Chronic intermittent hypercapnic hypoxia increases pulmonary arterial pressure and haematocrit in rats

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ABSTRACT: Sleep-disordered breathing is associated with pulmonary hypertension and raised haematocrit. The multiple episodes of apnoea in this condition cause chronic intermittent hypoxia and hypercapnia but the effects of such blood gas changes on pulmonary pressure or haematocrit are unknown. The present investigation tests the hypothesis that chronic intermittent hypercapnic hypoxia causes increased pulmonary arterial pressure and erythropoiesis.

Rats were treated with alternating periods of normoxia and hypercapnic hypoxia every 30 s for 8 h per day for 5 days per week for 5 weeks, as a model of the intermittent blood gas changes which occur in sleep-disordered breathing in humans. Haematocrit, red blood cell count and haemoglobin concentration were measured each week and systemic and pulmonary arterial blood pressure and heart weight were measured after 5 weeks.

In relation to control, chronic intermittent hypercapnic hypoxia caused a significant increase in systemic (104.3±4.7 mmHg *versus* 121.0±10.4 mmHg) and pulmonary arterial pressure (20.7±6.8 mmHg *versus* 31.3±7.2 mmHg), right ventricular weight (expressed as ratios) and haematocrit (45.2±1.0% *versus* 51.5±1.5%).

It is concluded that the pulmonary hypertension and elevated haematocrit associated with sleep-disordered breathing is caused by chronic intermittent hypercapnic hypoxia.
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Sleep disordered breathing affects 1–4% of adults [1]. It is associated with multiple episodes of hypoxia and hypercapnia due to hypopnoea or apnoea, which are either caused by intermittent collapse of the upper airway (UA), termed obstructive sleep apnoea (OSA; the most common form of sleep disordered breathing), or a reduced drive to breath, termed central apnoea [2]. The condition is associated with a number of cardiovascular changes, including increased pulmonary and systemic arterial blood pressure, right ventricular hypertrophy, cardiac arrhythmias and increased haematocrit. Diurnal pulmonary hypertension is present in 17–42% of OSA patients [3–7] and represents a serious threat to long-term outcome in these patients [8]. The cause of pulmonary hypertension in these patients is unknown. During an apnoeic event, pulmonary arterial pressure is acutely increased [9], presumably due to hypoxic pulmonary vasoconstriction, so that repetitive episodes of increased pressure may ultimately give rise to daytime pulmonary hypertension [10]. In support of this, it has recently been shown that chronic intermittent hypocapnic hypoxia in rats causes right ventricular hypertrophy, suggesting that pulmonary arterial pressure is increased [11]. However, apnoeas are accompanied by hypercapnia as well as hypoxia [12], but the effects of this combination of chronic intermittent blood gas changes on pulmonary arterial pressure are unknown. It is known that chronic continuous hypercapnia protects against the effects of continuous hypoxia on right ventricular weight and pulmonary arterial pressure [13]. Therefore, it is not clear whether prolonged intermittent hypercapnia in association with prolonged intermittent hypoxia would affect right ventricular weight or pulmonary arterial pressure. However, in view of the association of pulmonary hypertension with OSA, the present investigation tests the hypothesis that chronic intermittent hypercapnic hypoxia causes right ventricular hypertrophy and pulmonary hypertension.

Another contributor to increased pulmonary arterial pressure is a raised haematocrit. It has previously been shown that chronic intermittent hypocapnic hypoxia increases the haematocrit in rats [11]. A combination of chronic continuous hypoxia and hypercapnia greatly reduces the increase in haematocrit caused by chronic continuous hypoxia alone [13], but the effects of chronic intermittent hypoxia and hypercapnia on haematocrit have not been studied. As with pulmonary hypertension, the presence of increased haematocrit is variable in OSA patients. It has been suggested that an increased haematocrit only occurs in the presence of daytime hypoxaemia [4]. HOFFSTEIN *et al.* [14] reported no change or slight elevations in haematocrit in a large group of sleep apnoea patients without daytime hypoxaemia, whereas LAKS *et al.* [5] did observe an elevated haematocrit in the absence of daytime hypoxaemia.

Therefore, in the present investigation, the effects of chronic intermittent hypercapnic hypoxia on haematocrit, right ventricular weight and pulmonary arterial pressure are examined using a rat model which mimics the nocturnal pattern of hypercapnic hypoxia of sleep-disordered breathing in humans.

Materials and methods

Male Wistar rats (3 months old) were used in this study. Except during the treatment and experimental periods, the rats were housed, four to a cage, under a 12:12 h (light:dark) photoperiod and were given unrestricted access to food and water.

Chronic intermittent hypercapnic hypoxia treatment

Animals were placed in restrainers with their heads surrounded by hoods. Using timed solenoid valves, a mixture of nitrogen (N_2) and carbon dioxide (CO_2) was distributed into the hoods for 15 s at a flow that reduced the ambient fractional inspiratory concentration of oxygen (F_{I,O_2}) to 6–8% and increased the ambient fractional inspiratory concentration of CO_2 (F_{I,CO_2}) to 10–14%. This was followed by an infusion of air for 15 s allowing the F_{I,O_2} and the F_{I,CO_2} to return to nominal. This cycle was repeated twice per min, 8 h per day, for 5 days per week for 5 weeks. The control rats were given air for 15 s followed by air for 15 s at the same flow rates as the hypercapnic hypoxia rats. This cycle was repeated twice per min, 8 h per day, 5 days per week for 5 weeks. In all, 16 control and 13 hypercapnic hypoxia rats were treated in this way.

The ambient F_{I,O_2} and F_{I,CO_2} in the hoods were measured daily by placing the probes of the O_2 analyser (Engström Eliza Duo, Gambro-Engström AB, Bromma, Sweden) and CO_2 analyser (Capstar-100 end-tidal CO_2 analyzer, CWE Inc., Ardmore, PA, USA) as close as possible to the nose of the hypercapnic hypoxic rats for 5 min at 0, 4 and 8 h throughout the treatment period. These values at 0, 4 and 8 h were averaged for each day (fig. 1). The haematocrit, red blood cell count and haemoglobin concentration were measured on day 0, 7, 14, 21, 28 and 35 of the 5-week treatment period using blood collected from the rat tail artery in microcapillary tubes.

Pulmonary arterial pressure was measured in a separate group of control rats, treated as described above and in a group of hypocapnic hypoxia rats, treated as described elsewhere [11].

Measurement of arterial blood oxygen tension and end-tidal carbon dioxide tension

Untreated rats were used to measure systemic arterial oxygen tension (P_{a,O_2}) and end-tidal carbon dioxide tension (P_{ET,CO_2}) during normoxia/hypercapnic hypoxia treatment. Rats were anaesthetized intraperitoneally (*i.p.*) with 40 mg·kg^{-1} of pentobarbitone (Sagatal, Rhône Mérieux Ltd, Essex, UK). A
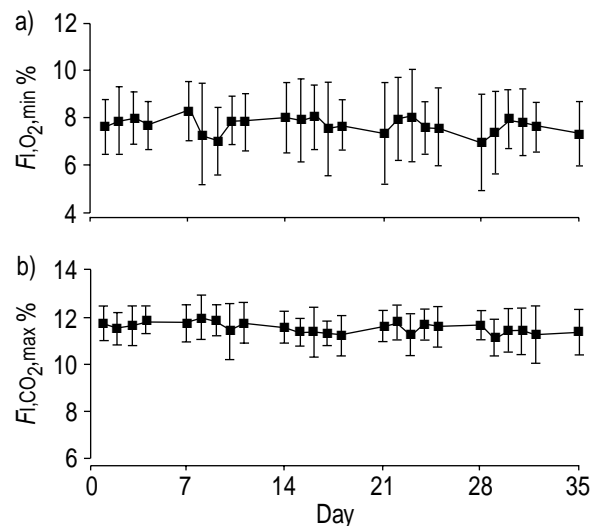

Fig. 1. – Values (measured over 5 min) for a) minimum fractional inspiratory concentration of O_2 (F_{I,O_2}) and b) maximum fractional inspiratory concentration of CO_2 (F_{I,CO_2}) measured three times per day in eight animals. Values are presented as mean±SD.

femoral artery and vein were cannulated and the incision was sutured closed. The cannulae were connected to a three-way tap which housed an O_2 needle electrode. A small ventral midline incision was made in the neck and a small hole was made in the trachea by using a hot needle. A cannula was inserted into the trachea through this hole. The hole was then sealed using nontoxic glue and the cannula was secured. The cannula was attached to a CO_2 end-tidal analyser (Capstar-100 end-tidal CO_2 analyzer, CWE Inc., Ardmore, PA, USA) with a response time of 0.33 s. The rats were allowed to recover and were placed into the treatment chambers to begin the intermittent hypercapnic hypoxia treatment. Heparin (1,000 U·kg^{-1}, *i.v.*) was given to ensure that blood flowed without clotting. Alternating periods of air for 15 s and hypercapnic hypoxia treatment (N_2 and CO_2) for 15 s were begun twice per min. P_{a,O_2} and P_{ET,CO_2} were continuously measured and F_{I,O_2} and F_{I,CO_2} in the hoods were also monitored. All signals were fed to a data acquisition system and recorded on a computer.

Measurement of systemic and pulmonary arterial blood pressure and heart weight

After the 5-week treatment, rats were anaesthetized with pentobarbitone (60 mg·kg^{-1}, *i.p.*). The carotid artery was exposed and cannulated, and the blood pressure was measured by attachment of a suitably calibrated pressure transducer. A tracheal cannula was inserted. The pulmonary arterial pressure was measured using the isolated-perfused lung technique [13, 15]. This technique has certain advantages over *in vivo* measurement, in that it is technically easier to use and yields results which are independent of cardiovascular and respiratory changes or reflex effects. Heparin (1 U·g^{-1}) was injected through the carotid

artery cannula. The chest wall was opened and the pulmonary artery was cannulated. The lungs were carefully removed and the tracheal cannula was connected to a small animal ventilator. The pulmonary artery cannula was perfused at constant flow ($0.02 \text{ mL·g}^{-1}\text{·min}^{-1}$) with physiological saline solution containing 2% Dextran, and pulmonary artery pressure was measured from a side-arm of the pulmonary artery cannula using a pressure transducer. The ventilator inflated and deflated the lungs with 5% CO_2 in air at a rate of 50 breaths min^{-1} and a volume of 2.5 mL.

The heart was removed and was placed in a 4% formalin solution for 3 days. After this time, the heart was removed from the formalin and the atria were trimmed away and discarded. The right ventricle was separated from the left ventricle and septum and these two portions were weighed separately. The right ventricular weight was expressed as a ratio of the left ventricular weight and also as a ratio of body weight.

Data analysis

Values for body weight, right and left ventricular weight, haematocrit, red blood cell count, haemoglobin concentration, pulmonary arterial pressure, systemic arterial pressure and heart rate were expressed as mean±SD and used to statistically compare the control, hypocapnic hypoxia and hypercapnic hypoxia groups using analysis of variance (ANOVA) and Fischer's least significant difference test, with p<0.05 taken as significant.

Results

In eight control and six hypercapnic hypoxia-treated rats, the food and water intake were measured over the 5-week treatment period. The control rats ingested $80.5\pm8.9 \text{ g·kg}^{-1}\text{·day}^{-1}$ of food and $90.2\pm8.1 \text{ mL·kg}^{-1}\text{·day}^{-1}$, of water. These values were similar to those of the hypercapnic hypoxia-treated rats ($78.3\pm11.4 \text{ g·kg}^{-1}\text{·day}^{-1}$ and $91.3\pm7.7 \text{ mL·kg}^{-1}\text{·day}^{-1}$, food and water, respectively). The body weight of the control and hypercapnic hypoxia-treated rats at the beginning of the 5-week treatment period was 343.7±28.5 g and 348.6±35.9 g respectively. The final body weights after 35 days are shown in table 1. The weight change in the control group was +6.3±4.0% (n=16) and in the hypercapnic hypoxia group was -5.6±4.6% (n=13). These differences were statistically significant.

Acute effects of intermittent hypercapnic hypoxia on arterial oxygen tension and end-tidal carbon dioxide tension

Pa,O_2 and PET,CO_2 were continuously monitored in a subgroup of rats exposed to identical conditions of intermittent hypercapnic hypoxia as the chronically treated groups. Figure 2 shows recordings of arterial blood Pa,O_2, PET,CO_2 and inspiratory oxygen tension (PI,O_2) while the rat was breathing air (before the start of the treatment) and then during the alternating periods of normoxia and hypercapnic hypoxia. The PET,CO_2 values for six rats measured during the air and hypercapnic hypoxia episodes were 35.7±8.5 mmHg and 63.6±8.6 mmHg, respectively.

Red blood cell count, haemoglobin concentration and haematocrit

Chronic intermittent hypercapnic hypoxia resulted in a significant increase in haematocrit, red blood cell count and haemoglobin concentration on day 7, 14, 21, 28 and 35 of the treatment period. There were no significant differences between the values on days 7, 14, 21, 28 or 35. These data are shown in figure 3. On day 35, the haematocrit was 51.5±1.5% in the hypercapnic hypoxia rats compared to 45.2+1.0% in the control animals.

Systemic arterial blood pressure, pulmonary arterial pressure and heart weight

Chronic intermittent hypercapnic hypoxia significantly increased mean systemic arterial blood pressure (104.3±4.7 mmHg *versus* 121.0±10.4 mmHg, control and hypercapnic hypoxia respectively) but had no effect on heart rate ($370\pm16 \text{ beats·min}^{-1}$, *versus* $371\pm11 \text{ beats·min}^{-1}$, control and hypercapnic hypoxia, respectively). Chronic intermittent hypercapnic hypoxia also significantly increased pulmonary arterial pressure from 20.7±6.8 mmHg in the control group to 31.3±7.2 mmHg in the hypercapnic hypoxia group.

Table 1 shows the right ventricular weight expressed as a ratio to the left ventricular weight and to body weight, left ventricular weight as a ratio to body weight and total heart weight as a ratio to body weight. The left ventricular to body weight ratio was unaffected, but the right ventricular to left ventricular weight and right ventricular to body weight ratios were significantly increased in response to chronic intermittent hypercapnic hypoxia, indicating right ventricular hypertrophy.

Table 1. – Effect of chronic intermittent hypercapnic hypoxia on heart weight

	Rats n	BW g	RV/LV	RV/BW mg·g^{-1}	LV/BW mg·g^{-1}	THW/BW
Control	16	349.6±34.0	0.25±0.03	0.53±0.05	2.16±0.15	2.69±0.17
Hypercapnic hypoxia	13	334.3±22.0	0.31*±0.03	0.63*±0.09	2.05±0.20	2.68±0.27

Values are presented as mean±SD. RV: right ventricular weight; LV: left ventricular plus septum weight; BW: body weight; THW: total heart weight. *: indicates significantly different from control, p<0.05, analysis of variance.
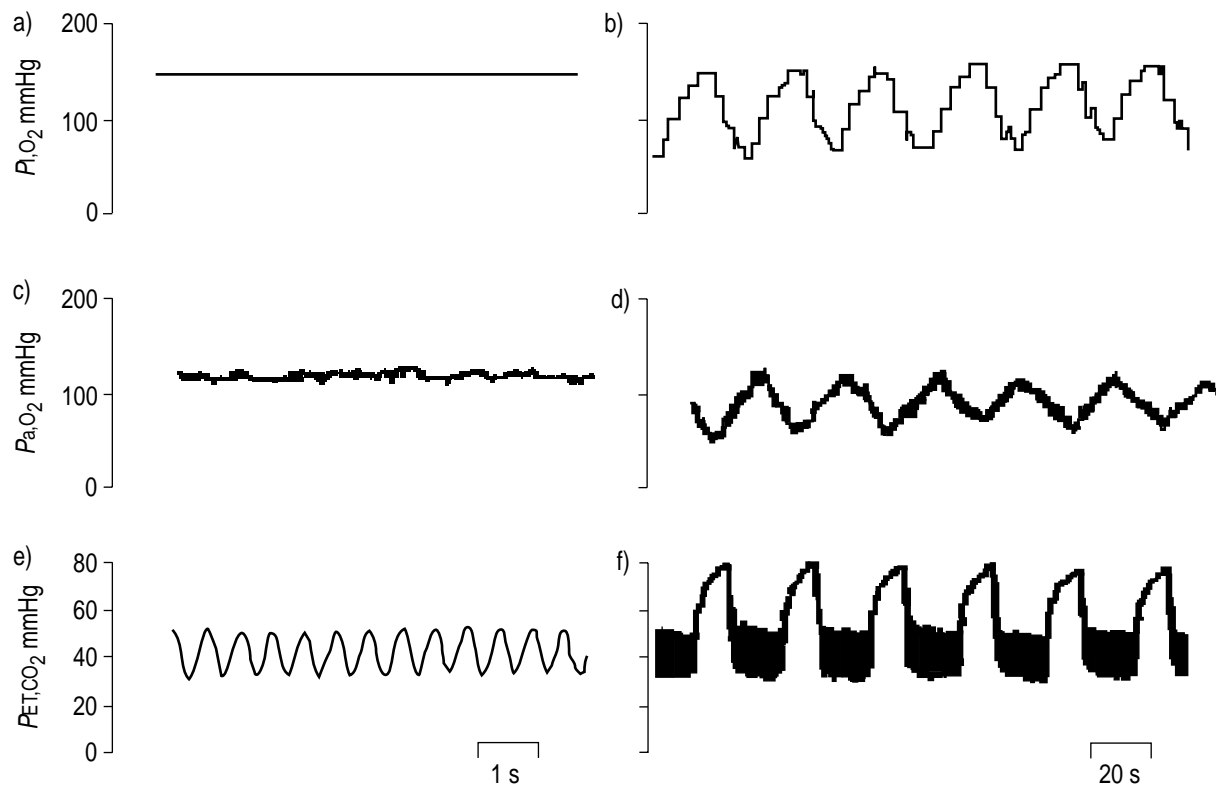
Fig. 2. – Recordings of a+b) inspiratory oxygen tension (P_{I,O_2}) c+d) arterial oxygen tension (P_{a,O_2}) and e+f) end-tidal carbon dioxide tension (P_{ET,CO_2}) while breathing air (a,c,e), and alternating periods of normoxia and hypercapnic hypoxia (b, d, f). All recordings are extracts of a continuous record.

Discussion

In a separate group of hypocapnic hypoxia rats (n=8), there was also a significant increase in pulmonary arterial pressure (39.1±18.5 mmHg) compared to controls (n=8, 22.7±7.9 mmHg).

In the present investigation, a rat model of the intermittent blood gas changes associated with sleep-disordered breathing in humans showed that chronic intermittent hypercapnic hypoxia causes systemic arterial and pulmonary hypertension, increased haematocrit and right ventricular hypertrophy. The fall in P_{a,O_2} to ~55 mmHg and the increase in arterial carbon dioxide tension (P_{a,CO_2}) to ~60 mmHg during the hypercapnic hypoxia episodes were similar to the values recorded by FLETCHER et al. [16] in rats exposed to repetitive episodes of hypercapnic hypoxia twice per min, 6–8 h per day for 35 consecutive days.

One major difference between the present model and patients with OSA is that during the obstructed breaths, marked negative intrathoracic pressures are generated [2]. This causes acute haemodynamic changes which may also result in chronic cardio-vascular changes [9]. Conversely, this model can be viewed as having an advantage that it allows the study of the effects of chronic blood gas changes independently of these mechanical changes. However, the model cannot mimic all of the blood gas changes which occur in OSA. These changes are complex, so that hypoxia can be accompanied by either normocapnia or hypercapnia during the apnoeas and hypocapnia following the apnoeas [12]. The body weights of the control and hypercapnic hypoxic rats at the end of the 5-week treatment were not significantly different. However, the actual weight gain of the hypercapnic hypoxic rats was impaired compared to the control rats, despite having ingested similar amounts of food and water. Chronic continuous hypoxia has been shown to impair normal weight gain in rats [17]. Whereas chronic continuous hypoxia alone and continuous hypercapnia alone impair weight gain, a combination of continuous hypoxia and hypercapnia causes weight loss [13]. For chronic intermittent hypercapnic hypoxia, FLETCHER et al. [16] showed that the body weights of the treated rats at the end of 5 weeks were not significantly different from controls, but the actual weight gain was somewhat impaired.

The mean arterial blood pressure was 16% higher in the hypercapnic hypoxia group compared to controls. This hypertensive effect of chronic intermittent hypoxia and hypercapnia has been well described previously [16, 18–22] and its importance stems from the clear association of OSA and systemic hypertension [23–25]. Systemic hypertension in the present study was not associated with left ventricular hypertrophy. Although FLETCHER and coworkers [16, 18–22] usually observed left ventricular hypertrophy,
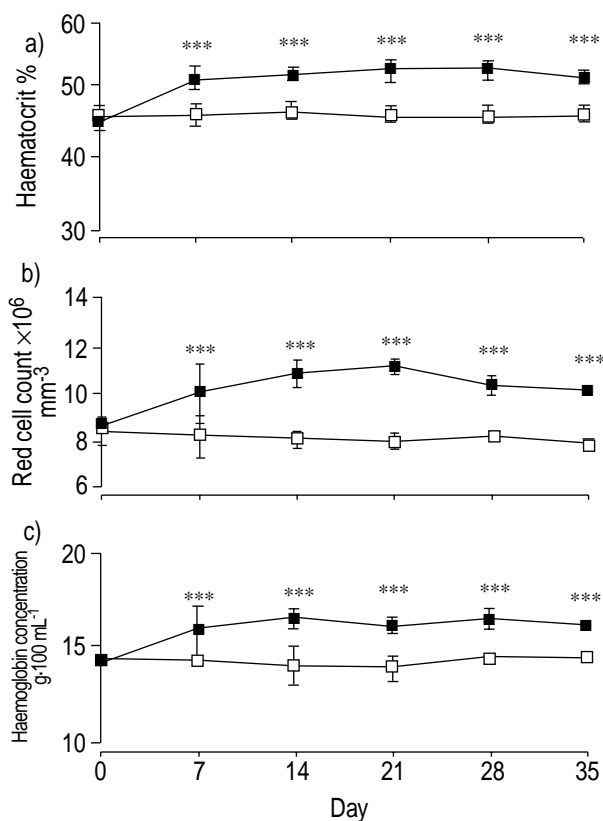
Fig. 3. – Effects of chronic intermittent hypercapnic hypoxia on a) blood haematocrit; b) red cell count; and c) haemoglobin concentration of control (□, n=16) and hypercapnic hypoxia (■, n=13) rats during the 5-week treatment period. Values are presented as mean±SD. ***: significantly different (p<0.001) from control using analysis of variance.

in one study they found that chronic intermittent hypocapnic hypoxia had no effect on left ventricular weight, despite increased systemic blood pressure [19]. In another study, sympathetic-denervated rats exposed to intermittent hypoxia showed an increase in left ventricular weight without an increase in systemic blood pressure [21]. This suggests that systemic hypertension may not cause left ventricular hypertrophy but that it is an effect of the intermittent hypoxia itself. FLETCHER and coworkers [20–22] have frequently observed hypertrophy of the chambers of the heart in response to chronic intermittent hypoxia without an increase in systemic blood pressure (when this was prevented by sympathectomy or carotid body denervation) and these workers have proposed that it is due to a direct effect of hypoxia. Further evidence for this stems from humans with an anomalous coronary arterial supply from the pulmonary artery [26]. In this condition, there is left ventricular hypertrophy but there is no increase in afterload, suggesting that the hypertrophy is due to the supply of hypoxic blood to the ventricle from the pulmonary artery.

The association of pulmonary hypertension and OSA is controversial with 17–42% of OSA patients showing the presence of diurnal pulmonary hypertension [3–7]. Right ventricular weight is linearly related to pulmonary arterial pressure in rats and thus right ventricular hypertrophy is an index of pulmonary hypertension [27]. Right ventricular weight is increased in rats exposed to continuous hypoxia [27], 2 h of hypoxia per day for 42 days [28] and 30 min of hypoxia per h, 8 h per day for 28 days [29]. However, none of these patterns of hypoxia resembles that of OSA. Using a rat model which does mimic the intermittent hypoxia of OSA, FLETCHER and co-workers [16, 18, 20, 21] found no change in right ventricular weight. However, in one study using Sprague-Dawley rats, these workers did show that chronic intermittent hypoxia resulted in right ventricular hypertrophy [22] and an unequivocal increase in right ventricular weight has previously been shown, using a similar model [11]. The present study shows that chronic intermittent hypercapnic hypoxia also results in an increased right ventricular weight, indicative of raised pulmonary arterial pressure. The effects of chronic intermittent hypoxia or hypercapnic hypoxia on pulmonary arterial pressure have not been previously investigated. It is well known that chronic continuous hypoxia has a significant effect on pulmonary arterial pressure and pulmonary vascular remodelling in animals [13, 27]. However, OOI et al. [13] have shown that hypercapnia combined with hypoxia markedly reduced the pulmonary hypertension and right ventricular hypertrophy observed in rats after chronic continuous hypoxia alone, and inhibited to some degree the pulmonary vascular remodelling that is associated with continuous hypoxia alone. It is thought that this effect of continuous hypercapnia might also occur in intermittent hypercapnic hypoxia and might therefore prevent an increase in pulmonary arterial pressure. Such a mechanism could then explain the absence of pulmonary hypertension in many patients with OSA. However, consistent with the finding of right ventricular hypertrophy, the present results show that chronic intermittent hypocapnic hypoxia and hypercapnic hypoxia cause pulmonary arterial hypertension. The importance of this stems from the suggestion that pulmonary hypertension does not develop in OSA unless it is accompanied by daytime hypoxaemia [3, 30]. However, pulmonary hypertension has been observed in some OSA patients without daytime hypoxaemia (P_{a,O_2} >80 mmHg) [5] or with only slight hypoxaemia (72 mmHg) [31] and the present results support the idea that nocturnal episodic hypercapnic hypoxia causes pulmonary hypertension in the absence of continuous hypoxia [32].

There is considerable controversy concerning the causes of increased haematocrit in OSA. HOFFSTEIN et al. [14] suggested that the intermittent nocturnal hypoxaemia of OSA patients did not lead to polycythaemia, although they did report slight elevations in their haematocrit values. BRADLEY et al. [3] showed raised haematocrit and pulmonary hypertension in OSA patients, but these also presented daytime hypoxaemia. GOLDMAN et al. [33] showed, in eight OSA patients, that there was no increase in haematocrit despite episodes of profound nocturnal hypoxaemia. CHAOUAT et al. [4] showed that OSA

patients with pulmonary hypertension also had raised haematocrit. This suggested that the causes for pulmonary hypertension were similar to those for raised haematocrit, namely the presence of daytime hypoxaemia [4, 7]. However, there are some OSA patients who present both pulmonary hypertension and raised haematocrit without daytime hypoxaemia [5]. It may be that in some cases, the intermittent nature of the hypoxia gives insufficient time to stimulate red blood cell production. Alternatively, since concomitant chronic continuous hypercapnia has been shown to greatly reduce the extent of the haematocrit increase caused by chronic hypoxia alone [13], it may be that the intermittent hypercapnia accompanying intermittent hypoxia diminishes the erythropoietic response. In the present investigation, chronic intermittent hypercapnic hypoxia caused a clear increase in haematocrit, which was apparent when first measured at day 7, and persisted for the duration of the treatment period. This shows that concomitant hypercapnia does not prevent the erythropoietic response to intermittent hypoxia.

The increase in haematocrit was apparent at day 7 of the hypercapnic hypoxia treatment. Chronic continuous hypoxia also increases haematocrit within this time period [13] and it has previously been shown that chronic intermittent hypocapnic hypoxia also increases haematocrit when measured at day 7 [11]. However, it is possible that the rapidity of this response may involve a change in plasma volume. Thus, a decrease in plasma volume without any change in erythropoiesis could cause an increase in haematocrit. However, the effects of hypoxia on plasma volume are highly controversial. In humans, for example, hypoxia has been reported to cause diuresis and natriuresis [34] or to cause antidiuresis and antinatriuresis [35]. Similarly, in rats, diuresis and natriuresis [36] or antidiuresis and antinatriuresis [37] have been described. However, more recent evidence suggests that acute hypoxia in rats causes antidiuresis and antinatriuresis [38, 39] and that chronic hypoxia results in normal renal function [40]. Therefore, it is believed that a decrease in plasma volume is an unlikely contributor to the increased haematocrit in the present experiments.

The present data show that exposure to chronic intermittent hypercapnic hypoxia induces a number of responses including erythropoiesis, right ventricular hypertrophy and pulmonary and systemic hypertension. These effects are probably due to altered patterns of gene expression and a major activator of the transcription of these genes is hypoxia-inducible factor 1 [41]. Thus, hypoxia-inducible factor 1 has been shown to participate in the pulmonary and systemic hypertensive, erythropoietic and right ventricular hypertrophic response [41]. Furthermore, mice which are partially deficient in hypoxia-inducible factor 1 show impaired development of polycythaemia, right ventricular hypertrophy, pulmonary hypertension and pulmonary vascular remodelling [42].

In conclusion, using a rat model of the chronic blood gas changes which occur during obstructive sleep apnoea in humans, it has been shown that chronic intermittent hypercapnic hypoxia causes an increase in haematocrit, pulmonary hypertension and right ventricular hypertrophy. However, it is unclear how these results in rats might transpose to humans with obstructive sleep apnoea, especially the degree and duration of hypoxia and hypercapnia which may cause such chronic cardiovascular changes.

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