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Contractility of papillary muscle from rats exposed to 28 days of hypoxia, hypercapnia, and hypoxia with hypercapnia

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ABSTRACT The effects of chronic respiratory failure (hypoxia and hypercapnia) on the contractile properties of cardiac muscle are not established. A study was performed of the isometric contractile properties of isolated papillary muscle removed from rats exposed in a normobaric environmental chamber to 28 days of hypoxia (fractional inspired oxygen (F_{IO_2}) 10%, fractional inspired carbon dioxide (F_{ICO_2}) < 1%), hypercapnia (F_{IO_2} 21%, F_{ICO_2} 5%), and hypoxia with hypercapnia (F_{IO_2} 10%, F_{ICO_2} 5%). Rats exposed to both hypoxia and hypoxia with hypercapnia developed selective right ventricular hypertrophy. Exposure to hypercapnia alone did not alter right ventricular weight. No change in right ventricular papillary muscle contractility per unit muscle mass was observed as measured by maximum active tension, maximum rate of rise or fall of tension, or time to peak tension. Rat cardiac muscle adapts successfully to the altered acid-base environment and increased work load associated with prolonged exposure to hypoxia and mild hypercapnia.

Introduction

The functional state of the right ventricle in patients with chronic bronchitis and emphysema is controversial, with evidence for both normal and reduced contractility.[1–5] Information about the contractile performance of the right ventricle is important in view of the suggestion that pulmonary vasodilators may benefit patients with chronic respiratory failure[6] by lowering pulmonary artery pressure when contractile function is compromised. Experimentally cardiac muscle dysfunction often occurs before the onset of pump failure,[7] but the direct study of cardiac muscle function in patients with chronic bronchitis is impossible. The cardiopulmonary circulation of the rat is similar to that of man[8,9] and its response to hypoxia resembles that in man. We have used the laboratory rat to study the effects of exposure to prolonged hypoxia, hypercapnia, and hypoxia with hypercapnia on cardiac muscle function.

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Methods

THE CHAMBER

A normobaric environmental chamber was constructed in which rats could be maintained in a hypoxic and hypercapnic environment.[10] The inspired fractions of oxygen (F_{IO_2}) and carbon dioxide (F_{ICO_2}) were measured continuously and kept at preset levels by feedback circuits. Temperature was maintained at 23 ± 1°C by thermostat control and freezer units. A 12 hour light-dark cycle was used.

EXPERIMENTAL GROUPS

Groups of male CSE Wistar rats (initial weight 340–380 g) were maintained in the chamber for 28 days in either a normoxic-normocapnic environment (F_{IO_2} 21%, F_{ICO_2} < 1%; n = 12), a hypoxic-normocapnic environment (F_{IO_2} 10%, F_{ICO_2} < 1%; n = 10), a normoxic-hypercapnic environment (F_{IO_2} 21%, F_{ICO_2} 5%; n = 8), or a hypoxic-hypercapnic environment (F_{IO_2} 10%, F_{ICO_2} 5%; n = 8). The exposure to chamber gases was continuous except for two periods of less than 20 minutes each week when the rats were weighed and the food and water replenished. Chamber gases were checked twice daily (Corning 168 pH/blood gas analyser).

TAIL ARTERY CANNULATION

At the end of the 28 days tail artery cannulation was performed under ether anaesthesia. After recovery the rats were returned to the chamber and arterial blood gas tensions were measured.

MUSCLE MECHANICS

The rats were killed with ether and their hearts removed rapidly and placed in oxygenated Kreb's solution. The right ventricle was opened and an anterior right ventricular papillary muscle was removed and mounted in an organ bath between a fixed hook and an isometric tension transducer (Statham MX2PO). The muscle was superfused with oxygenated (gas mixture 95% oxygen, 5% carbon dioxide; pH 7·4 [0·01]) Krebs' solution (constituents in mmol/l; NaCl 118, $NaHCO_3$ 24, KCl 4, $Na_2H_2PO_4$ 0·4, $MgCl_2$ 1·0, $CaCl_2$ 1·8, glucose 5·56, sodium pyruvate 5·0) maintained at 37°C by a thermostat controlled water bath. The muscle was stimulated at 60 beats/minute, pulse width 2 ms, and amplitude 1·5 times the threshold voltage for one hour. After this maximum active tension (Tmax) the maximum rate of rise of tension (dT/dt max), the maximum rate of fall of tension (−dT/dtmax), and the time to peak tension (TPT) were recorded and the superfusate pH measured.

HEART WEIGHTS

The great vessels, atria, and atrioventricular valves were dissected from the ventricles. The free wall of the right ventricle was separated by dissection at the line of reflection between the right ventricle and the interventricular septum. The left ventricular cavity was opened and blood clots removed by washing. The free wall and left ventricle were blotted dry by a standard technique and weighed. Ventricular weights were expressed as milligrams of muscle per gram of body weight.

MUSCLE WEIGHTS

At the end of the experiment muscle length was measured by a calibrated Vernier eye piece. The muscle was taken from the bath (muscle beyond the silk ties being removed), blotted dry, and weighed (Sartorius balance type 1712). Muscle cross sectional area was calculated on the assumption that the muscle was a uniform cylinder of specific gravity 1·00.

PAPILLARY MUSCLE OXYGENATION

We performed our experiments at physiological temperatures and a stimulation rate of 60 beats/min. The oxygenation of superfused papillary muscle depends on diffusion and might become inadequate as muscle cross sectional area increases,[11] leading to artefactual loss of contractility. We tested the viability of the muscles of the right ventricle under these conditions by exposing the muscle to a step reduction in the oxygen tension (Po_2) of the superfusate.[11] Right ventricular papillary muscles from six normoxic controls (weight 400–450 g; mean (SEM) cross sectional area 0·55 (0·07) mm^2), similar to the largest muscles used in the study (table 2), were mounted in the organ bath under the conditions described above and Tmax was recorded. By being superfused with a second, identical circuit bubbled with a gas mixture of 80% oxygen, 5% carbon dioxide, and 15% nitrogen the muscle was subjected to a step reduction in Po_2 for 10 minutes and active tension recorded. The change in tension was expressed as a percentage of the tension achieved with 95% oxygen and 5% carbon dioxide.

CALCULATIONS AND STATISTICAL ANALYSIS

Rat right ventricle muscle is not uniform in shape and we have expressed mechanical properties in terms of unit muscle weight.[12] Mechanical properties were compared by means of the unpaired Student's *t* test. Tensions after changes in muscle oxygenation were compared by using paired *t* tests. Values of $p < 0·05$ were taken as significant.

Results

BLOOD GAS TENSIONS

Satisfactory arterial samples were obtained from seven controls, nine hypoxic, six hypercapnic, and eight hypoxic-hypercapnic animals. In the remainder displacement of the cannula or occlusion occurred before sampling. The hypoxic group was hypocapnic and alkalotic, whereas both the hypercapnic groups had a mild respiratory acidosis (table 1). Both hypoxic groups were polycythaemic. These findings agreed with previously published results.[13]

HEART WEIGHTS

Selective right ventricular hypertrophy developed in both the hypoxic groups (table 2). No significant change in the weight of the left ventricle occurred in any group.

Table 1 *Arterial oxygen and carbon dioxide tensions (Pao_2, $Paco_2$) and pH (mean (SEM) values) and packed cell volume (PCV) for conscious rats breathing chamber gases*

	n	*Pao_2 (kPa)*	*$Paco_2$ (kPa)*	*pH*	*PCV (%)*
Controls	7	12·2 (0·5)	5·1 (0·1)	7·42 (0·01)	44 (1)
Hypoxia	9	5·3 (0·1)*	2·9 (0·1)*	7·48 (0·03)	63 (1)*
Hypercapnia	6	13·3 (0·3)	7·6 (0·1)*	7·33 (0·01)*	41 (1)
Hypoxia-hypercapnia	8	6·4 (0·3)*	6·7 (0·1)*	7·32 (0·01)*	57 (1)*

*$p < 0·001$ in the comparison with controls.

Table 2 *Mechanical properties (means (SEM) values) of right ventricular papillary muscles from control rats and rats exposed to 28 days of hypoxia, hypercapnia, and hypoxia with hypercapnia*

	Controls	*Hypoxia*	*Hypercapnia*	*Hypoxia-hypercapnia*
Number	12	10	8	8
Tmax (kN/kg)	8·9 (0·9)	7·8 (1·2)	9·8 (1·9)	8·9 (1·1)
+dT/dtmax (kN/s/kg)	49 (5)	64 (8)	62 (10)	46 (9)
−dT/dtmax (kN/s/kg)	36 (5)	44 (6)	47 (11)	45 (11)
TPT (ms)	94 (3)	101 (4)	96 (4)	101 (4)
Cross sectional area (mm^2)	0·42 (0·04)	0·59 (0·05)*	0·44 (0·06)	0·63 (0·10)*
Left ventricle weight (mg/g body weight)	1·93 (0·03)	1·89 (0·05)	2·03 (0·02)	1·93 (0·02)
Right ventricle (mg/g body weight)	0·45 (0·01)	0·75 (0·02)**	0·45 (0·01)	0·63 (0·01)**

*$p < 0.05$; **$p < 0.001$.
1 kilonewton = 10^8 dyne.

PAPILLARY MUSCLES

The cross sectional areas of the papillary muscles of the right ventricle from the hypoxic groups were significantly greater than those from the other groups (table 2).

MUSCLE OXYGENATION

Superfusate Po_2 fell from a mean (SEM) of 86·9 (3·6) to 66·9 (2·5) kPa. Active tension was unchanged at 100% of the initial value (95% confidence interval 96–104%; n = 6). No change in resting tension was observed.

MECHANICAL PROPERTIES

The mechanical properties of the control group (table 2) were similar to those reported by Fry and Poole-Wilson.[12] There was no significant difference in contractile properties between any of the acclimatised groups and the controls.

Discussion

In this paper we have shown that the isometric contractile properties of right ventricular papillary muscle from rats exposed to 28 days of hypoxia, hypercapnia, and hypoxia with hypercapnia are not depressed when studied in vitro. This result is surprising in view of previous work on experimental right ventricular hypertophy, where a fall in the contractility of the right ventricle has often been reported.[7 14–17]

We have found that exposure to chronic hypoxia results in selective right ventricular hypertrophy, and our findings are similar to those of previous reports.[8] Right ventricular hypertrophy may reduce cardiac muscle contractility as a result of inadequate capillary supply, poor energy reserves, and loss of mitochondria.[14 18] These observations have been made on cardiac muscle that has hypertrophied as a result of pulmonary artery banding.[7 14–17] This procedure causes very rapid and substantial changes in the morphology of the right ventricle and may lead to the death of an appreciable number of myocardial cells[19] so that, not surprisingly, the muscle function of the right ventricle is often compromised. Spann[20] has reviewed the factors governing the contractile function of hypertrophied muscle and concluded that the degree of hypertrophy is of major importance. In the rat exposure to chronic hypoxia produces only moderate hypertrophy of the right ventricle and does not result in the gross hypertrophy seen with pulmonary artery banding. The degree of right ventricular hypertrophy observed in our rats is similar to that reported in patients with chronic bronchitis and emphysema.[21] We conclude that exposure to chronic hypoxia results in only a moderate degree of right ventricular hypertrophy which is unlikely to depress cardiac function.

Exposure to chronic hypercapnia might also be expected to depress cardiac function. An acute rise in ambient carbon dioxide tension reduces extracellular pH (pHe) and rapidly reduces intracellular pH (pHi). The rise in $[H^+]_i$ depresses cardiac contractility.[12] We have measured contractility in vitro at a standard pHe of 7·40. In vivo cardiac muscle from the rats exposed to chronic hypercapnia was functioning in a more acidotic environment (pHe 7·33) and this could result in depressed contractility. We have previously shown that right ventricular muscle from rats exposed to chronic hypercapnia has an improved resistance to respiratory acidosis.[22] We have used these data to calculate the expected loss of contractility resulting from a change in pHe from 7·40 to 7·33 and found only a small fall in Tmax of 11%. The value of Tmax corrected for the in vivo respiratory acidosis of the hypercapnic group is 8·7 kN/kg and is not significantly different from that of the control muscles (8·9 kN/kg). The contractility of right ventricular papillary muscle from rats exposed to chronic hypercapnia is unlikely to be reduced even under in vivo acid-base conditions.

There has been little work on the mechanical properties of cardiac muscle exposed to chronic hypoxia and hypercapnia. Kentera *et al*[23] maintained rats in an environment of 10% oxygen and 5% carbon dioxide for 28 days and studied the isometric proper-

ties of right ventricular muscle. Arterial blood gas tensions were not recorded and no groups exposed to pure hypoxia or hypercapnia were studied. Isometric properties were recorded under less physiological conditions than in the present study. Despite these differences they also found no significant loss of contractile function and our findings support and extend their observations.

Rat cardiac muscle adapts in the medium term to the altered work load and metabolic environment associated with exposure to chronic hypoxia and mild hypercapnia with no loss of contractile function. If these results are applicable to the human myocardium, therapeutic interventions designed to improve the contractility of the right ventricle in early chronic respiratory failure may not be successful.

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