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Hypoxia, not hypercapnia, induces cardiorespiratory failure in rats

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ABSTRACT

Mechanical respiratory loads induce cardiorespiratory failure, presumably by increasing O_2 demand concurrently with decreases in O_2 availability (decreased PaO_2). We tested the hypothesis that asphyxia alone can cause cardiorespiratory failure ("failure") in pentobarbital-anesthetized rats. We also tested the hypothesis that hypoxia, not hypercapnia, is responsible by supplying supplemental O_2 during mechanical loading in a separate group of rats. Asphyxia (mean PaO_2 and $PaCO_2$ of 43 and 69 mmHg, respectively) resulted in failure, evident as a slowing of mean respiratory frequency (133–83 breaths/min) and a sudden and large drop in mean arterial pressure (71–47 mmHg), after 214 ± 66 min (n = 16; range 117–355 min). Neither respiratory drive nor heart rate decreased, indicating that failure was peripheral, not central. Of 8 rats tested after 3 h of asphyxia for the presence in blood of cardiac troponin T, all were positive. In an additional 6 rats, normocapnic hypoxia (mean $PaCO_2$ and PaO_2 were 39 ± 2.2 and 41 ± 3.1 mmHg, respectively) caused failure after an average 205 min (range 181–275 min), no different from that of asphyxic rats. In the 6 rats that breathed O_2 during an initially moderate inspiratory resistive load, endurances exceeded 7 h (failure occurring only because we increased the load after 6 h) and tracheal pressure and left ventricular dP/dt were maintained despite supercarbia ($PaCO_2$ > 150 mmHg). Thus, asphyxia alone can induce failure, the failure is due to hypoxia, not hypercapnia, and hypercapnia has minimal effects on cardiac and respiratory muscle function in the presence of hyperoxia.

1. Introduction

Mechanical respiratory loads have almost always been used to study the effects exclusively on the respiratory system, either in terms of gas exchange or respiratory muscle function. Recently, we showed that anesthetized rats breathing air against mechanical loads—inspiratory resistive (IRL) (Simpson and Iscoe, 2007), repeated inspiratory occlusions (Simpson et al., 2008), or expiratory threshold (ETL) (Simpson et al., 2009)—develop acute cardiorespiratory, rather than just respiratory, failure. (Unless qualified, 'failure' hereafter means 'cardiorespiratory failure'.) The loads cause both skeletal (presumably diaphragmatic) and cardiac injury, as indicated by the release of skeletal troponin I (Simpson and Iscoe, 2007; Simpson et al., 2004) and cardiac troponin T (Simpson and Iscoe, 2007; Simpson et al., 2009), respectively.

Fatigue and eventual failure of a muscle, possibly accompanied by injury, occur when oxygen delivery is inadequate to meet its metabolic demands. Load-induced hypoventilation decreases arterial O_2 content and, therefore, O_2 delivery if perfusion does not increase sufficiently to compensate. All loads increase the diaphragm's workload but a mechanical load increases it more because of the combined effects of, for example, increased resistance or decreased resting length and increased chemical respiratory drive. However, the effect on the heart depends on both the type of load and changes in arterial blood gases. Inspiratory loads, by decreasing intrathoracic pressure, increase left ventricular afterload (Pinsky, 2005) but this can be offset by peripheral vasodilation induced by arterial hypercapnia and hypoxemia. An ETL, however, increases intrathoracic pressure and therefore decreases left ventricular workload; in contrast, the same load increases right ventricular workload because of stretch-induced narrowing of pulmonary blood vessels and hypoxic pulmonary vasoconstriction, accounting for right ventricular injury (Simpson et al., 2009). Again, these effects on afterload can be offset by changes in arterial blood gases.

Because ETL causes failure despite a decreased left ventricular afterload, reduced O_2 delivery, not increased metabolic demand, is likely responsible. We therefore tested the hypothesis that asphyxia (hypercapnic hypoxia) alone, not a mechanical load, induces failure in anesthetized rats. We also tested the hypothesis that the hypoxia, not the hypercapnia, is responsible by having another group of rats breathing a normocapnic hypoxic gas

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mixture and by providing supplementary O_2 to a third group of rats breathing against a severe IRL.

2. Methods

Experiments, approved by the Animal Care Committee of Queen's University and in conformity with the guidelines of the Canadian Council on Animal Care, were conducted on 16 pentobarbital-anesthetized Sprague-Dawley rats (300–460 g; 65 mg/kg i.p., supplemented as required to prevent a pedal reflex) prepared as described previously (Simpson et al., 2004). In brief, after a surgical plane of anesthesia was established, the rat was placed supine. Body temperature was maintained with a servo-controlled heating pad. One port of a tracheal cannula was connected to a pressure transducer to measure tracheal pressure (P_{tr}). The right carotid artery (for measuring blood pressure (Cybersense CyQ BPM01, Nicholasville, KY) and sampling arterial blood gases (Radiometer ABL-5, Copenhagen, Denmark)) and jugular vein (for administration of additional anesthetic) were cannulated. The left phrenic nerve was isolated and its activity recorded en passant, amplified and filtered (100–10,000 Hz; Grass P-511, Quincy, MA) and integrated (time constant 50 ms). We recorded transdiaphragmatic pressure (P_{di}) as the difference between abdominal (P_{ab}) and esophageal (P_{es}) pressures (Millar SPR 524, Houston, TX). All signals (blood pressure, integrated phrenic activity ($\int$Phr), and P_{tr}, P_{es}, P_{ab}, and P_{di}) were acquired to computer (CED Spike2, Cambridge, UK).

2.1. Chemical loading

A two-way valve (Hans Rudolf 2300, Kansas City, MO) was attached to the other port of the tracheal cannula. Before loading, a blood sample was taken to ensure that PaO_2 and $PaCO_2$ were >70 and <50 mmHg, respectively. To induce asphyxia, we connected the output of a gas blender (Voltek Enterprises, Toronto, ON, Canada) to a bag on the inspiratory port of the valve. We adjusted the fractional concentrations of inspired N_2, O_2 and CO_2 to yield asphyxic arterial blood gases with a PO_2 and PCO_2 in the low 40 s and high 60 s, respectively, values similar to those obtained when breathing air against a moderate IRL (Simpson et al., 2004). In other words, these rats were subjected to the same chemical load but without the mechanical load. The duration of loading (endurance) was measured as the time from reaching the target blood gas values until mean arterial pressure (MAP) fell below 50 mmHg (cardiovascular failure). Because of inter-animal variations in endurance, load durations were normalized; onset was considered as normalized time = 0 and load removal as normalized time = 1.

In eight of the rats, we measured cardiac troponin T before and after chemical loading (Roche E170, Laval, Quebec, Canada). In a different subset of four rats, we evaluated the effect of asphyxia-induced failure on myocardial contractile reserve by infusing dobutamine after the termination of loading (Simpson et al., 2008).

To differentiate the role of hypoxia from hypercapnia, we studied six additional rats breathing a gas mixture that produced normocapnic hypoxia. In another six rats, we supplied 100% O_2 during a severe IRL (75% of the peak P_{tr} generated during the 30 s occlusion administered before loading) in order to produce hyperoxic hypercapnia. Because of the anticipated increase in load endurance, we simplified the experimental preparation by eliminating recordings of both phrenic activity and P_{di}; in four, however, we measured left ventricular (LV) pressure and its rate of change (LV dP/dt) using a transducer-tipped miniature catheter (Millar SPR-407, Houston, TX).

2.2. Blood and cardiorespiratory analysis

Analysis was done as previously described (Simpson et al., 2004, 2008, 2009; Simpson and Iscoe, 2007). In brief, before and within 5 min after termination of loading, plasma samples of eight rats were tested for the presence of cardiac troponin T (a cardiac-specific marker of myocardial injury; Roche E170; Roche Diagnostics, Laval, QC). Diaphragmatic contractility was measured as the ratio of its output, P_{di}, divided by its input, peak $\int$Phr ($P_{di}/\int$Phr). Respiratory timing (frequency, f) and the durations of inspiration (T_I) and expiration (T_E) along with the fraction of the total cycle spent in inspiration (T_I/T_{TOT}) were measured from the tracing of $\int$Phr except before inhalation of the asphyxic gas mixture when the $\int$Phr signal was too small; in this case, we used the P_{tr} tracing. Respiratory drive was represented by two indices, the rate of rise of $\int$Phr during inspiration ($\int$Phr/T_I) and the minute phrenic activity (MinPhr; $\int$Phr × f).

2.3. Statistics

At each decile of load duration, at least 30 breaths, excluding sighs and the post-sigh breath, were analyzed. Data are presented as means ± SD or SEM, as indicated. Normality and equal variance were determined before analysis of raw, not normalized, data. Paired t tests or one-way ANOVA, Holm–Sidak corrected, for multiple comparisons was used to compare data; $p < 0.05$ was considered significant. Because most parameters changed from control to the first and all subsequent loaded values (indicated by the dagger (†) in the figures), we determined when, during loading, a given parameter changed by comparing all loaded values to those at $t = 0.1$; these are indicated by asterisks, unless otherwise indicated. For analysis of cardiac troponin T data, Mann–Whitney rank sum test was used.

3. Results

A sample tracing from a representative asphyxic (hypoxic and hypercapnic) rat is shown in Fig. 1. Inhalation of asphyxic gas modestly increased $\int$Phr, P_{di} and P_{tr}; pulse pressure increased. Changes in these parameters are more evident in the lower panels; they reveal that the larger deflections in the top tracing were due to sighs. Failure was evident as a slowing in f and a sudden and large drop in blood pressure (panel d). Average endurance on the asphyxic gas mixture was 214 ± 66 (SD) min ($n = 16$; range 117–355 min); these times were normally distributed (Kolmogorov–Smirnov).

Changes in average blood gases and pH over time are depicted in Fig. 2. PaO_2, $PaCO_2$, and pH were stable, PaO_2 and $PaCO_2$ averaging 43 ± 0.8 and 66 ± 1.8 mmHg over the last five samples. Asphyxia increased f due to a decrease in T_E; as a result, T_I/T_{TOT} increased (Fig. 3); at failure, f decreased due to increases in both T_I and T_E.

Diaphragmatic contractility ($P_{di}/\int$Phr) decreased ~21 min after load onset and remained at this level until failure (Fig. 4A). Both indices of respiratory drive, $\int$Phr/T_I and MinPhr, increased immediately after load onset and remained stable until failure when both increased again (Fig. 4B and C).

Changes in heart rate (HR) and mean arterial pressure (MAP) are shown in Fig. 5. There was a gradual increase in HR and a steady fall in MAP, until a sudden decline at the time of failure. Just before cardiorespiratory failure, both P_{tr} and MAP fell rapidly; in 11 of the rats, the onsets of these falls were coincident.

Of the eight rats tested for the presence of cardiac troponin T, six were positive (*i.e.*, greater than the upper limit of a (human) reference population) after 3 h of asphyxic loading (<0.01–0.072 ng/L [25% = 0.046, 75% = 0.364], $p = 0.008$).