Cardiovascular changes in anesthetized rats during exposure to 30 bar

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Stuhr LEB, Ask JA, Tysssebotn I. Cardiovascular changes in anesthetized rats during exposure to 30 bar. Undersea Biomed Res 1990; 17(5):383–393.—The effect of exposure to 30 bar (PHe = 29.0 bar, PN₂ = 0.8 bar, PO₂ = 0.2 bar) on left ventricular pressure, cardiac contractility, heart rate (HR), and arterial pressure was studied in anesthetized rats. During compression there was a progressive increase in maximal left ventricular pressure (LVPₘₚ), maximal velocity of LVP rise (+dP/dtₘₚ), and fall (−dP/dtₘₚ), systolic pressure (APSys), and pulse pressure (ΔAP). The greatest increase in contractility per bar was found between 1 and 5 bar. Immediately after 30 bar was reached, LVPₘₚ (19%), +dP/dtₘₚ (69%), and −dP/dtₘₚ (22%), APSys (19%), and ΔAP (43%) were significantly increased from pretive values, with an additional increase detected for all these variables after 60 min at 30 bar. An increase in estimated oxygen consumption (work load) of the heart was also found during compression to and at 30 bar. No changes in HR, mean arterial pressure, and end-diastolic pressure were observed during the high-pressure exposure, indicating that the inotropic changes were not due to changes in peripheral hemodynamics.

A number of changes in cardiac function, such as decreased heart rate, slowed impulse propagation, and decreased excitation-contraction coupling, have been observed during exposure to high ambient pressure (60–200 bar) surpassing the depths reached in manned diving (1–5). Despite the fact that hydrostatic pressure has been shown to compromise the electrical activity in cardiac cells, pressure has a positive inotropic action on the myocardium at high ambient pressure (100–200 bar) (6, 7).

Limited information is available concerning cardiac contractility and blood pressure changes during hyperbaric exposure within depths of present diving activity and in particular during a substantial time period at pressure. A recent in vivo experiment on rats has shown that cardiac contractility is markedly enhanced even during an elevation of the ambient pressure to 5 bar (8). However, the heart rate, end diastolic pressure, and mean arterial pressure were unchanged in this experiment. A study performed on anesthetized cats exposed to 305 msw for a few minutes (5), and one
study performed on auricular preparations (rats) hydrostatically compressed to 5, 30, and 60 bar (9) for 60 min, showed similar elevations in contractility. However, the in vitro study showed that the maximal effect in contractility was reached at 30 bar, and that further elevation of the ambient pressure gave no additional increase in cardiac contractility.

The aim of the present study was therefore: a) to describe the cardiovascular changes in pentobarbital-anesthetized rats during compression to and at normoxic 30 bar (60 min), and b) to estimate the oxygen consumption at the different ambient pressures.

METHODS

The investigation was undertaken on male Wistar rats weighing 300–400 g.

Pressure chamber

The experiments were performed in a 30-liter pressure chamber with an internal diameter of 25 cm. An external water jacket regulated the chamber gas temperature to keep the rat thermally neutral. The chamber atmosphere was continuously circulated to provide adequate gas mixing and to eliminate CO₂ by ventilating the gas atmosphere through soda lime. The frontal port window allowed visual inspection of the animal. The rear port contained electrical penetrator plugs and the gas inlet and outlet. For detailed description see (8).

Surgery and blood pressure measurements

The rats were intraperitoneally anesthetized with pentobarbital (50 mg/kg bw) and immediately placed on a heating pad after induced anesthesia. The core temperature was recorded with a thermistor inserted 4 cm into the rectum and maintained at 37° ± 0.5°C by the heating pad during surgery and by the chamber gas temperature at high ambient pressure (32°–35°C).

A 6-cm long, saline-filled PE-50 catheter (Clay Adams, Persepany, NJ) was placed 4 cm upstream in a femoral artery and connected to an AME-840 pressure transducer (AME, Horten, Norway) to record arterial pressure (AP), pulse pressure (ΔAP), and heart rate (HR) on a HP recorder (model 7754 A, Hewlett Packard, MA) running at a speed of 100 mm · sec⁻¹.

The right common carotid artery was carefully dissected free avoiding damage to the vagal nerve. A high-fidelity micromanometer (Millar Micro Tip pressure transducer, Millar Instruments, Houston, TX), with a diameter of 0.78 mm, was gently introduced into the left ventricle via the carotid artery for continuous recording of the left ventricular pressure (LVP). The entrance into the ventricle was demonstrated by pressure readings from 0 to systolic pressure. The catheter was fixed in the position at which the ventricular pressure was recorded without artifacts. The maximal LVP during cardiac systole was termed LVP_max. The maximal velocity of the LVP increase (+ dP/dt_max) and decrease (− dP/dt_max) was determined from the LVP curves by placing tangents along the steepest portion of the curve. The reported dP/dt was the mean of three consecutive measurements. All calculations were performed by the
same person. Comparison of manually drawn tangents correlated within ±2% of electrically differentiated curves (Hewlett Packard derivator computer 8814A) (9). The capacity of the pressure transducer (Millar Micro Tip) to measure rapid changes in blood flow was previously tested using a "multifunction pressure generator" (model NGA-200, Millar Instruments). Constant amplitude of generated sinus and sawtooth pressure waves was found up to 90 Hz by the transducer. The deflection of differentiated pressure signals and frequency of sawtooth and sinus waves were found to be linear up to 80 and 70 Hz, respectively. Theoretical calculations using the measured differentiated deflections of generated waves indicate that the values of 12,000 mmHg · sec⁻¹ could be detected correctly by this setup.

A saline-filled PE-50 catheter was introduced into the right femoral vein and connected to a cannula that penetrated the rear chamber port.

Experimental procedure

Two series of experiments were performed.

Series 1 (n = 4), control experiments

Control experiments were performed for 180 min at 1 bar to evaluate the surgical level of the pentobarbital and its effect on blood pressure parameters and HR over time.

Series 2 (n = 7), pressure experiments

The animals were placed in the pressure chamber with the transducers and the thermistor connected to electrical connections inside the chamber. The rats were monitored for 15 min after instrumentation to ensure stable hemodynamic predive values. These animals were compressed to 30 bar by He at a rate of 1 bar · min⁻¹ to 5 bar, and then 2 bar · min⁻¹ for the rest of the compression phase. A stop of 1 min on each bar was made before blood pressure measurements were performed at 5, 10, 20, and 30 bar. The rats were exposed to 30 bar for 60 min and blood pressure was recorded every 5 min at 30 bar. During compression and stable elevated pressure, the partial pressure of O₂ was kept at the normal level of 0.21 bar. The rats were killed after 60 min at pressure with a lethal dose of pentobarbital, injected through the cannula penetrating the rear chamber port and into the right femoral vein, before rapid decompression.

Estimation of oxygen consumption (\( \dot{V}O_2 \))

The \( \dot{V}O_2 \) was estimated by using the factors: HR, LVP\(_{max}\), and \(+dP/dt\)\(_{max}\) (10).

Statistics

All results are given as means ± SE. Statistical evaluation within a series was made with Wilcoxon’s signed rank test (two tailed). Statistical differences between the results of control (series 1) and test experiments (series 2) and comparison of the
increase in dP/dt with other variables were calculated by use of Student’s t test. P values less than 0.05 were considered significant.

RESULTS

Control measurements, series 1, 1 bar

Measurements of the LVP\textsubscript{max}, +dP/dt\textsubscript{max}, −dP/dt\textsubscript{max}, and HR at normal ambient pressure over a period of 80.5 min (corresponding to the time needed for the experiments to 30 bar, series 2) are in Fig. 1, open squares. No significant changes were

Fig. 1. Changes in maximal left ventricular pressure (LVP\textsubscript{max}, top left), maximal velocity of LVP rise (+dP/dt\textsubscript{max}, top right), maximal velocity of LVP fall (−dP/dt\textsubscript{max}, bottom left), and HR (bottom right) in control experiments at 1 bar (open squares), predive (open circles), and at 30 bar (solid circles). Asterisk = P < 0.01 vs. predive values. Dagger = P < 0.01 vs. control values (series 1).
found in the measured parameters during this time nor during the rest of the total 180-min recording period.

**Predive control measurements, series 2, \( n = 7 \)**

Recordings of the LVP and AP from one representative rat after the stabilization period are given in Fig. 2, *left*, and all average predive values are presented in Table 1, *left column*.

**Pressure exposure measurements, series 2**

*Compression phase (1–30 bar).* The gradual increase in LVP and AP from 1 representative experiment is shown during compression to 30 bar in Fig. 2. All average values are presented in Table 1. Figure 3 shows that the greatest increase in cardiac inotropic variables was found during elevation from 1 to 5 bar (LVP_{\text{max}} = 15\%, \( P < 0.01 \); +dP/dt_{\text{max}} = 32\%, \( P < 0.01 \); -dP/dt_{\text{max}} = 12\%, \( P < 0.05 \)). No significant increase in any of the variables was found between 5 and 10 bar. Between 5 and 20 bar, only LVP_{\text{max}} increased significantly. However, LVP_{\text{max}}, +dP/dt_{\text{max}}, -dP/dt_{\text{max}}, and systolic pressure (APsys) were enhanced between 5 and 30 bar (5–14\%, \( P < 0.05 \)). Additionally, Fig. 3 shows a significantly (\( P < 0.01 \)) higher elevation of +dP/dt_{\text{max}} than of -dP/dt_{\text{max}} and LVP_{\text{max}} throughout the compression. An increase in oxygen consumption (\( \dot{V}_{O_2} \)) of 40\% was estimated when 30 bar was reached.

**Stable elevated pressure, series 2, 30 bar**

Immediately after reaching 30 bar, LVP_{\text{max}}; +dP/dt_{\text{max}}; -dP/dt_{\text{max}}; APsys; and \( \Delta \text{AP} \) were enhanced by 19, 60, 22, 19, and 43\%, respectively, compared to predive control values at 1 bar (Table 1, Fig. 3). During stable hyperbaric exposure, gradual elevations of these pressure parameters were observed (Table 1, Figs. 1, *top left and right and bottom left, and 2*). After 60 min, LVP_{\text{max}}; +dP/dt_{\text{max}}; -dP/dt_{\text{max}}; APsys; and \( \Delta \text{AP} \) were increased by 36, 80, 42, 30, and 52\%, respectively, compared to

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**Fig. 2.** Recording of (LVP) and (AP) in one single experiment at 1, 5, 10, 20, and 30 bar (0–60 min).
<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>CARDIOVASCULAR CHANGES DURING EXPOSURE TO HIGH AMBIENT PRESSURE N = 7</td>
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<table>
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<tr>
<th>Pressure (bar)</th>
<th>15</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
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<td>Minutes</td>
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<tr>
<td>LVP(_{\text{max}}), mmHg</td>
<td>110 ± 6</td>
<td>126 ± 6(^a)</td>
<td>129 ± 5</td>
<td>131 ± 5</td>
<td>131 ± 6(^a)</td>
<td>135 ± 7(^b,c)</td>
<td>137 ± 6(^b,c)</td>
<td>140 ± 6(^b,c)</td>
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<tr>
<td>+dP/dt(_{\text{max}}), mmHg · sec(^{-1})</td>
<td>5871 ± 385</td>
<td>7746 ± 491(^a)</td>
<td>8350 ± 435</td>
<td>8832 ± 365</td>
<td>9395 ± 495(^a,b)</td>
<td>9395 ± 495(^b,c)</td>
<td>9395 ± 495(^b,c)</td>
<td>9475 ± 475(^b,c)</td>
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<tr>
<td>-dP/dt(_{\text{max}}), mmHg · sec(^{-1})</td>
<td>4904 ± 425</td>
<td>5495 ± 380(^a)</td>
<td>5599 ± 435</td>
<td>5875 ± 456</td>
<td>5992 ± 449(^a,b)</td>
<td>6375 ± 388</td>
<td>6210 ± 438(^b,c)</td>
<td>6558 ± 498(^b,c)</td>
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<tr>
<td>APr, mmHg</td>
<td>108 ± 5</td>
<td>124 ± 5(^a)</td>
<td>127 ± 6</td>
<td>129 ± 5</td>
<td>129 ± 7(^a,b)</td>
<td>133 ± 7</td>
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<td>APd, mmHg</td>
<td>66 ± 4</td>
<td>69 ± 6</td>
<td>67 ± 5</td>
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<td>ΔAP, mmHg</td>
<td>42 ± 3</td>
<td>55 ± 3(^a)</td>
<td>57 ± 3</td>
<td>55 ± 3</td>
<td>60 ± 4(^b)</td>
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<td>AER, mmHg</td>
<td>80 ± 8</td>
<td>88 ± 3</td>
<td>89 ± 7</td>
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<td>EDP, mmHg</td>
<td>3.9 ± 0.2</td>
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<td>3.8 ± 0.2</td>
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<td>HR min(^{-1})</td>
<td>360 ± 20</td>
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<td>Temp, °C</td>
<td>37.7 ± 0.3</td>
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Key: LVP\(_{\text{max}}\) = maximal ventricular blood pressure; +dP/dt\(_{\text{max}}\) = maximal velocity of LVP raise; -dP/dt\(_{\text{max}}\) = maximal velocity of LVP fall; APr = systolic arterial pressure; APd = diastolic arterial pressure; AP = pulse pressure; AER = mean arterial pressure; EDP = end-diastolic pressure; HR = heart rate, Temp = rectal temperature.

All values are given as means ± SE. *P < 0.02 comparing 2 and 5 bar, 1 and 30 bar (0 min); **P < 0.05 comparing 5 and 30 bar; ***P < 0.05 when comparing different times at 30 bar to 1 min at 30 bar.
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Fig. 3. Change in LVP_max, +dP/dt_max, and −dP/dt_max during compression to 30 bar (5, 10, 20 bar) and at 30 bar (0 and 60 min) in percent of control (1 bar).

predive values, which are significantly higher (P < 0.05) than values obtained immediately after 30 bar was reached.

The LVP_max, +dP/dt_max, and −dP/dt_max were significantly (P < 0.01) increased during the 30 bar experiment (series 1) compared to the control experiments (series 2). On the other hand, the ΔAP, diastolic arterial pressure, end diastolic pressure (EDP), and HR (Fig. 1, bottom right) remained at control levels during compression and at stable elevated pressure (Table 1). The Vo2 increased by 60% from control values after 60 min at 30 bar.

DISCUSSION

These experiments have shown an increased cardiac contractility (60–80%), based on the findings of enhanced +dP/dt_max, whereas HR was unchanged in pentobarbital-anesthetized rats exposed to 30 bar for 60 min. The +dP/dt_max has proved to be a useful index for cardiac contractility measurement (11–15). This index correlated well with changes in force of contraction of the heart measured by a gauge arch (11), and quantitative changes in +dP/dt_max, have been shown to occur after injection of catecholamines (16, 17). Absolute LVP_max and the ratio of dP/dt to LVP have also been used for measuring cardiac contractility (18, 19). However, regardless of which index we used in the present experiments, an increased cardiac contractility was found, although the relative increase differed to some extent. Our conclusion is based on the use of a special catheter-tip micromanometer without possible gas bubbles, thoroughly calibrated and evaluated (8), and capable of sensing pulses directly within the cardiac chamber.

The +dP/dt_max is insensitive to changes in afterload but moderately sensitive to preload (20). In our study, an elevated +dP/dt_max was found without changes in preload (EDP). This finding, together with the constant ΔAP and HR, indicates that the increased +dP/dt_max found during pressure exposure is not caused by changed blood pressure, but most likely by an elevated contractile state (inotropy) of the myocardium. This hypothesis is supported by results from recent in vitro experiments where a corresponding increase in the tension and velocity of tension (~70%) during
unchanged preload and beating frequency was observed in auricular preparations from rats during exposure to 30 bar (9).

During the compression phase, a rapid initial increase in cardiac contractility followed by a gradual increase was found. There was a similar, but not as rapid and extensive, response in \(-\frac{dP}{dt_{\text{max}}}\) as in \(+\frac{dP}{dt_{\text{max}}}\) to elevated ambient pressure, indicating that the contraction and relaxation phases of the ventricular myocardium are both stimulated by the hyperbaric environment, although not to the same extent.

The present study showed a 30% increase in cardiac contractility when the ambient pressure was increased to 5 bar and a further 30% increase in contractility at 30 bar, which means that the greatest increase in cardiac contractility per bar is observed nearest to surface pressure (between 1 and 5 bar). During compression there might be a phase lag between contractility changes and ambient pressure changes reflected in the results at 5, 10, and 20 bar. However, the consequence of this would be an underestimation of the contractility during compression in the present study.

Thermal stress might have influenced the measurements at 30 bar due to the necessity for high ambient temperature during pressure exposure using He as inert gas. Experiments on Wistar rats (21) exposed to a He-O₂ atmosphere at 1 bar, documented pronounced metabolic and hemodynamic disturbances at ambient temperatures of 23°–28°C. On the other hand, these changes were almost completely absent at 33°C. This agrees with findings at 71 bar (22), where the chamber temperature was held within a temperature range similar to that of our study, showing no change in the level of stress-related parameters like free fatty acids and corticosterone. Thus, there is little reason to believe that the blood pressure parameters in the anesthetized rats exposed to 30 bar were influenced by the relatively high temperature within the pressure chamber.

During stable pressure at 30 bar a gradual increase in left ventricular contractility was observed. Such a rise was not found in the experiments to 5 bar, where a constant contractility was observed during 60 min (8). A theoretical explanation could be that the surgical level of the anesthesia was reduced at the end of the experiments. However, this is not likely because the control experiments with equal pentobarbital doses showed no change in the blood pressure measurements and HR for a period of 3 h. High ambient pressure is known to antagonize the effect of anesthesia (23, 24). However, the observed increase in the blood pressure parameters LVP, \(+\frac{dP}{dt}\), and \(-\frac{dP}{dt}\) found in the present study did not differ from those previously found in conscious rats of the same strain (25). Furthermore, hydrostatically compressed heart preparations from rats (9) have shown increases in peak tension and velocity of tension at 30 bar similar to those observed in the present study, giving no evidence that reversibility of anesthesia at pressure influenced the results in the present study. The cardiac contractility remained elevated during the exposure to 30 bar. Theoretically this could be a delayed lagging pressure effect more noticeable at 30 bar than at 5 bar. However, the 60-min measuring time at 30 bar is probably too long for a lagging effect to account for this inotropic enhancement after 60 min.

Sympathetic activation of the adrenoceptors may be one factor explaining the increased contractility found in the present study. However, since recent in vitro studies (9) have shown an equal increase in cardiac contractility at 30 bar, although general α, β, and muscarine receptor blockers were used, it is not likely that cardiac adrenoceptor activation is involved. The unchanged HR found in the present study also supports this suggestion. Therefore other factors, such as increased calcium
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sequestration, might be involved. This suggestion is supported by the recording of an increased duration of the plateau phase of the action potential when cardiac tissue was subjected to hyperbaric conditions (26). This might increase the calcium flux through the myocardial cell membrane and trigger a greater calcium release from the sarcoplasmic reticulum (27).

As a consequence of the enhanced cardiac contractility and pumping pressure (LVP) found in the present study, the oxygen need of the rat heart is likely to increase. This is supported by the increase in oxygen consumption (workload) found. The increased workload on the heart may not be limited to rats because an increase in tension and velocity is found in human heart preparations exposed to 5 bar (28).

Bradycardia is a common finding in hyperbaric environments both in rat (2, 6), guinea-pig, rabbit, dog, and mouse in vitro (2) and in man (29), cat (5), and mice (30) in vivo. However, bradycardia was not detected in the present study, or with in vitro or in vitro studies on rats at pressures of 5, 30, 60, and 71 bar during normoxic conditions (8, 9, 27). In addition, inconsistent cardiac responses have been reported in man, showing no change in beating frequency (31, 32) or gradual disappearance of bradycardia in mice at high ambient pressure (30). The dominant single factor reducing HR is increased Po2 (33), and the normoxic conditions in the present study might explain the unchanged frequency.

In our study, APsys and ΔAP were increased during compression to 30 bar, possibly evoking the baroreceptor responses and inducing HR reduction. Inasmuch as we found a constant HR, that is not likely. An increased stroke volume would theoretically also be a reason for this, but similar experiments on rats have shown an unchanged stroke volume (34).

In our experiments, APsys increased concomitant with elevation of LVPmax during both compression to and at 30 bar. The observation of an enhanced APsys is reflected by an unchanged AP and increased ΔAP. Since other experiments to elevated ambient pressure on rats (34) have shown unchanged cardiac output, there is no reason to believe that the volume ejected was increased in our study. These findings might be explained by an increased velocity of blood ejected from the left ventricle or possibly an increased stiffness of the aorta due to smooth muscle involvement.

In conclusion, this study has shown a progressive increase in cardiac inotropic variables during compression to 30 bar, with the greatest inotropic increase per bar found nearest the surface. The elevation in cardiac inotropic variables persisted during 60 min at 30 bar. The oxygen consumption increased by 60% during the 60 min at 30 bar. No changes in chronotropy, mean arterial pressure, or end-diastolic pressure were detected.

REFERENCES


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