min at 2500 rpm, and

the supernatant was aspirated. This process was repeated twice using acidified ethanol as the wash reagent. The microsphere containing sample pellets were dried by evaporation at room temperature overnight. The colored dye was eluted from the microspheres by the addition of 850 µl of DMF (N,N- Dimethylformide). The tubes were vortexed, allowed to stand for 15 min, vortexed again, and centrifuged for 5 min at 2500 rpm. The supernatant containing the extracted dye was analyzed by absorbance spectrophotometry (Zeiss PM6) using 200 µl microcuvettes at the absorption spectrum peaks of the yellow (448 nm) and red (535 nm) dyes, respectively. In this study the distribution of regional pulmonary blood flow to the neoplumo (NEO) and paleopulmo (PALEO) was measured as the absorbance/g tissue weight of yellow (control) or red (experimental) dyes in neopulmonic and paleopulmonic tissue samples for control (normoxic) and experimental (hypoxic or hypercapnic) conditions respectively and is expressed as the ratio NEO/PALEO.

RESULTS

As shown in Figure 1 administration of the hypoxic gas mixture containing 16.0% O_2 to Group I animals produced a significant increase in the distribution of blood flow to the neopulmo relative to the paleopulmo. In this group NEO/PALEO = 0.833 ± 0.06 (mean ± SEM) during the normoxic control period and increased to 0.945 ± 0.07 ($P \leq 0.05$) during the hypoxic period representing a 13.4% increase in the relative distribution of pulmonary blood flow to the neopulmo. In Group ll animals NEO/PALEO = 0.931 ± 0.07 during the normoxic control period and increased significantly to 1.190 ± 0.09 ($P \leq 0.05$) during the hypercapnic period produced by the administration of 5.0% CO_2 representing a 27.8% increase in the relative distribution of pulmonary blood flow to the neopulmo. The relative distribution of pulmonary blood flow did not significantly change in Group lll sham control animals with NEO/PULMO = 0.877 ± 0.09 during the normoxic control period

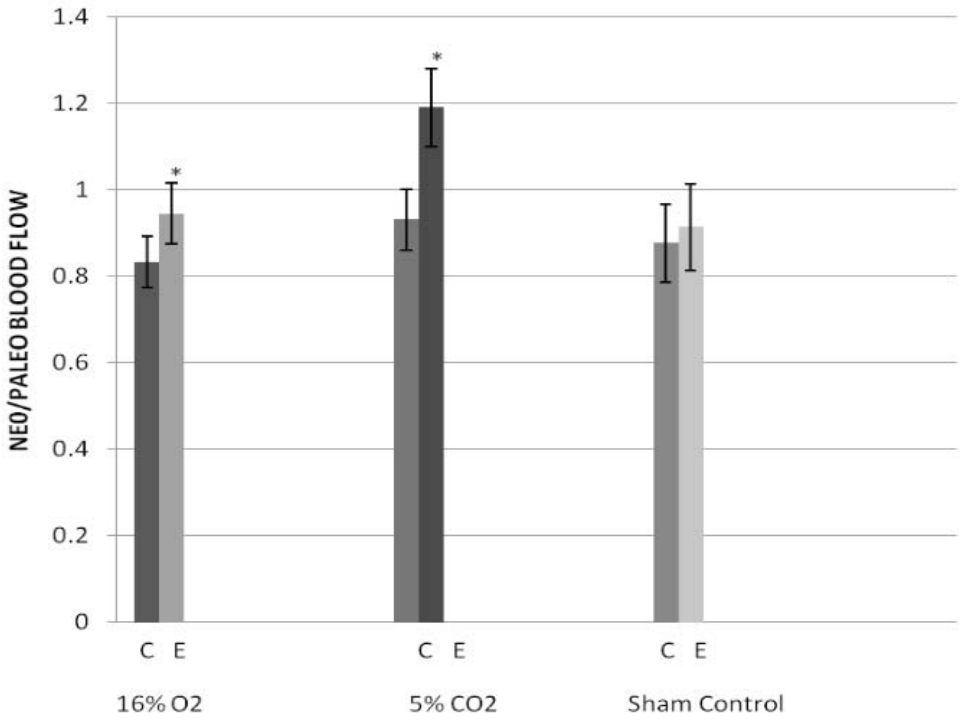

Figure 1. Effects of hypoxia (16% O_2) and hypercapnia (5% CO_2) on the relative regional distribution of pulmonary blood flow (NEO/PALEO) in experimental and normoxic sham control chickens during control (C) or experimental (E) periods. *P < 0.05 compared to control period.

and 0.914 ± 0.10 during the subsequent normoxic sham experimental period.

PaO_2, $PaCO_2$, and mean Psa were not significantly changed by the administration of either the hypoxic or hypercapnic gas mixtures, nor did these parameters change significantly in sham control animals. In Group l PaO2 = 90.2 ± 2.1 mmHg during the normoxic control period and 86.5 ± 3.1 mmHg following the administration of the experimental hypoxic gas mixture; control PaCO2 = 36.2 ± 1.7 mmHg and 34.4 ± 2.2 mmHg during the hypoxic period; and control Psa = 93.5 ± 2.9 mmHg and 90.1 ± 3.4 mmHg during the hypoxic period. Group ll PaO_2 = 92.2 ± 2.4 mmHg during the normoxic control period and 87.2 ± 3.0 mmHg following the administration of the hypercapnic gas mixture; control $PaCO_2$ = 36.6 ± 2.0 mmHg and 37.4 ± 2.5 mmHg during the hypercapic period, and control Psa = 89.8 ± 2.7 mmHg and 85.6 ± 3.8 mmHg during the hypercapnic period. Group lll PaO2 = 90.7 ± 2.3 mmHg during the normoxic control period and 86.0 ± 2.8 mmHg during the normoxic sham experimental period; PaCO2 = 35.8 ± 2.1 mmHg and 34.7 ± 2.6 mmHg and Psa = 92.4 ± 3.2 mmHg and 87.3 ± 4.1 mmHg for the same periods respectively. Moderate hyperpnea was observed following administration of hypoxic and hypercapnic gas mixtures.

DISCUSSION

It is well established that reflex and local mechanisms act in the mammalian lung to match pulmonary ventilation and perfusion. Among these mechanisms alterations in the O_2 and CO_2 content of respired gases are known to effect the regional distribution of mammalian pulmonary blood flow [4, 5]. In the avian lung, mechanisms which operate to match ventilation and perfusion have been less studied than in mammals, and many existing studies on the distribution of pulmonary blood flow in the avian lung have considered intraparabronchial blood flow distribution [6, 10, 11] rather than the effects of respired gases on regional distribution of pulmonary blood flow, which could have the more profound effect on ventilation and perfusion matching. Several studies suggest that hypoxia may influence the distribution of avian pulmonary blood flow [6, 7, 11, 12]. Holle *et al.* (1978) used a microsphere technique to examine the effects of respired gases on regional lung perfusion in spontaneously breathing anesthetized ducks (*Cairina moschata*). Their data show that hypoxia decreased both PALEO and NEO blood flow, with the effect being more pronounced in the NEO, and that hypercapnia had little effect on PALEO blood flow, but resulted in an increase in NEO blood flow. Jones (1982) used a microsphere technique to measure the regional distribution of pulmonary blood flow in the lungs of unanesthetized ostriches (*Struthio camelus*) during heat induced panting. While blood gases were not measured in this study, the results showed that the distribution of NEO blood flow fell relative to PALEO blood flow during panting.

Corresponding data on the regional distribution of pulmonary blood flow in the chicken have not been reported. In the present study, the NEO was relatively underperfused compared to the PALEO during normoxic control periods (NEO/PULMO < 1.0). This finding is similar to the observations of Song *et al.* (1978) who, using a microsphere technique in geese, reported lower perfusion of the NEO during normoxic control conditions, but differs from the observations reported by Jones (1982), who found that the NEO was better perfused than the PALEO during resting conditions in spontaneously breathing ostriches, and by Holle *et al.* (1978) and Scheid and Holle (1978), who found a greater distribution of blood flow to the NEO than to the PALEO in ducks during control ventilation with a gas mixture containing 17% O_2. In the present study, administration of both the hypoxic and the hypercapnic gas mixtures resulted in a significant increase in the distribution of pulmonary blood flow to the NEO relative to the PALEO (NEO/PALEO > 1.0). Our results differ from those of Holle *et al.* (1978) whose data show that while perfusion of the NEO was increased by hypercapnia, perfusion of the NEO fell as a result of hypoxia. Jones (1982) observed decreased perfusion of the NEO relative to the PALEO in response to panting which he attributed to local hypoxia. In the present study PaO_2 and $PaCO_2$ did not change significantly during either hypoxia or hypercapnia, which suggests that the observed redistribution of pulmonary blood flow was mediated by local mechanisms arising in the airways. Such a mechanism is supported by

the data of Holle *et al.* (1978) and Scheid and Holle (1978) and was suggested by King *et al.* (1978) to involve changes in vascular resistance in interparabronchial arterioles.

The differing methodologies and species used may in part explain the differences between our observations and those of the other studies sited, however compared to other birds, the domestic fowl is an outlier with respect to pulmonary function in many respects. Maina *et al.* (1989) in a seminal allometric study of lung morphometric parameters in birds, found the domestic fowl to be "consistently atypical" of the many other species studied, particularly with regard to those parameters which determine pulmonary diffusing capacity. However, as Maina *et al.* (1989) pointed out, the domestic fowl is the single species from which most of our understanding of the avian lung is based because it is the species in which most aspects of pulmonary physiology and anatomy have been thoroughly studied. It is interesting to note that, despite its sheltered and non- flying mode of life, the chicken belongs to that group of birds having the most highly developed NEO. Perhaps in the domestic fowl a highly developed NEO has been selected to compensate for low pulmonary diffusing capacity and is an important component of the matching of ventilation to perfusion, particularly during periods of physical stress or increased activity.

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