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Cardiovascular changes during acute episodic repetitive hypoxic and hypercapnic breathing in rats

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Cardiovascular changes during acute episodic repetitive hypoxic and hypercapnic breathing in rats. M. Bakehe, J.L. Miramand, B. Chambille, Cl. Gaultier, P. Escourrou. ©ERS Journals Ltd 1995.

ABSTRACT: It has been shown that chronic repetitive ambient hypoxia, simulating pulmonary gas disturbances observed in apnoea, leads to systemic hypertension in rats. However, the relative roles of hypoxia (HO), hypercapnia (HC), gas stress and vigilance on the cardiovascular changes have not yet been demonstrated.

The aim of this study was to investigate the acute haemodynamic changes observed during the repetitive inhalation of various gas mixtures in rats for HO alone and HO + HC, and to analyse the effects of vigilance and of the stress of gas administration.

We studied 6 unanaesthetized Wistar rats chronically instrumented with an aortic catheter. Nitrogen, nitrogen + CO_2 mixtures and compressed air were randomly administered in a Plexiglass chamber for 10 s and then flushed by compressed air for 20 s. Two cycles were repeated every min for 10 to 12 min. The inhaled gas fractions (F_{I,O_2}, F_{I,CO_2}) were monitored by O_2 and CO_2 analysers. Blood pressure (BP) was measured by a P23XL transducer. The blood gases were analysed by a 1306 IL meter.

In control experiments, with compressed air alone, there were no significant acute changes in heart rate (HR) and BP. During HO there were no changes in HR or BP at F_{I,O_2} values from 0.05–0.14, whilst at F_{I,O_2} values from 0–0.05 systolic blood pressure (SBP) rose significantly (+25.3±25.7 (SD) mmHg) and HR decreased (-93.8±124.1 bpm). During HOHC, SBP rose (+35.1±26.4 mmHg) and HR decreased (-139.3±75.7 bpm), significantly more than in HO alone. SBP was linearly correlated with P_{a,O_2} during HO (r=0.53) and also during HOHC (r=0.44) and was not directly related to P_{a,CO_2} which has, nevertheless, an additive effect to HO. SBP rose with each challenge significantly more when the rats were awake than when asleep (behavioural sleep).

We conclude that in this acute repetitive inhalation model, the rise in SBP is not related to gas stress or to P_{a,CO_2} but to a decrease in P_{a,O_2} and is enhanced by wakefulness.

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Sleep Apnoea Syndrome (SAS) is the consequence of repetitive obstructive apnoea due to the collapse of the upper airways. These apnoea are responsible for repetitive hypoxaemia, sleep disruptions and blood pressure swings. The acute postapnoeic blood pressure elevation is correlated with the degree of hypoxia during apnoea [1]. HEDNER *et al*. [2] have suggested that hypoxaemia and sympathetic activity mediate blood pressure changes in SAS. However, prevention of hypoxia during obstructive sleep apnoea by supplementary oxygen did not decrease the systemic pressure rise associated with the termination of apnoea [3, 4]. Another potential mechanism which may contribute to the rise in blood pressure in sleep apnoea is arousal, which is usual at apnoea termination. It has been shown that arousal from normal sleep raises blood pressure to a level similar to that seen at the resumption of breathing after sleep apnoea [5]. The role of acute episodic hypercapnia has not been evaluated up to now, although hypercapnia is known to be a potent arousing stimulus and is increased during long apnoeas in humans [6]. Each of these three mechanisms may participate in cardiovascular changes associated with acute SAS. This study examines the relationship between hypoxia, hypercapnia, stress and vigilance on the haemodynamic changes observed during simulated repetitive apnoeas in rats, in order to define the mechanisms of these acute haemodynamic changes in SAS.

Materials and methods

Six Wistar male rats (400–500 g) were anaesthetised using sodium pentobarbital (30 $mg \cdot kg^{-1}$ *i.p.*). Except during the experimental periods, the animals were housed in their usual cages where they ate and drank water *ad libitum*.

Inhalation chamber

Severe HO (SHO) was defined as fractional concentration of inspiratory O_2 0–0.05 (F_{I,O_2}), and mild HO (MHO) as 0.051–0.14 F_{I,O_2}. Mild HOHC (MHOHC) and severe HOHC (SHOHC) were similarly defined from fractional concentration of inspiratory CO_2 (F_{I,CO_2}) ranges. The inhalation chamber was custom made in our laboratory (fig. 1). The animal was housed in a cylindrical Plexiglass chamber (inner diameter: 92 mm; length: 320 mm; total volume: 1,993 cm^3) during daytime for 1–2 hours. The gases (ambient air, N_2(100%), N_2(95%) + CO_2(5%)) were distributed in the inhalation chamber by manual valves. The animal received a turbulent flow of gases across a filtration device. The rat was totally free in the chamber. Two openings allowed sampling of the chamber gas for analysis of O_2 and CO_2 fractions (Gauthier, France), (Datex Normocap, FIN) and a catheter allowed blood pressure measurement.

Catheterisation

At least 2 days before the experiment, a PE 10 catheter (Portex, France) in PE 50 (Biotrol, France) was placed in the abdominal aorta *via* a femoral artery, tunnelled under the skin of the back and coming out at the neck in the chronically instrumented conscious rats. Blood pressure was measured by a P23XL Gould transducer (Gould Electronics, USA) connected to a Gould pressure Processor (Gould Electronics, USA). Blood pressure, heart rate, F_{i,O_2} and F_{i,CO_2} were recorded on a Data Management System (DMS) 1000 (Graphtec, USA).

Blood gas measurement

Blood was sampled from the aortic catheter between the 7th and 8th min during each recording session. A sample of 150 μL of blood was removed in a heparinised glass capillary tube which took about 1 min to be completed. Blood gases were analysed by an IL 1306 pH/blood gas analyser (Instrumentation Laboratory, Italy).

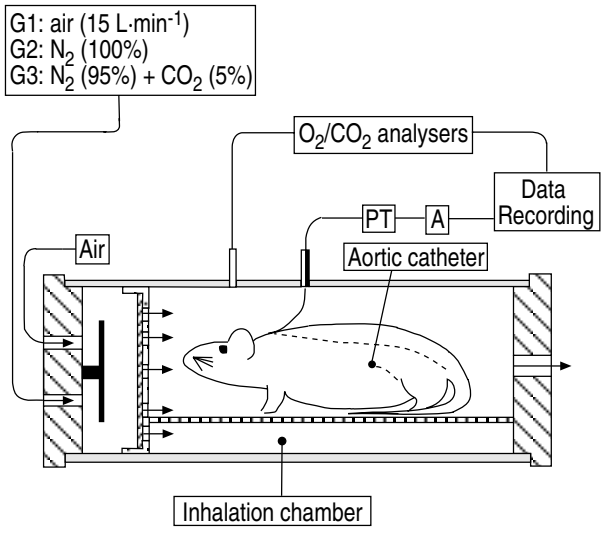

Fig. 1. – Experimental set up. PT: pressure transducer; A; amplifier; G: gases or gas mixtures.

Protocol

Once a day, before the first recording session, the animal was acclimatised to the inhalation chamber. The test gas was randomly chosen (N_2, N_2 + CO_2 and compressed air) for between 10–12 min periods. During each period the test gas was administered in the inhalation chamber for 10 s then flushed by compressed air for 20 s. Two cycles were repeated every min for 10–12 min. Sleep status was evaluated by the analysis of behavioural sleep to differentiate between wakefulness and sleep status (quiet sleep and active sleep periods were added together).

Data analysis

For each cycle, values of heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) have been measured on the recording by visual averaging over the whole part of the cycle in air breathing (20 s) and at the maximum of the response during the 10 s of gas challenge. For each variable, the difference between the maximal response and the air breathing average has been computed for each cycle. These differences have been averaged for 12–14 cycles in the same experimental period for each rat. Then, these differences were averaged for all rats exposed to an identical challenge.

Statistical methods

The results are expressed as mean±SD; $p<0.05$ was considered as significant. The effects of different levels of hypoxia and hypoxia + hypercapnia on the variations in SBP, DBP and HR were compared by two-sided paired t-test. Regression analysis between SBP and P_{a,O_2} or P_{a,CO_2} was performed using the least squares method. BP and HR were compared between sleeping (behavioural sleep) and waking animals by two-way analysis of variance (ANOVA) with gas challenge and vigilance as factors. Mean values have been compared by t-test using Bonferroni correction for multiple comparisons. P_{a,O_2} and P_{a,CO_2} were compared using two-sided paired t-test between each gas challenge.

Results

Typical tracings

The BP and HR changes stabilised after 2–3 min of challenge and remained so during the 10–12 min period. Figure 2 illustrates a recording for the same animal in

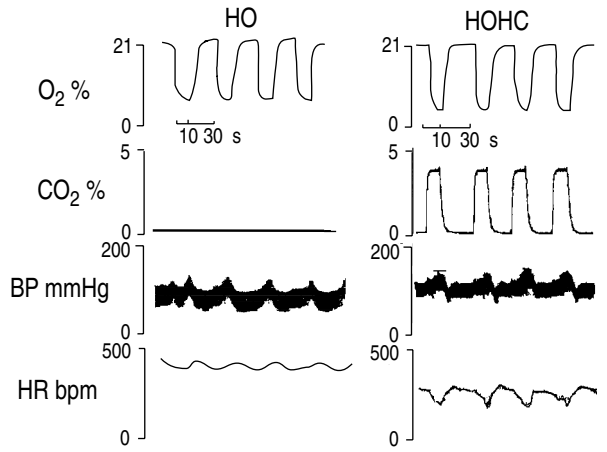

Fig. 2. – Typical tracings illustrating a recording for the same animal in HO (hypoxia alone) and HOHC (hypoxia + hypercapnia). There was an increase in blood pressure (pressor response) and a decrease in HR (bradycardia) diminished during the 10 s of the gas challenge during hypoxia (left panel). The HR and BP changes were greater when hypercapnia was added to HO (right panel). BP: blood pressure; HR: heart rate.

HO (hypoxia alone) and HOHC (hypoxia + hypercapnia). There was an increase in blood pressure (pressor response) and a decrease in HR (bradycardia) during the 10 s of the gas challenge during hypoxia (left panel). The HR and BP changes were greater when hypercapnia was added to HO (right panel).

Effect of compressed air

Four rats were studied. There were no significant differences in SBP values between the baseline (131.8±39.5 mmHg) and compressed air (131.9±13.4 mmHg). Similarly, there was no significant change in HR (319.7±4.2 bpm *vs.* 317.6±45.4 bpm respectively).

Blood gases (table 1)

Ambient hypoxia (HO) led to hypoxaemia and hypocapnia in the waking and sleeping states. P_{a,O_2} was lower and P_{a,CO_2} higher during sleeping ($p<0.05$). In HOHC, P_{a,CO_2} was identical to air during the waking state and was slightly lower in the sleeping state ($p<0.05$).

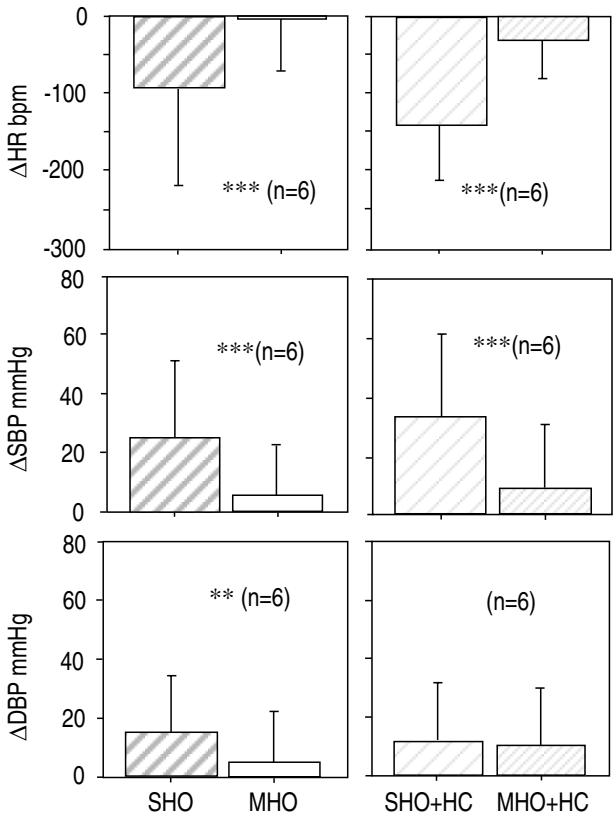

Fig. 3. – Variations in heart rate and blood pressure. SHO: severe hypoxia, 0–5% O_2/0% CO_2; MHO: mild hypoxia, 5.1–14% O_2/0% CO_2; SHO + HC: severe hypoxia + hypercapnia, 0–5%O_2/5–3% CO_2; MHO + HC: mild hypoxia + hypercapnia, 5.1–14%O_2/2.9–1% CO_2; DPB: diastolic blood pressure; SPB: systolic blood pressure; HR: heart rate. **: $p<0.01$; ***: $p<0.0001$ for comparison SHO *vs.* MHO and SHO+HC *vs.* MHO+HC.

Heart rate

Six rats were studied during HO and HOHC. HR decreased significantly more during severe HO (-93.8±124.1 bpm) or severe HOHC (-139.3+75.7 bpm) ($p<0.0001$) than during mild HO (-3.1±67.8 bpm) or mild HOHC (-28.5±52.0 bpm) (fig. 3). HR decreases more during HOHC compared to HO.

Table 1. – Blood gases in waking and sleeping animals during hypoxia (HO) and hypercapnia (HOHC).

		HO		HOHC	
	Air (n=6)	Awake (n=3)	Asleep (n=3)	Awake (n=3)	Asleep (n=3)
P_{a,O_2}					
torr	86.3±7.1	66.6±10.1*+	57.2±14.8*	78.5±15.7+	70.9±11.6*
kPa	11.5±0.9	8.9±1.3	7.6±2.0	10.4±2.1	9.4±1.5
P_{a,CO_2}					
torr	36.4±4.1	31.3±2.8*+	33.9±7.1	36.2±3.3+	33.4±3.1*
kPa	4.8±0.5	4.2±0.4	4.5±0.9	4.8±0.4	4.4±0.4
pH	7.38±0.06	7.40±0.07+	7.44±0.04*	7.38±0.01+	7.41±0.04

The values are mean±SD. (+) denotes a significant difference ($p<0.05$) between waking and sleeping values. (*) denotes a significant difference ($p<0.05$) between Air (Baseline) values and HO or HOHC values.

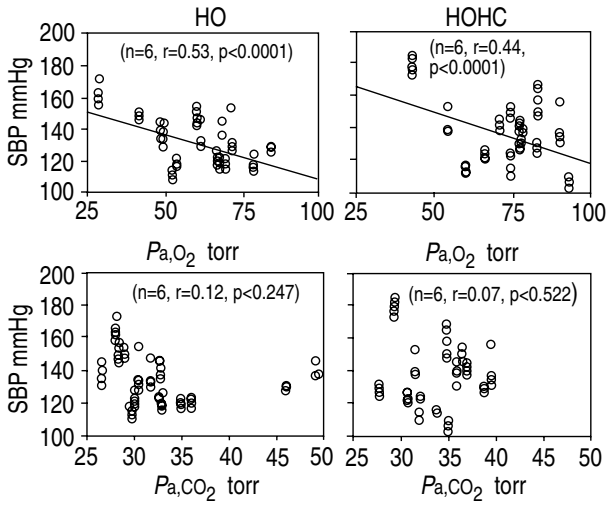

Fig. 4. – Correlations between SBP *vs.* P_{a,O_2} (HO, HOHC) and P_{a,CO_2} (HO, HOHC). P_{a,O_2}: arterial oxygen tension; P_{a,CO_2}: arterial carbon dioxide tension. For further abbreviations see legend to figure 3.

Blood Pressure

All the SBP increases were statistically significant at p<0.0001 for severe HO (+25.3±25.7 mmHg) and severe HOHC (+35.1±26.4 mmHg) compared respectively to mild HO (+5.9±16.9 mmHg) and mild HOHC (+10.4±21.1 mmHg) (fig. 3). There was a significant difference between the SBP rise in severe and mild HO compared respectively to the SBP rise in severe and mild HOHC (p<0.001). DBP increase was significant in SHO compared to MHO (p<0.01).

Relationship between SBP and Pa,O_2 and Pa,CO_2

Figure 4 shows the relationship between SBP during gas challenge and the corresponding values of P_{a,O_2} and P_{a,CO_2}. There were significant correlations between SBP and P_{a,O_2} in HO (r=0.53; p<0.0001) and in HOHC (r=0.44; p<0.0001) with similar slopes but significantly different intercepts. There was no relationship between SBP and P_{a,CO_2} in HO or in HOHC.

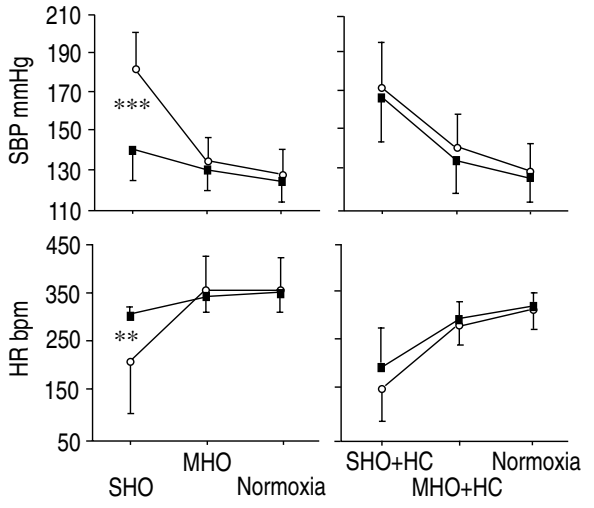

Fig. 5. – Effect of vigilance on SBP and HR. SHO: Severe hypoxia 0–5% O_2/0% CO_2; MHO: mild hypoxia 5.1–14% O_2/0% CO_2; SHO + HC: severe hypoxia + hypercapnia 0–5% O_2/5–3% CO_2; MHO + HC: mild hypoxia + hypercapnia 5.1–14% O_2/2.9–1% CO_2; normoxia: 21% O_2. ○: awake (n=3); ■: behavioural sleep (n=3). **: p<0.01; ***: p<0.0001. For further abbreviations see legend to fig 3.

Effect of vigilance

Three rats slept throughout the experiment (behavioural sleep) and three others remained awake. In HO, all the SBP and HR changes were significantly higher during wakefulness than during sleep (p<0.001) (ANOVA). In HOHC, HR was only slightly higher during sleep (p<0.06) (fig. 5).

Discussion

Our data confirms that acute repetitive periods of HO or acute HO combined with HC for 10–12 min significantly increase SBP and DBP, and decrease HR. The new findings in this study are that: 1) the control experiment to test the gas stress (compressed air) showed no difference for HR and SBP compared to their baselines; 2) the haemodynamic changes observed in this acute repetitive inhalation model in rats were higher when HC was superimposed by HO; 3) the increase in SBP and the moderate decrease in HR were enhanced by wakefulness during HO.

In this acute inhalation model the gas stress is not responsible for the observed haemodynamic changes. This finding is in agreement with a recent study demonstrating that the chronic blood pressure of rats exposed for 5 weeks to episodic compressed air was unchanged [7].

The increases in SBP and DBP and the decreases in HR were significantly related to the level of hypoxia but not hypercapnia. However, the sampling for blood gas determination provided only averaged values for 1 min, therefore, including two full cycles of gas challenge and compressed air flushing. In agreement with this finding, STOOHS and GUILLEMINAULT [8] demonstrated in five men with severe obstructive sleep apnoea, that mean blood pressure always increased during the obstructive period and that the changes in HR were correlated with the degree of hypoxaemia. In experimental models of repetitive airway obstruction in anaesthetised dogs, IWASE *et al*. [9] showed that the main factor in the increase in systemic arterial pressure during repetitive airway obstruction was hypoxaemia and that hypercapnia and acidosis had no significant effects. Increases in apnoeic and postapnoeic blood pressure correlate with the degree of hypoxia during apnoea [1]. Periods of 35 days of episodic hypoxia in rats, mimicking the episodic hypoxaemia of sleep apnoea in humans, cause a diurnal increase in mean blood pressure of 14 mmHg [7]. The bradycardia that occurs during apnoea in adults has been found to be proportional to the degree of oxygen desaturation and the duration of apnoea [10]. BARKER *et al*. [11] demonstrated that oxygen administration during obstructive apnoea can attenuate bradycardia. However, in contrast to these data, the prevention of hypoxia during sleep apnoea syndrome

by supplementary oxygen does not appear to blunt the elevation of systemic pressure associated with apnoea termination [3]. Furthermore, our model shows arterial blood pressure elevation during the hypoxaemic period of the apnoea whereas ALI *et al*. [3] gave BP values only during the resumption of breathing after the apnoea. The pressor response during apnoea may be due to hypoxaemia whilst the postapnoeic pressor response may have other origins such as an increase in venous return due to mechanical changes. In another study, PELLETIER [12] showed, in dogs, that the pressor response to the stimulation of carotid chemoreceptors with hypoxic blood is augmented by hypercapnic acidosis. On the other hand, increasing P_{a,CO_2} by 1.4 kPa in anaesthetised dogs increased arterial pressure only by about 5 mmHg [13] when blood pressure increased more than 20 mmHg during the obstructive period in sleep apnoea [1]. These results [3, 5, 7] conflict with previous data [1, 8–10] and our finding showing the predominant role of hypoxaemia over hypercapnia in acute changes in SBP, DBP and HR. In our study, increases in SBP were higher with HOHC (+35.1±26.4 mmHg) than with HO (+25.3±25.7 mmHg) and HR decreased more with HOHC, (severe, -139.3±75.7 bpm; mild, -28.5±52.0 bpm) than HO (severe, -93.8±124.1 bpm; mild, -3.1±67.8 bpm) (fig. 3). These results may be explained by the interaction of CO_2 on the hypoxic response so that the magnitude of the BP rise with HO is shifted upwards by HC.

Another finding in our study is that the haemodynamic changes are significantly enhanced by wakefulness. Previous work by PINTO *et al*. [14] showed, in a porcine model of airway obstruction, that mean arterial pressure (MAP) increased by 5±2 mmHg on arousal during non-rapid-eye-movement (NREM) sleep. However, during rapid-eye-movement (REM) sleep, MAP was increased fourfold (22±2 mmHg) on arousal compared to NREM. Furthermore, it has been demonstrated that blood pressure increases are correlated with graded arousals in non-rapid-eye-movement (NREM) sleep [4, 5]. In our study, the SBP of waking animals was significantly increased ($p<0.0001$) in SHO compared to sleeping animals. The HR of sleeping animals decreased less, although significantly ($p<0.001$) compared to waking animals in HO (fig. 5).

The mechanisms by which hypoxaemia increases blood pressure and decreases HR are still the subject of debate. For blood pressure elevation, hypoxaemia may act through activating peripheral chemoreceptors which results in increases in systemic vascular resistance [15, 16]. On the other hand, WALKER and BRIZZEE [17] showed that baroreflexes are important components in cardiovascular responses to both hypercapnic and hypoxic stimuli in conscious rats. Cardiac brady-arrhythmias are mediated by vagal activity [18] and bradycardia through arterial chemoreceptor action [19]. However, the local vascular effect of hypoxia is vasodilatory. This effect seems to depend on the vessel type [20]. The mechanism of this vasodilator action by hypoxia is not yet clear. In recent studies vasoregulators derived from the endothelium, including prostaglandins [21] and nitric oxide [21, 22] may be implicated. This suggests that SAS may be associated with increased autonomic nervous system activity and endothelium derived activity.

The present acute, repetitive, inhalation model in rats, simulating gas changes observed during acute obstructive sleep apnoea in humans, may provide a new instrument for exploring and understanding the early mechanisms implicated in arterial hypertension associated with apnoea.

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