Surgical procedure affects physiological parameters in rat myocardial ischemia: need for mechanical ventilation

GEORG HORSTICK, 1 OLIVER BERG, 1 AXEL HEIMANN, 2 HARALD DARIUS, 1 HANS ANTON LEHR, 4 SUCHARIT BHAKDI, 3 OLIVER KEMPSKI, 2 AND JÜRGEN MEYER 1

12nd Medical Clinic, 2 Institute for Neurosurgical Pathophysiology, 3 Institute for Microbiology and Hygiene, and 4 Institute for Pathology, Johannes Gutenberg-University Mainz, 55101 Mainz, Germany

Horstick, Georg, Oliver Berg, Axel Heimann, Harald Darius, Hans Anton Lehr, Sucharit Bhakdi, Oliver Kempski, and Jürgen Meyer. Surgical procedure affects physiological parameters in rat myocardial ischemia: need for mechanical ventilation. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H472–H479, 1999.—Several surgical approaches are being used to induce myocardial ischemia in rats. The present study investigated two different operative procedures in spontaneously breathing and mechanically ventilated rats under sham conditions. A snare around the left coronary artery (LCA) was achieved without occlusion. Left lateral thoracotomy was performed in spontaneously breathing and mechanically ventilated rats (tidal volume 8 ml/kg) with a respiratory rate of 90 strokes/min at different levels of O2 supplementation (room air and 30, 40, and 90% O2). All animals were observed for 60 min after thoracotomy. Rats operated with exteriorization of the heart by left lateral thoracotomy while breathing spontaneously developed severe hypoxia and hypercapnia despite an intrathoracic operation time of <1 min. Arterial O2 content decreased from 18.7 ± 0.5 to 3.3 ± 0.9 vol% O2. Lactate increased from 1.2 ± 0.1 to 5.2 ± 0.3 mmol/l. Significant signs of ischemia were seen in the electrocardiogram up to 60 min. Mechanically ventilated animals exhibited a spectrum ranging from hypoxia (room air) to hyperoxia (90% O2). In order not to jeopardize findings in experimental myocardial ischemia–reperfusion injury models, stable physiological parameters can be achieved in mechanically ventilated rats at an O2 application of 30–40% at 90 strokes/min.

reperfusion; hypoxia

Experimental models of myocardial ischemia and reperfusion are essential for the study of pathophysiological mechanisms and cardioprotective pharmacological effects. In 1946, models for studying myocardial infarction in the rat were first established by Heimburger (17) and later modified for other small animals in 1954 by Johns and Olson (20). Johns and Olson (20) performed thoracotomy, and respiration with 90% O2 was maintained under positive pressure for up to 30 min with the use of a tight-fitting face mask.

In 1960, Selye et al. (40) published a modified technique, the hallmark of which was the rapid access to the heart in spontaneously breathing rats by anterior thoracotomy and transection of the sixth costal cartilage. The time required was 2–3 min from skin incision to completion of wound closure, with only 60–90 s required for the intrathoracic part of the operation. The immediate postoperative mortality rate for this surgical procedure was given as 10%. Deloche et al. (11) extended the experimental protocol by introducing a reperfusion step in their experiments. Operative mortality in their control group was given as 35%.

In 1976, Madea et al. (25) presented a different surgical technique involving a left lateral thoracotomy and exteriorization of the heart by gentle pressure on the right side of the thorax (25). The operative mortality was given as 21% (24). This technique has gained popularity, and many experimental studies on myocardial infarction and reperfusion have been performed using this model (1, 9, 12, 19, 28, 36). Attempts to improve the technique led to the introduction of mechanical ventilation, using either room air or 90–95% O2 (18, 22). The mortality rate was given as 13% (21).

Despite the widespread use of these techniques and their variations, little information is available on the effects of the different surgical conditions on respiratorv and metabolic parameters, which in turn may profoundly influence the pathophysiology of ischemic and reperfused myocardium and also affect coronary blood flow of the collateral circulation. In the present study, we compared two different surgical procedures in a sham-operative situation and analyzed the respiratory, metabolic, and myocardial effects.

METHODS

Experimental conditions. Twenty-nine Wistar rats of either sex (250–400 g body wt) were maintained on standard rat chow and water ad libitum until the beginning of the experiment. After intraperitoneal anesthesia with chloral hydrate (360 mg/kg) was administered, the carotid artery was cannulated with a small polyethylene (PE) tube for arterial blood gas analysis (ABG) and measurement of arterial blood pressure. ABG [arterial Po2 (PaO2), S02 (Sao2), PCO2 (PaCO2), pH, and base excess (BE)], hemoglobin (Hb), hematocrit (Hct), lactate, potassium, and sodium were analyzed with Arterial Blood Gas Laboratory Radiometer Copenhagen 615, which contains the oximeter of the OSM 3 (Radiometer Copenhagen) (6, 30); PaO2, Sao2, and arterial pH, Hb, lactate, potassium, and sodium are measured, whereas BE and Hct are calculated from measured values. Hepatized blood (150 µl) was drawn for each set of analyses. In addition, a six-lead electrocardiogram (EKG) was registered during the surgical procedure with a Siemens Mingograph at 100 mm/s. Rectal temperature was kept constant at 38.0 ± 0.5°C by means of a feedback-controlled homeothermic blanket control unit (Harvard, South Natick, MA).
Two surgical techniques were used. First, the method published by Maclean et al. (25) was applied, which involves left lateral thoracotomy and exteriorization of the heart by gentle pressure applied to the right side of the thorax. While the beating heart was held outside the thorax between two fingers, a 6-0 suture was placed around the left coronary artery (LCA) near its origin. For the purpose of this study, myocardial ischemia was not intended and the LCA was not occluded by tightening the ligation. The heart was repositioned, the chest was compressed to remove air from the thorax, and the muscle and skin layers were closed with a purse-string suture. In accordance with Selye et al. (40), two thoracotomies were performed with a Harvard apparatus 683. Observation period lasted 60 min after thoracotomy.

The basic difference of the second surgical procedure was that animals in these groups (3–6, n = 4 in each group) were mechanically ventilated for 60 min with open chest and modification of a method described by Hale et al. (14). These rats were intubated with a PE tube under direct vision and were ventilated with a Harvard apparatus 683. The tidal volume of the ventilated animals was maintained constant at 8 ml/kg body wt. Respiratory rate at baseline was 2 min (group 1, n = 4) or 1 min (group 2, n = 4). These rats left to breathe spontaneously as in the original protocol of Selye and Maclean (25, 40). The rats were left to breathe spontaneously with open-chest time after thoracotomy (Student-Newman-Keuls test) was used. Statistical analysis was performed with Sigma Stat (Jandel). Statistical significance of changes from baseline values within each group was tested with ANOVA for repeated measures. Differences among groups were statistically analyzed with one-way ANOVA comparing several groups. If values did not show a normal distribution, ANOVA for nonparametric values (Kruskal-Wallis test) with the multiple-comparison method (Student-Newman-Keuls test) was used. Statistical significance was accepted at an error probability of P < 0.05 after pairwise testing.

RESULTS

Animal survival. All animals in group 1 with an intrathoracic operation time of 2 min developed hypoxic respiratory arrest and expired within 3 min after thoracotomy and closure of the chest. Rats in the remaining groups survived, and the collective data obtained for PaO2, SaO2, PaCO2 arterial pH, and lactate are depicted in Figs. 1–5. Baseline data for PaO2, SaO2,

Table 1. Baseline values of hemodynamic and arterial blood gas data in anesthetized rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Respiratory Management</th>
<th>OT, min</th>
<th>R-R Interval HR, ms</th>
<th>MAP, mmHg</th>
<th>PaO2, mmHg</th>
<th>SaO2, %</th>
<th>CaO2, vol%</th>
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</thead>
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<tr>
<td>1</td>
<td>4</td>
<td>Spont RA</td>
<td>2</td>
<td>161 ± 11</td>
<td>75 ± 2</td>
<td>77.8 ± 2.9</td>
<td>92.6 ± 1.6</td>
<td>18.8 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Spont RA</td>
<td>1</td>
<td>152 ± 6</td>
<td>76 ± 2</td>
<td>80.3 ± 2.2</td>
<td>93.3 ± 1.6</td>
<td>18.7 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>Vent RA</td>
<td>60</td>
<td>163 ± 7</td>
<td>79 ± 2</td>
<td>76.2 ± 4.4</td>
<td>92.8 ± 2.7</td>
<td>19.1 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Vent 30% O2</td>
<td>60</td>
<td>165 ± 8</td>
<td>75 ± 2</td>
<td>71.1 ± 0.9</td>
<td>88.9 ± 0.3</td>
<td>17.0 ± 0.6</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>Vent 40% O2</td>
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<td>165 ± 9</td>
<td>76 ± 3</td>
<td>75.2 ± 4.4</td>
<td>89.1 ± 3.0</td>
<td>16.7 ± 0.8</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>Vent 90% O2</td>
<td>60</td>
<td>171 ± 12</td>
<td>78 ± 3</td>
<td>77.2 ± 1.8</td>
<td>90.5 ± 0.9</td>
<td>16.8 ± 1.2</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>Vent 30% O2 and exteriorization</td>
<td>60</td>
<td>153 ± 8</td>
<td>77 ± 4</td>
<td>83.5 ± 3.6</td>
<td>93.5 ± 1.5</td>
<td>18.3 ± 0.7</td>
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</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>PaO2, mmHg</th>
<th>Arterial pH</th>
<th>BE, mmol/l</th>
<th>Lactate, mmol/l</th>
<th>K+, mmol/l</th>
<th>Na+, mmol/l</th>
<th>Baseline Hb, g/dl</th>
<th>Hb after 10ABGs, g/dl</th>
<th>Baseline Hct, %</th>
<th>Hct after 10ABGs, %</th>
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<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>38.4 ± 2.1</td>
<td>7.4 ± 0.01</td>
<td>-0.4 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>137.0 ± 9</td>
<td>14.4 ± 0.2</td>
<td>14.3 ± 0.2</td>
<td>44.2 ± 0.4</td>
<td>44.0 ± 0.5*</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>39.8 ± 1.3</td>
<td>7.4 ± 0.02</td>
<td>-0.8 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>136.5 ± 15</td>
<td>14.2 ± 0.4</td>
<td>12.9 ± 0.4</td>
<td>43.6 ± 0.4</td>
<td>39.6 ± 1.3</td>
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<tr>
<td>3</td>
<td>4</td>
<td>37.7 ± 1.7</td>
<td>7.4 ± 0.01</td>
<td>-0.6 ± 0.1</td>
<td>1.5 ± 0.4</td>
<td>4.3 ± 0.2</td>
<td>131.3 ± 12</td>
<td>13.1 ± 0.4</td>
<td>12.7 ± 0.4</td>
<td>44.9 ± 1.3</td>
<td>40.2 ± 1.3</td>
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<tr>
<td>4</td>
<td>4</td>
<td>40.7 ± 0.8</td>
<td>7.4 ± 0.01</td>
<td>-0.3 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>4.6 ± 0.4</td>
<td>135.1 ± 14</td>
<td>13.1 ± 0.5</td>
<td>12.7 ± 0.4</td>
<td>40.4 ± 1.6</td>
<td>38.8 ± 1.4</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>38.7 ± 1.3</td>
<td>7.4 ± 0.02</td>
<td>-0.8 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>3.9 ± 0.4</td>
<td>140.1 ± 18</td>
<td>12.6 ± 0.6</td>
<td>11.6 ± 0.2</td>
<td>38.6 ± 1.7</td>
<td>35.7 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>37.4 ± 1.3</td>
<td>7.4 ± 0.01</td>
<td>-1.5 ± 0.7</td>
<td>2.1 ± 0.4</td>
<td>4.3 ± 0.2</td>
<td>139.0 ± 6</td>
<td>13.2 ± 0.8</td>
<td>12.8 ± 0.5</td>
<td>40.5 ± 2.5</td>
<td>39.3 ± 1.4</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>42.3 ± 0.9</td>
<td>7.4 ± 0.01</td>
<td>0.5 ± 0.8</td>
<td>1.2 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>137.2 ± 12</td>
<td>13.8 ± 0.4</td>
<td>12.6 ± 0.4</td>
<td>42.4 ± 1.2</td>
<td>38.8 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; data are from 29 rats. OT, open-chest time after thoracotomy; HR, heart rate; MAP, mean arterial blood pressure; PaO2, SaO2, CaO2, andPaCO2, arterial PO2, saturation, O2 content, and PaCO2, respectively; BE, base excess; Hb, hemoglobin; ABGs, arterial blood gas analyses; Hct, hematocrit; Spont, spontaneously breathing; Vent, mechanically ventilated; RA, room air. *Hb after 5ABGs.
shortening the operation period to 60 s (not recover. As originally demonstrated by Selye et al. developed severe hypoxia at 0.5–2 min from which they did not recover. Results for PaO2 in group 1 dramatically decreased from baseline levels (80.3 $\pm$ 2.2 mmHg) at 30 s (25.2 $\pm$ 2.2 mmHg) and at 1 min (20.8 $\pm$ 3.0 mmHg) but then returned to 51.3 $\pm$ 3.9 mmHg and 58.9 $\pm$ 1.8 mmHg at 2 and 3 min, respectively, after thoracotomy (Fig. 1). At 15, 30, and 45 min, PaO2 in group 2 was still significantly lower than at baseline levels (59.6 $\pm$ 2.9, 60.0 $\pm$ 3.8, and 67.0 $\pm$ 2.7 mmHg, respectively) and reached 72.5 $\pm$ 6.1 mmHg at 60 min.

Intubation and ventilation with 90 strokes/min after thoracotomy totally prevented the initial drop of PaO2 (Fig. 1). Ventilation on room air led to hypoxic levels below baseline between 15 and 60 min. In contrast, ventilation with 30% O2 maintained baseline PaO2 levels, 40% O2 raised PaO2 levels slightly above baseline, and 90% O2 markedly increased PaO2 (Fig. 1).

Exteriorization under mechanical ventilation with 30% O2 (group 7) resulted in a significant decrease in PaO2 lasting until 3 min after thoracotomy (Table 2).

PaO2 measurements. The results of PaO2 measurements are depicted in Fig. 2 and followed a similar pattern to that observed for PaO2. Nonventilated animals (groups 1 and 2) displayed a steep drop of SaO2 between 30 s and 1 min after thoracotomy. SaO2 levels could be maintained at baseline values by ventilating the animals with 30 or 40% O2. Ventilation on room air resulted in a significant decrease in SaO2, between 15 and 60 min in group 3, whereas ventilation with 90% O2 produced significantly elevated SaO2 levels (Fig. 2). Group 7 showed no significant changes compared with baseline values (Table 2).

PaCO2 measurements. Nonventilated animals presented with a steep rise of PaCO2 directly after thoracotomy. Under mechanically ventilated conditions with 90 strokes/min after thoracotomy (Fig. 3), PaCO2 was maintained at 30–40 mmHg throughout the experiment, irrespective of the O2 concentration in the ventilation gas. Manual exteriorization and ventilation with 30% O2 did not affect PaCO2 throughout the experiment.

Arterial pH. The PaCO2 findings were mirrored by results of pH analysis (Fig. 4). Nonventilated rats developed a significant decrease in pH between 0.5 and 3 min, but values in group 2 (1-min thoracotomy time) recovered to slightly below baseline between 15 and 60 min (Fig. 4). In contrast, mechanically ventilated animals displayed values within physiological range throughout the experiment. Group 7, however, presented a decrease in pH that was signifi-

Table 2. Blood gas values of animals in group 7 after thoracotomy and exteriorization of hearts under mechanical ventilation with 30% O2

<table>
<thead>
<tr>
<th>Time After Thoracotomy and Exteriorization, min</th>
<th>Baseline</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2, mmHg</td>
<td>83.5 $\pm$ 3.6</td>
<td>63.7 $\pm$ 1.9*</td>
<td>68.4 $\pm$ 1.9*</td>
<td>66.7 $\pm$ 2.4*</td>
<td>68.1 $\pm$ 1.9*</td>
<td>71.8 $\pm$ 3.6</td>
<td>75.1 $\pm$ 3.6</td>
<td>73.6 $\pm$ 3.6</td>
<td>77.3 $\pm$ 4.9</td>
</tr>
<tr>
<td>SaO2, %</td>
<td>93.5 $\pm$ 1.5</td>
<td>83.2 $\pm$ 3.4</td>
<td>86.9 $\pm$ 2.8</td>
<td>83.9 $\pm$ 2.6</td>
<td>85.3 $\pm$ 2.0</td>
<td>87.1 $\pm$ 2.3</td>
<td>88.6 $\pm$ 1.4</td>
<td>88.2 $\pm$ 1.5</td>
<td>89.4 $\pm$ 1.4</td>
</tr>
<tr>
<td>PaCO2, mmHg</td>
<td>42.3 $\pm$ 0.9</td>
<td>43.8 $\pm$ 2.5</td>
<td>43.9 $\pm$ 1.9</td>
<td>43.4 $\pm$ 1.9</td>
<td>43.2 $\pm$ 2.0</td>
<td>41.2 $\pm$ 0.8</td>
<td>39.4 $\pm$ 1.1</td>
<td>39.0 $\pm$ 1.3</td>
<td>39.3 $\pm$ 0.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 $\pm$ 0.01</td>
<td>7.34 $\pm$ 0.02*</td>
<td>7.34 $\pm$ 0.01*</td>
<td>7.35 $\pm$ 0.01*</td>
<td>7.34 $\pm$ 0.02</td>
<td>7.36 $\pm$ 0.02</td>
<td>7.37 $\pm$ 0.01</td>
<td>7.37 $\pm$ 0.01</td>
<td>7.37 $\pm$ 0.01</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>1.2 $\pm$ 0.1</td>
<td>3.2 $\pm$ 0.2</td>
<td>3.1 $\pm$ 0.4</td>
<td>4.0 $\pm$ 0.7*</td>
<td>3.3 $\pm$ 0.2</td>
<td>2.5 $\pm$ 0.6</td>
<td>2.6 $\pm$ 0.7</td>
<td>2.6 $\pm$ 0.7</td>
<td>2.5 $\pm$ 0.7</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; n = 5 animals. Baseline values were measured before exteriorization. *P < 0.05 compared with baseline values.

Fig. 1. Arterial PaO2 (PaO2) measurements after thoracotomy. Rats in groups 1 and 2 were spontaneously breathing room air (Spont RA) with an open-chest time (OT) of 2 and 1 min, respectively. Rats in groups 3–6 were mechanically ventilated (Vent) as follows: group 3, RA; group 4, 30% O2; group 5, 40% O2; and group 6, 90% O2. Data are means $\pm$ SE (n = 4 animals per group). Values are significantly different (P < 0.05) between groups 1 and 2 and all mechanically ventilated groups at 0.5 and 1 min, between group 2 and all ventilated and oxygenated animals (groups 4–6) at 2–45 min, and within all mechanically ventilated groups (groups 3–6) at all time points.
significantly lower than baseline values until 2 min after thoracotomy (Table 2).

Arterial lactate measurements. The results of arterial lactate measurements are summarized in Fig. 5. Lactate in the nonventilated animals showed a steep increase at 2–3 min after thoracotomy. Arterial lactate concentration in group 1 rose to 3.93 ± 0.37 mmol/l at 2 min and 5.10 ± 0.25 mmol/l at 3 min. Values in group 2 similarly increased to 4.60 ± 0.27 mmol/l at 2 min and 5.18 ± 0.29 mmol/l at 3 min. At 15 min, lactate values in group 2 returned to baseline levels. Lactate values in groups 3–6 increased up to the third minute of thoracotomy and then returned to baseline levels in groups 4–6. However, in group 3 (ventilation on room air), lactate levels rose continuously. The increase in group 3 (room air, 90 strokes/min) was statistically significant between 15 and 60 min after the chest was opened (Fig. 5).

Manual handling of the heart and ventilation with 30% O₂ led to a significant increase in lactate at 2 min after thoracotomy. Maximum values reached in group 7 were lower than those presented in group 2 (Table 2).

Arterial BE measurements. Baseline BE in all groups was within physiological range (Table 1). Nonventilated animals in group 2 developed a dramatic decrease to −5.2 ± 0.5 mmol/l at 2 min and −5.5 ± 0.7 mmol/l at 3 min after thoracotomy from which they recovered to levels slightly below baseline. Mechanical ventilation on room air caused a significant decrease in BE from 15 to 60 min of open chest. In contrast, arterial BE remained unchanged in all animals receiving 30, 40, and 90% O₂. BE in group 7 did not show significant changes throughout the experiment.

Arterial potassium and sodium chloride values. Preoperative levels of potassium and sodium chloride did not differ among groups (Table 1). During the operative procedure there was no significant change compared with baseline values except for group 1, which showed an increase in potassium in the moribund animals at 3 min after thoracotomy (6.0 ± 0.2 mmol/l).

Hb and Hct. Values (means ± SE) of Hb and Hct for each group at baseline and at the end of the experiments are depicted in Table 1. The decrease in Hb and Hct was not significant for any group (repeated-measures ANOVA). Comparison among all groups at all time points for Hb and Hct did not show any significant differences in the ANOVA.

Arterial blood pressure, heart rate, and ECG morphology. No significant differences in heart rate and mean arterial blood pressure at baseline were observed among animals in the different groups (Table 1). Blood pressure of rats in group 2 was not different before and after the operative procedure. However, there was a significant increase in heart rate 5 min after the chest was opened and the heart repositioned in group 2. The R-R interval in group 2 was 152 ± 4 ms 15 min before...
thoracotomy, 152 ± 6 ms immediately before thoracotomy, and 134 ± 8.5 ms 5 min after thoracotomy. Similarly, the R-R interval in group 7 decreased significantly to 126 ± 9 ms at 5 min after exteriorization of the heart. At 15, 30, 45, and 60 min the R-R interval returned to baseline values in groups 2 and 7. Blood pressure values and heart rate measured at 5, 15, 30, 45, and 60 min after thoracotomy did not differ significantly from baseline in the ventilated animals.

ECG analysis of the animals in groups 4–6 shows no signs of myocardial ischemia (Fig. 6A, Table 3). Pathological ECG findings were defined as S-T segment elevation >0.1 mV, S-T segment depression >0.1 mV, and ventricular arrhythmias of Lown class III and higher. All animals in group 2 demonstrated signs of myocardial ischemia from 5 to 60 min after thoracotomy (Fig. 6, B–D, Table 3). Specifically, all animals had S-T segment elevations, and one of four animals presented ventricular arrhythmias until 30 min after thoracotomy. From 45 to 60 min, significant S-T segment elevation was seen in three of four animals (Table 3). The ECG in Fig. 6B (group 2) shows S-T segment elevation and ventricular arrhythmias with bigeminus up to 30 min and recovery toward the end of the experiment. The next animal in group 2 in Fig. 6C presents changes of QRS morphology and S-T segment elevation up to 60 min. ECG depicted in Fig. 6D for group 2 shows significant, persistent S-T segment elevation in leads II, III, and aVF after surgical intervention. Two animals in group 3 (room air) presented signs of S-T segment depression starting 15 min after thoracotomy until the end of the experiment (not shown). In contrast, animals in group 7 showed S-T segment elevation and ventricular arrhythmias at 5 min after thoracotomy, and the incidence of S-T segment elevation gradually declined from 15 (4 of 5 animals) to 60 min (1 of 5 animals; Table 3).

DISCUSSION

This study addressed the influence of the surgical approach and ventilation conditions on respiratory and metabolic parameters during experimental myocardial manipulation. The results indicate that mechanical ventilation with 30–40% O\textsubscript{2} is required in rats undergoing thoracotomy for experimental myocardial ischemia to guarantee stable conditions during the period of open-chest surgery. Otherwise, severe alterations in cardiorespiratory function occur with consecutive additional damage to the myocardium as documented via ECG alteration (Fig. 6, B–D). It can be assumed that these changes will affect coronary blood flow (5) and, hence, influence the outcome of experimental myocardial ischemia and reperfusion.

Hypoxia develops because of atelectasis due to the elastic recoil of the lung after thoracotomy and loss of the negative pressure in the chest cavity. The physiological response of the pulmonary vasculature to atelecta-
sis is an increase in pulmonary vascular resistance selectively in the atelectatic lung. This increase, thought to be due almost entirely to hypoxic pulmonary vasoconstriction, diverts blood flow from the atelectatic lung toward the remaining lung (2, 34). If only small areas of the lung are hypoxic, the overall effects on blood oxygenation are negligible, and PaO₂ remains normal because the shunt volumes are small (26). In our experiments, nonventilated animals presented severe transient hypoxia. Likewise, mechanical ventilation on room air resulted in continual hypoxia after 15 min. In both cases a decrease in standard BE and an increase in lactate ensued. Lactate increase in group 2 was compensated to baseline levels because the chest was closed 60 s after thoracotomy and spontaneous respiration could be maintained at higher PaO₂ levels in contrast to group 3. These alterations could be entirely prevented by the application of 30–40% O₂ (Fig. 5). Hypercapnia could be completely prevented by hyperventilation at 90 strokes/min (Fig. 3). These findings are in perfect accord with the recommendation of Benumof and Alfery (3), who have pointed out that the respiratory rate during the open-chest period must be increased by 20–30% to maintain CO₂ homeostasis.

An experienced investigator can place the coronary suture within 30 s using the operative method de-

Table 3. Electrocardiogram analysis of groups 2–7

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
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Data are no. of animals per group with pathological electrocardiogram (ECG) changes. Pathological ECG findings are defined as S-T segment elevation ≥0.1 mV and ventricular arrhythmias of Lown class III or higher.
scribed by Maclean et al. (25), in which the heart is exteriorized by gentle pressure on the right side of the thorax. The entire procedure from thoracotomy to wound closure lasted ~60 s in group 2 of our experiments. The time required for the operative procedure obviously depends on the experience of the surgeon, and our results suggest that it must not exceed 90–120 s. In the experimental group of spontaneously breathing animals (group 2), arterial O2 content decreased 30 s after thoracotomy to 5.1 ± 1.0 vol% and after 60 s to 3.3 ± 0.9 vol%. In addition, due to the operative technique, there was a brief period of complete cardiac arrest with a supravalvular increase in afterload (41).

This may be another cause of global myocardial hypoxia due to the operative technique, as seen in the ECGs of the rats in group 2 (Fig. 6, B–D). Similar ECG alterations were seen in group 7, although PaO2 was only marginally altered during the first 3 min after exteriorization of the heart. As an alternative explanation of the prolonged ECG changes (despite transient ischemia), mechanical traumatization of the muscle tissue during exteriorization as shown in group 7 and/or the release of inflammatory mediators should be considered.

The effect of these basic physiological or metabolic changes may be far reaching and may affect the outcome in the applied models of ischemia and reperfusion injury.

Myocardial hypoxia results in coronary vasodilation and a decrease in coronary vascular resistance (4, 5). The O2 content of 3.3 ± 0.9 vol% in spontaneously breathing animals of our experiments was below the levels observed by Berne et al. (5), which result in maximum vasodilation and increase of coronary blood flow. Several mediators may be responsible for the vasodilation, including bradykinin, O2, CO2, cytokines, serotonin, histamine, H+, lactate, K+, and prostaglandins (13, 33). Even brief periods of coronary occlusion cause release of adenosine (38, 39). Likewise, there is a close inverse relationship among cytoplasmic phosphorylation potential, O2 consumption, and coronary blood flow (32, 37). Hypercapnia itself is a stimulus for an increased coronary flow (8), and when combined with hypoxia, an even more pronounced decrease in coronary vascular resistance is observed (7). Coronary vasodilation is known to be complete within 15 s after an abrupt disturbance of homeostasis, such as that given in groups 1 and 2 (42). Hyperoxegenation with 90% O2 results in high arterial O2 tensions and could affect reperfusion (31, 35), i.e., through the excessive production of O2 free radicals (10, 29).

In both groups of spontaneously breathing rats, hypoxia and hypercapnia reached levels that must be expected to affect coronary vascular resistance and blood flow (Figs. 1–3). There was a significant decrease in standard BE in group 2, and lactate production increased significantly after 3 min (Fig. 5). The decrease in lactate levels of the surviving animals after 15 min in group 2 indicates the possibility for metabolic compensation in the face of signs of myocardial ischemia prevailing in the ECG up to 30–60 min (Fig. 6, B–D). Even a brief period of exteriorization of the heart from the chest cavity under ventilation with 30% O2 caused intermittent lactate increase, which did not reach the levels of spontaneously breathing rats, however.

Induction of experimental myocardial ischemia in the rat may appear attractive because of the simplicity and rapidity of the procedure. One of the well-known limitations of this model is the relation of infarct size to the true area at risk (16). The latter will obviously be liable to marked variation if metabolic disturbances coupled to variation in collateral blood flow occur (23). Published data of Maxwell et al. (27) demonstrate that the collateral blood flow as a percentage of flow to the nonischemic myocardium of rats is 6.1%. Rats, therefore, range between pigs (0.6%) on one hand and cats (11.8%) and dogs (15.9%) on the other hand, underlying the variety of collateral blood flow between different mammalian species (27). In the study of Hale and Kloner (14), the subendocardial blood flow in the ischemic zone of rat myocardium is 13% of that in the normal subendocardium, and the ischemic subepicardial flow is 9%. This problem of not normalizing infarct size to the size of the risk zone can occur in any infarct model. Now that it is possible to provide stable physiological conditions before and after ischemia, additional alterations of coronary blood flow due to the manual handling of the heart can be avoided and the dual perfusion technique or applying microspheres will permit the area at risk to be precisely defined (15). In this way, ischemia-reperfusion experiments should acquire an additional basis for their reproducibility in acute and chronic rat heart models.

In conclusion, our study demonstrates that stable physiological respiratory and metabolic parameters can be achieved in experimental myocardial ischemia through open-chest surgery with the use of mechanical hyperventilation and with oxygenation defined at 30–40% O2. Maintenance is thereby guaranteed for the operative access inducing left ventricular myocardial infarction by occlusion of the left coronary artery with the use of a minimally invasive surgical procedure. The results are discussed in relation to the potential risk of hypoxia or hyperoxia arising from inappropriate surgical regimens.

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