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BLOOD GASES AND RESPIRATORY PATTERN IN EXERCISING FOWL: COMPARISON IN NORMOXIC AND HYPOXIC CONDITIONS

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SUMMARY

1. Clavicular air sac pressure, arterial blood gases and pH, and rectal temperature were measured in treadmill-exercised cockerels breathing air, 10% O_2 in N_2 or a mixture of 10% O_2/3% CO_2 in N_2. Air sac pressures were used to estimate changes in the rate and the relative depth of breathing.

2. In air-breathing conditions exercise took place at two intensities corresponding to treadmill speeds of 3·2 and 5·0 km h^{-1}, respectively. Rectal temperature increased by 0·5°C but there was no sign of thermal hyperventilation and arterial P_{CO_2} remained constant. Increased ventilation was mainly brought about by changes in respiratory rate, with relatively small increases in depth.

3. During exercise at 3·2 km h^{-1} inhalation of 10% O_2 in N_2 produced a 35% increase in ventilation and breathing became faster and shallower. Arterial P_{CO_2} fell by 3–4 Torr, apparently as a result of lung hyperventilation. Addition of 3% CO_2 to the hypoxic gas restored normal arterial P_{CO_2} and reversed the trend to polypneic breathing. However, it failed to produce an exact matching of respiratory characteristics with those observed during isocapnic exercise hyperpnea.

4. It is concluded that rapid, shallow breathing during hypocapnic hypoxia in running birds serves as a mechanism to minimize lung hyperventilation and CO_2 washout. This reflex, which may stem from the intrapulmonary CO_2 receptors, occurs in the face of a severe hypoxic challenge. Failure to match respiratory characteristics during isocapnic hypoxia and isocapnic exercise may be due to an inibitory effect of the inhaled CO_2 on these receptors.

INTRODUCTION

Recent studies on the respiratory responses of ducks (Kiley, Kuhlmann & Fedde, 1979, 1982) and domestic fowl (Brackenbury, Gleeson & Avery, 1982; Brackenbury & Gleeson, 1983, 1986) exercised on treadmills have provided information about factors that govern changes in the rate and depth of breathing during spontaneous hyperpnea. It is clear that the respiratory rhythm generator is highly sensitive to

Key words: birds, respiration, exercise.

variations in intrapulmonary and arterial P_{CO_2}. Experiments on anaesthetized birds have demonstrated the importance of specifically CO_2-sensitive intrapulmonary chemoreceptors (IPCs) in the regulation of breathing pattern (Miller & Kunz, 1977; Tallman & Kunz, 1982; Tallman & Grodins, 1982) and the results from the exercise studies support this role. In chickens it has been suggested that the IPCs regulate the depth of breathing in order to match lung ventilation to CO_2 flux across the respiratory epithelium, thereby preserving normal arterial P_{CO_2} (Gleeson, Haigh, Molony & Anderson, 1985*b*). When, as during thermal hyperventilation, minute ventilation rises in excess of CO_2 production the development of a rapid, shallow breathing pattern, under the influence of the IPCs, ensures that most of the excess ventilation is shunted away from the parabronchi into the anatomical dead space.

When chickens are subjected to a range of exercise loads of light to moderate intensity, lung ventilation is precisely matched to increased CO_2 production and P_{CO_2} remains constant (Brackenbury & Gleeson, 1983). This happens only if rises in body temperature are kept to a minimum and in these circumstances it has been shown that increased ventilatory demand is met by coupled increased in tidal volume and respiratory rate, with the emphasis strongly on the latter variable. Exercise, however, also imposes an increased demand for oxygen as well as for the elimination of CO_2. In normal air-breathing conditions the systemic P_{CO_2} receptors do not appear to be involved in the control of ventilation during exercise hyperpnea in chickens (Brackenbury *et al.* 1982). However, if the birds were subjected to exercise in hypoxic conditions, the demand for lung ventilation would exceed the optimum required to maintain normal intrapulmonary P_{CO_2}. In order to investigate the way in which the respiratory rhythm generator would respond to these potentially competing demands, experiments were designed to measure relationships between respiratory pattern and arterial P_{CO_2} during exercise in normal and hypoxic conditions.

MATERIALS AND METHODS

Animal training and experimental methods

The experiments were carried out on six adult, male domestic fowl (Light Sussex breed, body mass 2·7–3·5 kg), which had been trained for several months to run on a treadmill at a maximum speed of 5·0 km h^{-1} for periods of 15–20 min. The birds were also trained to wear a loose-fitting plastic mask attached to a 1-m length of plastic tube (external diameter 0·5 cm) which was used to deliver either air or experimental gas mixtures into the mask. Before the experiments began each bird was anaesthetized with a 1:1 mixture of 30% Urethane and sodium pentobarbitone (60 mg ml^{-1}), administered intravenously, and cannulae were sewn into the clavicular air sac and a carotid artery. The free end of the arterial cannula was externalized just caudal to the outer ear and the cannula was flushed daily with heparinized saline. Experiments began 24–48 h after recovery from the anaesthetic.

Arterial blood pH, P_{CO_2} and P_{O_2} were measured using a Radiometer BMS MK2 blood-gas analyser thermostatted at 41·0 °C, close to resting rectal temperature (T_{re}).

The electrodes were calibrated with a precision gas supply and with precision buffers after each set of readings. The measured values were corrected to actual T_{re} using factors from Severinghaus (1965). The blood samples were drawn using a double syringe technique, one syringe being used to remove dead-space blood and saline from the cannula, the other to draw the sample. Approximately 1·5 ml of blood was drawn on each occasion and transferred immediately to the blood gas analyser. This process took less than 1 min.

Air sac pressure was monitored using a Grass PT5 manometer connected to a Grass Model 7D pen recorder. Air sac pressure was used to obtain an indication of respiratory airflow rate since there is a direct relationship between respiratory airflow and pressure changes within the lung–air sac system (Brackenbury, 1973) although this relationship is slightly non-linear. It had originally been intended to monitor airflow directly using the mask technique developed in previous studies using hens (Brackenbury *et al.* 1982; Brackenbury & Gleeson, 1983) but, as Gleeson, Barnas & Rautenberg (1985*a*) also found, the comb and wattles of the cockerel made it almost impossible to obtain an airtight seal around the mask. It was felt that the hood technique adopted by the latter authors would be unwieldy and would certainly interfere with normal respiratory movements, particularly during high-speed running. The method used to assess the rate and depth of breathing from the air sac pressure trace is shown in Fig. 1. The raw pressure trace was displayed directly and a second parallel output was led into a Grass 7P10 integrator which summated the pressure trace over both the inspiratory and the expiratory phases and automatically returned to baseline at the end of each minute. Since air sac pressure is proportional to airflow, the height of the integrator trace is proportional to the minute volume. Dividing the minute volume by the respiratory rate gave a value proportional to the tidal volume. Since airflow was not measured directly in these experiments, and in view of the slight non-linearity of the relationship between airflow and air sac pressure, no attempt was made to obtain absolute values for minute ventilation and tidal volume. However, the data can be used to obtain estimates of the relative values of these variables.

Paired sets of data were compared for significance of differences at the 5 % level using two statistical tests. First, a one-tailed *t*-test was used to compare blood gas/pH values in birds breathing air and 10 % O_2 in N_2, respectively, since it was expected that the hypoxic birds would also become hypocapnic. Second, in order to avoid type II errors in the comparison of data from birds breathing air and 10 % O_2/3 % CO_2 in N_2, respectively, a two-tailed Scheffé test was employed.

Experimental protocols

Series I

These experiments were all performed at a treadmill speed of 3·2 km h^{-1} (0·9 m s^{-1}). At the beginning of each experiment the bird was fitted with the mask, the air sac and arterial cannulae were connected and the animal was placed on the stationary treadmill. The mask was continuously ventilated with a measured stream of tanked

air (flow rate 10–15 l min^{-1}) in order to remove end-expired gas. The bird remained standing on the treadmill until the air sac pressure trace indicated steady breathing and the first blood sample was taken. The treadmill was then started and exercise took place for 6–8 min in air-breathing conditions. A steady-state ventilatory response to exercise was usually achieved within 4–5 min. A second blood sample was taken from the running animal before replacing the airstream with one of the experimental gas mixtures delivered to the mask at the same rate. Exercise continued for a further 4–5 min until a new steady-state ventilatory response had been achieved and a final blood sample was drawn just before the treadmill was stopped. Therefore a total of 4·5 ml of blood was drawn per experiment. This represented less than 2 % of the estimated blood volume of the animals and since only one experiment was performed on each animal during any single day, it was assumed that the sampling procedure had no significant effect on haematocrit. Two experimental gas mixtures were used: 10 % O_2 in N_2 and a mixture of 10 % O_2/3 % CO_2 in N_2. Preliminary experiments had indicated that during inhalation of the hypoxic gas alone the birds became hypocapnic. The addition of 3 % CO_2 to the second gas mixture was intended to forestall this drop in P_{CO_2} and permit ventilatory measurements to be made in normocapnic hypoxic conditions. T_{re} was measured with a mercury-in-glass thermometer before and immediately after exercise. This process took 1–2 min and it was assumed that the handling procedure had no significant effect on the measured temperature.

Series II

In these experiments the birds breathed only air and exercise took place at a speed of 5·0 km h^{-1} (1·4 m s^{-1}). Exercise lasted 6–8 min and blood was sampled before, and during the final seconds of exercise. Both series of experiments were performed at relatively low environmental temperatures (10–15 °C) in order to minimize any thermal response from the animals. Previous experiments had demonstrated that in such conditions lung ventilation was precisely matched to increased CO_2 production and arterial and intrapulmonary P_{CO_2} remained at their control values (Brackenbury & Gleeson, 1983). By measuring ventilatory characteristics in air at two exercise intensities it was therefore hoped to establish the graphical relationships between the rate and depth of breathing during isocapnic hyperpnea. This would then be used as a baseline against which to compare the ventilatory responses to hypoxia in isocapnic and hypocapnic conditions.

RESULTS

Ventilation and blood gases during normoxic exercise

T_{re} rose significantly ($P < 0·05$) during exercise from 41·8 ± 0·08 °C to 42·3 ± 0·06 °C. However, this rise produced no apparent effect on ventilation since arterial P_{CO_2} remained unchanged from resting at both exercise intensities (Table 1), suggesting that lung ventilation and CO_2 production were matched. In addition there was no indication of the rapid, shallow panting which has been observed in

Table 1. *Mean air sac pressure (P_{CS}) and blood gas values during rest and exercise*

		Exercise			
	Resting	(3·2 km h^{-1})	(5·0 km h^{-1})	(3·2 km h^{-1})	(3·2 km h^{-1})
Inhaled gas	air	air	air	10 % O_2	10 % O_2/3 % CO_2
Arterial P_{CO_2} (Torr)	31·1 ± 0·4	30·3 ± 0·6	31·9 ± 0·5	26·9 ± 0·7*	32·3 ± 0·5**
Arterial P_{O_2} (Torr)	93·2 ± 0·9	89·3 ± 1·6	94·1 ± 1·7	58·5 ± 1·7*	62·5 ± 2·4*
Arterial pH	7·511 ± 0·005	7·523 ± 0·007	7·478 ± 0·03	7·548 ± 0·01*	7·511 ± 0·02
P_{CS} (cmH_2O)	0·6 ± 0·04	2·6 ± 0·1*	4·2 ± 0·3*	3·6 ± 0·2*	4·7 ± 0·2*

Mean values ± S.E. Data from six birds.
* Significantly different from resting value ($P < 0·05$).
** Significantly different from exercise in air at 3·2 km h^{-1}.
1 cmH_2O = 98·1 Pa.

chickens exercised at much higher environmental temperatures (Brackenbury & Gleeson, 1983). Increased minute ventilation during exercise was achieved mainly by increasing the respiratory rate with relatively small effects on the depth of breathing (Figs 1, 2). Representative traces of air sac pressure changes are shown in Fig. 1. The small spikes shown on the trace were synchronized with leg movements and appeared to represent small air pulsations caused by impact of the legs on the body wall enclosing the lung–air sac system.

Effects of hypoxia

When the running birds inhaled 10 % O_2 in N_2, minute ventilation, as indicated by the mean air sac pressure, increased by approximately 35 %. This increase was due entirely to an increased respiratory rate and the tidal volume, given by mean air sac pressure divided by respiratory rate, was reduced in value (Fig. 2). As a result of these changes the ventilatory pattern during hypocapnic hypoxia was faster and shallower than would have been the case if the same increment in ventilation had been achieved by an increase in exercise intensity in isocapnic conditions. The ventilatory characteristics in the latter instance can be estimated from Fig. 2 by projecting the isoventilatory line which passes through the hypoxic/hypocapnic exercise point, towards the line connecting the isocapnic exercise data points. The point of intersection of these two lines then gives the required rate and depth values. Arterial P_{O_2} fell by approximately 30 Torr and the blood was slightly but significantly alkalotic compared with its control value at rest (Table 1). Inhalation of 10 % O_2/3 % CO_2 in N_2 brought about a similar drop in arterial P_{O_2} but this time arterial P_{CO_2} was held at its resting value. However, it was marginally but significantly higher than the arterial P_{CO_2} value measured in air-breathing conditions during exercise (Table 1). Ventilation increased by 80 % compared with its value in air-breathing conditions and the increase was mostly due to a rise in tidal volume coupled with a small rise in respiratory rate (Fig. 2). Thus the resultant ventilatory pattern during hypoxia was deeper and slower than would have been the case if the same increment

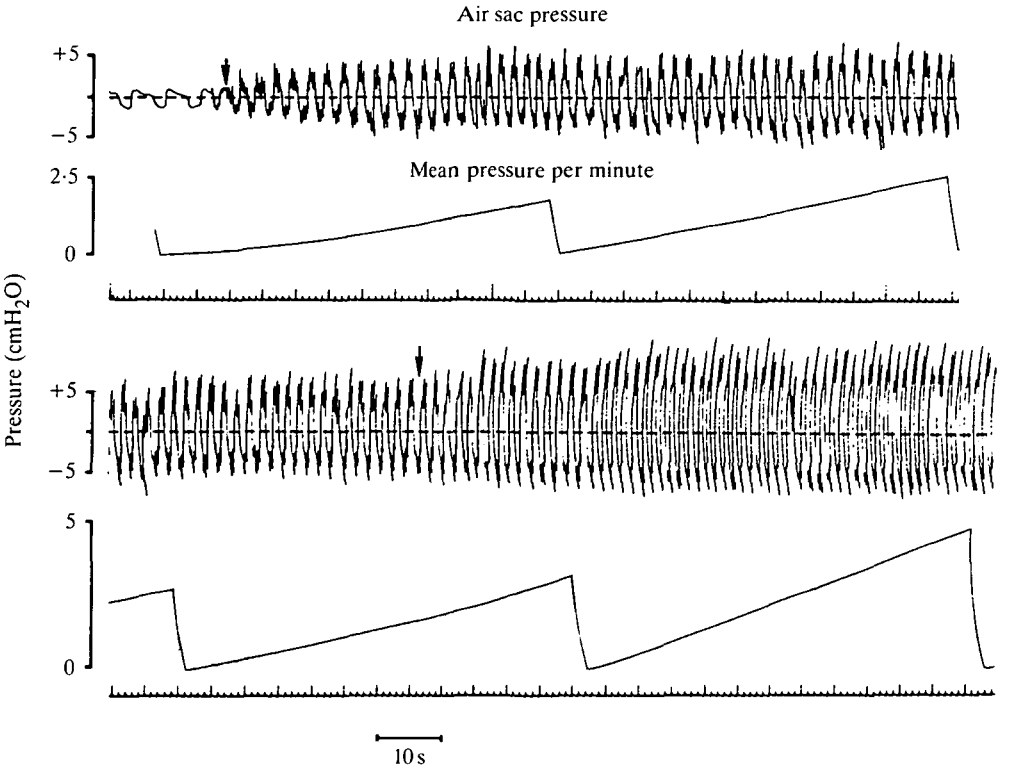

Fig. 1. Original recordings of air sac pressure during the same experimental run. Exercise began at the point marked by the first arrow. The second arrow marks the point at which the gas flowing into the mask was switched from air to 10 % O_2/3 % CO_2 in N_2. The upper trace in each section shows the raw signal, the second trace shows the integrated signal the height of which indicates the mean air pressure recorded during each minute. The spikes on the carrier trace are probably air pulses caused by the movements of the legs. 1 cmH_2O = 98·1 Pa.

in ventilation had been brought about by isocapnic exercise. Again the ventilatory characteristics in the latter instance can be estimated from Fig. 2 by projecting the isoventilatory line, which passes through the data point for inhalation of the 10 % O_2/3 % CO_2 gas mixture, towards the line connecting the isocapnic exercise data points.

DISCUSSION

Control of rate and depth of breathing

The effects of exercise on the rate and depth of breathing (Fig. 2) were qualitatively similar to those previously described in running hens (Brackenbury & Gleeson, 1983, 1986) and in running cockerels (Gleeson *et al.* 1985*a*), that is ventilation is increased by rises in both tidal volume and respiratory rate with the emphasis on the latter variable. These studies used mask techniques to measure

airflow directly and the agreement between them and the present study supports the use of air sac pressure as an indication of relative changes in ventilation. The relationships between minute ventilation, tidal volume and respiratory rate in exercising chickens are strongly dependent on arterial and intrapulmonary P_{CO_2}. Thermal hyperventilation can lead to a fall in P_{CO_2} which is compensated for by a shift towards a more rapid and shallow breathing pattern. In order, therefore, to establish the precise relationships between rate and depth of breathing during isocapnic hyperpnea it is necessary to minimize rises in body temperature. In the present experiments, although it was not possible to prevent a 0·5 °C rise in T_{re}

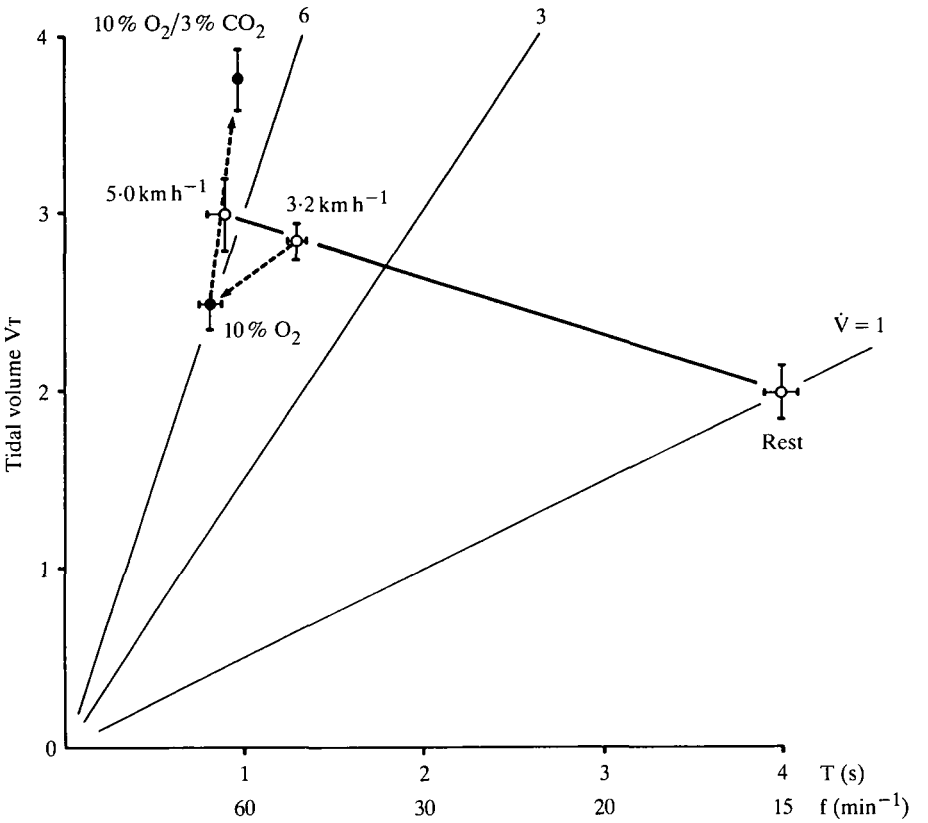

Fig. 2. Ventilatory parameters recorded during rest and exercise. Open symbols represent the values obtained when the birds breathed air. Filled symbols represent values obtained during inhalation of the experimental gas mixtures. Since airflow was not measured directly, tidal volume and minute volume are given as values relative to resting. Minute volume was assumed to be proportional to mean air sac pressure, tidal volume to mean air sac pressure divided by respiratory rate, as described in the text. Actual values for air sac pressure are given in Table 1. The thick, solid line connects data points measured at rest and during normoxic normocapnic exercise at 3·2 km h^{-1} and 5·0 km h^{-1} respectively. The arrowed dotted lines connect data points measured during exercise at 3·2 km h^{-1} during inhalation of air, 10% O_2 in N_2 or 10% O_2/3% CO_2 in N_2, respectively. $\dot{V}$, minute volume; V_T, tidal volume; T, respiratory period; f, respiratory rate.

during exercise, this was not sufficient to provoke a thermoregulatory breathing response and arterial P_{CO_2} remained at its control value.

Control of respiratory pattern in ducks exercised on treadmills appears to differ somewhat from that in the domestic fowl. In ducks increased respiratory rate was responsible for the entire increase in ventilation, and tidal volume actually fell even when exercise took place in isothermic conditions (Kiley *et al.* 1979, 1982; Kiley, Faraci & Fedde, 1985). Gleeson *et al.* (1985*a*) have criticized the tracheal cannulation technique used in the duck studies on the grounds that it might alter the normal pattern of breathing, and it is still not certain to what extent chickens and ducks differ in their responses to exercise. However, a recent study by Woakes & Butler (1986) on swimming tufted duck, *Aythya fuligula*, suggests that these differences may not be great. These authors measured ventilation using a non-invasive mask technique and found that, over a range of swimming speeds, the main increases in ventilation were due to increased respiratory rate, but with significant increases in tidal volume as well at the highest work loads. Geiser, Gratz, Hiramoto & Scheid (1984) artificially increased metabolic rate and ventilatory demand in ducks by injecting them with 2,4-dinitrophenol and found large increments in respiratory rate coupled with small but significant increments in tidal volume. Some of these birds were reported to be hyperthermic, as was reflected by a 4 Torr drop in arterial P_{CO_2}, and it is therefore likely that the observed increases in tidal volume were an underestimate of the isocapnic response. Gleeson (1986) also examined the effects of 2,4-dinitrophenol as well as cold exposure on metabolic rate and ventilation in unanaesthetized chickens. In these experiments arterial P_{CO_2} did not change and there was strong evidence that the alterations in respiratory pattern caused by both of these stimuli were of an identical nature to those produced by isothermic exercise.

Effects of hypoxia

In earlier studies on domestic fowl it was found that inhalation of hypoxic gas brought about increased ventilation almost entirely through increased respiratory rate in resting (Brackenbury & Gleeson, 1985) and exercise (Brackenbury *et al.* 1982) conditions. However, blood gases were not measured in these studies and it was not clear to what extent the changes in respiratory pattern during hypoxia may have been influenced by secondary alterations in P_{CO_2}. Bouverot & Sébert (1979) found that inhalation of 12 % O_2 in N_2 by resting, fully-conscious chickens produced a 40 % increase in ventilation accompanied by a 9 Torr drop in arterial P_{CO_2} and breathing became more rapid and shallow. In the present experiments lung hyperventilation during inhalation of 10 % O_2 in N_2 evidently led to a 3–4 Torr drop in arterial P_{CO_2} and presumably a similar fall in intrapulmonary P_{CO_2}. This may well account for the development of a more rapid and shallow breathing pattern than the control pattern produced by isocapnic exercise (Fig. 2). The observed drop in arterial P_{CO_2} was not large, but even smaller levels of hypocapnia are capable of evoking very dramatic alterations in ventilatory pattern during hyperthermic exercise. It was anticipated that the addition of 3 % CO_2 to the inhalation gas would have restored the ventilatory pattern to a point on the isocapnic exercise line. It can

be seen from Fig. 2 that although restoration of an arterial P_{CO_2} close to normal did result in an appropriate adjustment in respiratory pattern, this was clearly an overcompensation. Although arterial P_{CO_2} after inhalation of the 10% O_2/3% CO_2 gas mixture was the same as its resting value in air, it was significantly higher than the value measured in exercising, air-breathing conditions (Table 1). In view of the sensitivity of the respiratory pattern generator to P_{CO_2} it is possible that even an increase of 1–2 Torr could have a large influence on the depth/rate ratio.

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