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

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[1]*2nd 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 O_2 supplementation (room air and 30, 40, and 90% O_2). All animals were observed for 60 min after thoracotomy. Rats operated with exteriorization of the heart through left lateral thoracotomy while breathing spontaneously developed severe hypoxia and hypercapnia despite an intrathoracic operation time of <1 min. Arterial O_2 content decreased from 18.7 ± 0.5 to 3.3 ± 0.9 vol%. 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% O_2). 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 O_2 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% O_2 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, Maclean 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% O_2 (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 respiratory 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 P_{O_2} (Pa_{O_2}), S_{O_2} (Sa_{O_2}), P_{CO_2} (Pa_{CO_2}), 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 oxymeter of the OSM 3 (Radiometer Copenhagen) (6, 30); Pa_{O_2}, Sa_{O_2}, Pa_{CO_2}, and arterial pH, Hb, lactate, potassium, and sodium are measured, whereas BE and Hct are calculated from measured values. Heparinized blood (150 µl) was drawn for each set of analyses. In addition, a six-lead electrocardiogram (ECG) 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).

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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 groups were compared with an intrathoracic operative time of either 2 min (*group 1*, $n = 4$) or 1 min (*group 2*, $n = 4$). These rats were left to breathe spontaneously as in the original protocol of Selye and Maclean (see Table 1) (25, 40). The observation period lasted 60 min after thoracotomy.

The basic difference of the second surgical procedure was that animals in these groups (*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 70 strokes/min. To gain access to the LCA in the mechanically ventilated groups, a small pericardial window at the site of the left atrium was opened after thoracotomy between the fourth and fifth intercostal spaces. The heart was left in place and, under sterile conditions, the first one-third of the LCA originating from the aorta was visualized by turning the atrial appendage with a left cranial oblique view. A 6-0 suture was placed around the artery but not tied.

After the thorax was opened, the respiratory rate was increased to 90 strokes/min to avoid hypercapnia. The difference among animals in *groups 3–6* pertained to the inhaled O_2 content; ventilation was with room air (*group 3*) or 30% O_2 (*group 4*), 40% O_2 (*group 5*), or 90% O_2 (*group 6*). To differentiate between the effects of transient hypoxia and those of manual handling with exteriorization of the heart, five animals underwent a combined operative procedure (*group 7*). Under ventilation with 30% O_2, the heart was exteriorized as described for the spontaneously breathing rats. Inspired O_2 concentration was recorded with a Heyer Artema MM 205 (Artema Medical). The thorax remained open during the entire surgical procedure (Table 1).

The animals were randomly assigned to the different treatment groups by lots drawn after rats had been anesthetized. All investigative procedures and animal facilities conformed with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee.

Experimental protocol. Baseline ABG was performed 15 min before thoracotomy, after animals had reached stable body temperature for at least 15 min. The second control ABG was drawn immediately before thoracotomy. Further ABGs were performed at 0.5, 1, 2, 3, 15, 30, 45, and 60 min after the chest was opened under the different ventilatory regimens. A six-lead ECG and arterial blood pressure were examined 15 min before and immediately before thoracotomy. After the chest had been opened, ECGs and arterial blood pressure were documented at 5, 15, 30, 45, and 60 min.

Statistical analysis. Data are presented as means ± SE. 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 $P \leq 0.05$ 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 Pa_{O_2}, Sa_{O_2}, Pa_{CO_2}, arterial pH, and lactate are depicted in Figs. 1–5. Baseline data for Pa_{O_2}, Sa_{O_2},

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

Groups	*n*	Respiratory Management	OT, min	R-R Interval HR, ms	MAP, mmHg	Pa_{O_2}, mmHg	Sa_{O_2}, %	Ca_{O_2}, vol%
1	4	Spont RA	2	161 ± 11	75 ± 2	77.8 ± 2.9	92.6 ± 1.6	18.8 ± 0.5
2	4	Spont RA	1	152 ± 6	76 ± 2	80.3 ± 2.2	93.3 ± 1.6	18.7 ± 0.5
3	4	Vent RA	60	163 ± 7	79 ± 2	76.2 ± 4.4	92.8 ± 2.7	19.1 ± 1.0
4	4	Vent 30% O_2	60	165 ± 8	75 ± 2	71.1 ± 0.9	88.9 ± 0.3	17.0 ± 0.6
5	4	Vent 40% O_2	60	165 ± 9	76 ± 3	75.2 ± 4.4	89.1 ± 3.0	16.7 ± 0.8
6	4	Vent 90% O_2	60	171 ± 12	78 ± 3	77.2 ± 1.8	90.5 ± 0.9	16.8 ± 1.2
7	5	Vent 30% O_2 and exteriorization	60	153 ± 8	77 ± 4	83.5 ± 3.6	93.5 ± 1.5	18.3 ± 0.7

Groups	*n*	Pa_{CO_2}, mmHg	Arterial pH	BE, mmol/l	Lactate, mmol/l	K^+, mmol/l	Na^+, mmol/l	Baseline Hb, g/dl	Hb after 10 ABGs, g/dl	Baseline Hct, %	Hct after 10 ABGs, %
1	4	38.4 ± 2.1	7.4 ± 0.01	−0.4 ± 0.4	1.1 ± 0.2	4.8 ± 0.3	137 ± 0.9	14.4 ± 0.2	14.3 ± 0.2*	44.2 ± 0.4	44.0 ± 0.5*
2	4	39.8 ± 1.3	7.4 ± 0.02	0.8 ± 0.8	1.2 ± 0.1	4.7 ± 0.1	136 ± 1.5	14.2 ± 0.2	12.9 ± 0.4	43.6 ± 0.4	39.6 ± 1.3
3	4	37.7 ± 1.7	7.4 ± 0.01	−0.6 ± 1.2	1.5 ± 0.4	4.3 ± 0.2	138 ± 1.3	14.6 ± 0.4	13.1 ± 0.4	44.9 ± 1.3	40.2 ± 1.3
4	4	40.7 ± 0.8	7.4 ± 0.01	−0.3 ± 1.4	2.0 ± 0.2	4.6 ± 0.4	135 ± 1.4	13.1 ± 0.5	12.7 ± 0.4	40.4 ± 1.6	38.8 ± 1.4
5	4	38.7 ± 1.3	7.4 ± 0.02	−1.0 ± 0.8	1.3 ± 0.1	3.9 ± 0.4	140 ± 1.8	12.6 ± 0.6	11.6 ± 0.2	38.6 ± 1.7	35.7 ± 0.5
6	4	37.4 ± 1.3	7.4 ± 0.01	−1.5 ± 0.7	2.1 ± 0.4	4.3 ± 0.2	139 ± 0.6	13.2 ± 0.8	12.8 ± 0.5	40.5 ± 2.5	39.3 ± 1.4
7	5	42.3 ± 0.9	7.4 ± 0.01	0.5 ± 0.8	1.2 ± 0.1	4.4 ± 0.1	137 ± 1.2	13.8 ± 0.4	12.6 ± 0.4	42.4 ± 1.2	38.8 ± 1.1

Values are means ± SE; data are from 29 rats. OT, open-chest time after thoracotomy; HR, heart rate; MAP, mean arterial blood pressure; Pa_{O_2}, Sa_{O_2}, Ca_{O_2}, and Pa_{CO_2}, arterial P_{O_2}, O_2 saturation, O_2 content, and P_{CO_2}, respectively; BE, base excess; Hb, hemoglobin; ABGs, arterial blood gas analyses; Hct, hematocrit; Spont, spontaneously breathing; Vent, mechanically ventilated; RA, room air. *Hb after 5 ABGs.

Table 2. *Blood gas values of animals in group 7 after thoracotomy and exteriorization of hearts under mechanical ventilation with 30% O_2*

	Baseline	Time After Thoracotomy and Exteriorization, min							
		0.5	1	2	3	15	30	45	60
Pa_{O_2}, mmHg	83.5 ± 3.6	63.7 ± 1.9*	68.4 ± 1.9*	66.7 ± 2.4*	68.1 ± 1.9*	71.8 ± 3.6	75.1 ± 3.6	73.6 ± 3.6	77.3 ± 4.9
Sa_{O_2}, %	93.5 ± 1.5	83.2 ± 3.4	86.9 ± 2.8	83.9 ± 2.6	85.3 ± 2.0	87.1 ± 2.3	88.6 ± 1.4	88.2 ± 1.5	89.4 ± 1.4
Pa_{CO_2}, mmHg	42.3 ± 0.9	43.8 ± 2.5	43.9 ± 1.9	43.4 ± 1.9	43.2 ± 2.0	41.2 ± 0.8	39.4 ± 1.1	39.0 ± 1.3	39.3 ± 0.7
pH	7.39 ± 0.01	7.34 ± 0.02*	7.34 ± 0.01*	7.35 ± 0.01*	7.34 ± 0.02	7.36 ± 0.02	7.37 ± 0.01	7.37 ± 0.01	7.37 ± 0.01
Lactate, mmol/l	1.2 ± 0.1	3.2 ± 0.2	3.1 ± 0.4	4.0 ± 0.7*	3.3 ± 0.2	2.5 ± 0.6	2.6 ± 0.7	2.6 ± 0.7	2.5 ± 0.7

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

Pa_{CO_2}, and arterial pH and lactate showed no differences among groups (Table 1). Results for *group 7* are presented in Table 2. Because we used an occlusive technique for intratracheal intubation, the rats showed no evidence of gastric content aspiration. Oral inspection at the end of the experiment showed no signs of gastric reflux.

Pa_{O_2} measurements. Nonintubated animals with an intrathoracic operation time of 2 min (*group 1*) developed severe hypoxia at 0.5–2 min from which they did not recover. As originally demonstrated by Selye et al. (40), shortening the operation period to 60 s (*group 2*) improved survival. Pa_{O_2} in *group 2* dramatically decreased from baseline levels (80.3 ± 2.2 mmHg) at 30 s (25.2 ± 2.2 mmHg) and at 1 min (20.8 ± 3.0 mmHg) but then returned to 51.3 ± 3.9 mmHg and 58.9 ± 1.8 mmHg at 2 and 3 min, respectively, after thoracotomy (Fig. 1). At 15, 30, and 45 min, Pa_{O_2} in *group 2* was still significantly lower than at baseline levels (59.6 ± 2.9, 60.0 ± 3.8, and 67.0 ± 2.7 mmHg, respectively) and reached 72.5 ± 6.1 mmHg at 60 min.

Intubation and ventilation with 90 strokes/min after thoracotomy totally prevented the initial drop of Pa_{O_2} (Fig. 1). Ventilation on room air led to hypoxic levels below baseline between 15 and 60 min. In contrast, ventilation with 30% O_2 maintained baseline Pa_{O_2} levels, 40% O_2 raised Pa_{O_2} levels slightly above baseline, and 90% O_2 markedly increased Pa_{O_2} (Fig. 1).

Exteriorization under mechanical ventilation with 30% O_2 (*group 7*) resulted in a significant decrease in Pa_{O_2} lasting until 3 min after thoracotomy (Table 2).

Sa_{O_2} measurements. The results of Sa_{O_2} measurements are depicted in Fig. 2 and followed a similar pattern to that observed for Pa_{O_2}. Nonventilated animals (*groups 1* and *2*) displayed a steep drop of Sa_{O_2} between 30 s and 1 min after thoracotomy. Sa_{O_2} levels could be maintained at baseline values by ventilating the animals with 30 or 40% O_2. Ventilation on room air resulted in a significant decrease in Sa_{O_2} between 15 and 60 min in *group 3*, whereas ventilation with 90% O_2 produced significantly elevated Sa_{O_2} levels (Fig. 2). *Group 7* showed no significant changes compared with baseline values (Table 2).

Pa_{CO_2} measurements. Nonventilated animals presented with a steep rise of Pa_{CO_2} directly after thoracotomy. Under mechanically ventilated conditions with 90 strokes/min after thoracotomy (Fig. 3), Pa_{CO_2} was maintained at 30–40 mmHg throughout the experiment, irrespective of the O_2 concentration in the ventilation gas. Manual exteriorization and ventilation with 30% O_2 did not affect Pa_{CO_2} throughout the experiment.

Arterial pH. The Pa_{CO_2} 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-

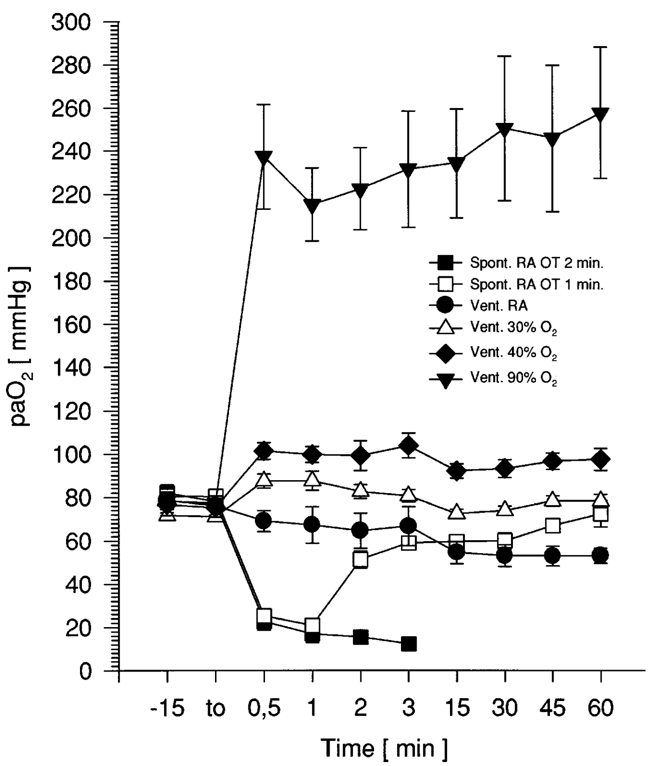

Fig. 1. Arterial P_{O_2} (Pa_{O_2}) measurements after thoracotomy. Rats in *groups 1* and *2* were spontaneously breathing room air (Spont RA) with an open-chest time (OT) of 2 and 1 min, respectively. Rats in *groups 3–6* were mechanically ventilated (Vent) as follows: *group 3*, RA; *group 4*, 30% O_2; *group 5*, 40% O_2; and *group 6*, 90% O_2. Data are means ± SE ($n = 4$ animals per group). Values are significantly different ($P \leq 0.05$) 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.

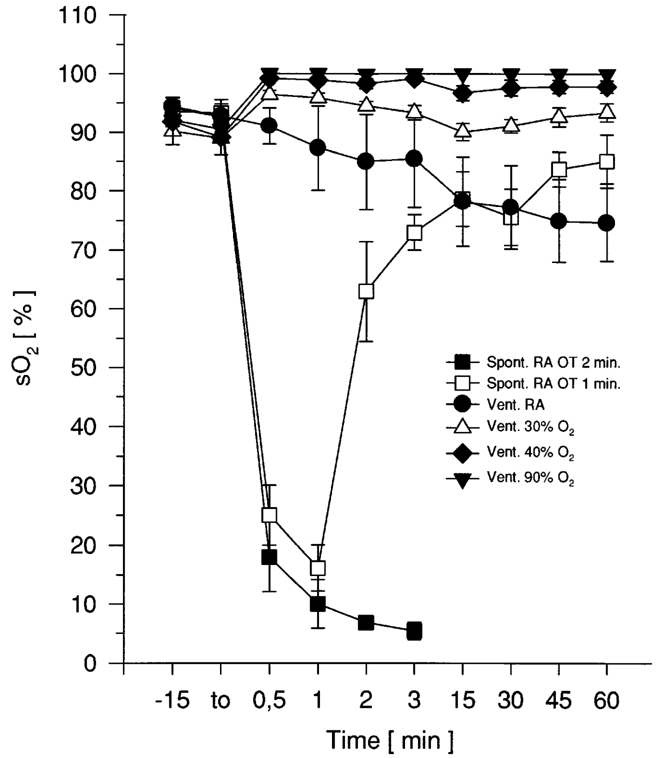

Fig. 2. Sequential changes in arterial S_{O_2} expressed as a percentage. Data are means ± SE. Values between *groups 1* and *2* and all mechanically ventilated groups (*groups 3–6*) are significantly different ($P \leq 0.05$) at 0.5 and 1 min and between *group 2* and all other groups at 2 min after thoracotomy. The decrease in S_{O_2} in *group 3* (Vent RA) is significantly different from values for all ventilated and oxygenated animals (*groups 4–6*) from 30 to 60 min. Values of S_{O_2} in *group 4* (Vent 40% O_2) are significantly different from those in *groups 2, 3, 5,* and *6* ($P \leq 0.05$) from 30 to 60 min.

cantly 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 values.

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% O_2 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% O_2. 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

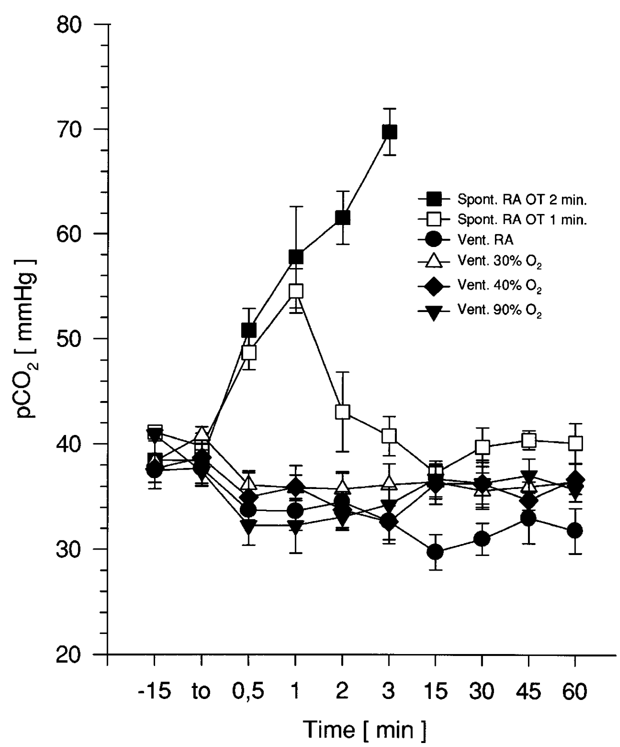

Fig. 3. Time course of arterial P_{CO_2} after thoracotomy. Data are means ± SE. Values are significantly different ($P \leq 0.05$) between *groups 1* and *2* and all mechanically ventilated animals (*groups 3–6*) at 0.5 and 1 min. Data within mechanically ventilated groups (*groups 3–6*) present no significant differences at all time points except *group 3* at 15 min.

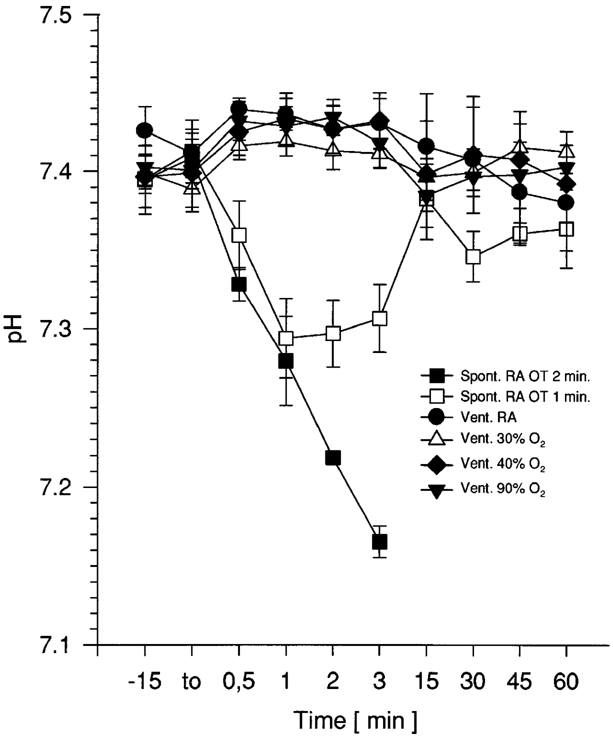

Fig. 4. Alterations in arterial pH changes after thoracotomy. Data are means ± SE. Values are significantly different ($P \leq 0.05$) between *group 2* and all mechanically ventilated animals (*groups 3–6*) at 0.5–3 min. Data within mechanically ventilated groups (*groups 3–6*) present no significant differences at all time points.

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. 6*A*, 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 arrythmias 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. 6*B* (*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. 6*C* presents changes of QRS morphology and S-T segment elevation up to 60 min. ECG depicted in Fig. 6*D* 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_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-

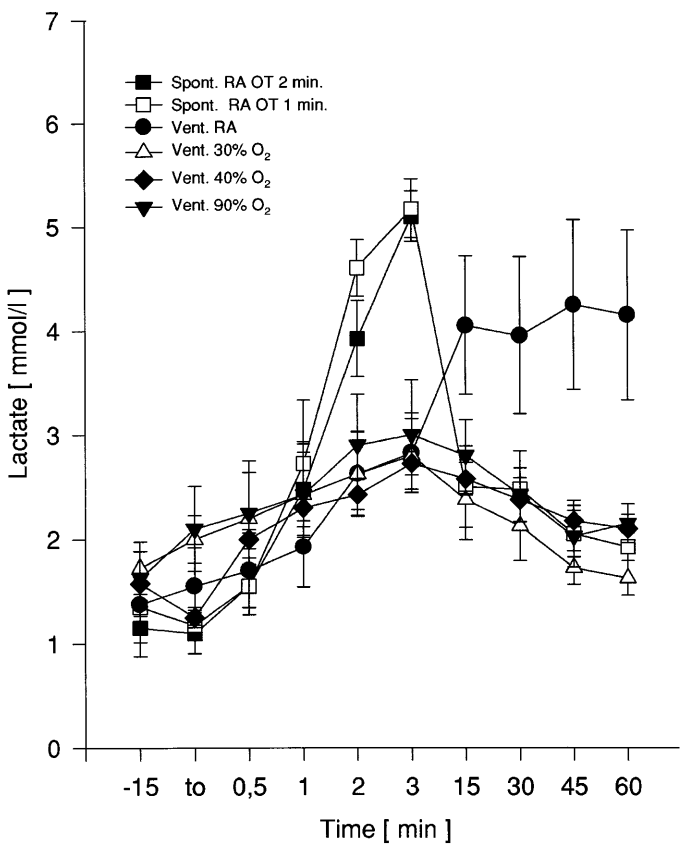

Fig. 5. Time course of arterial lactate concentration. Data are means ± SE. Values are significantly different ($P \leq 0.05$) between *groups 1* and *2* and all mechanically ventilated groups (*groups 3–6*) at 3 min. Lactate values in *group 3* (Vent RA) were significantly increased from 30 to 60 min vs. all other groups.

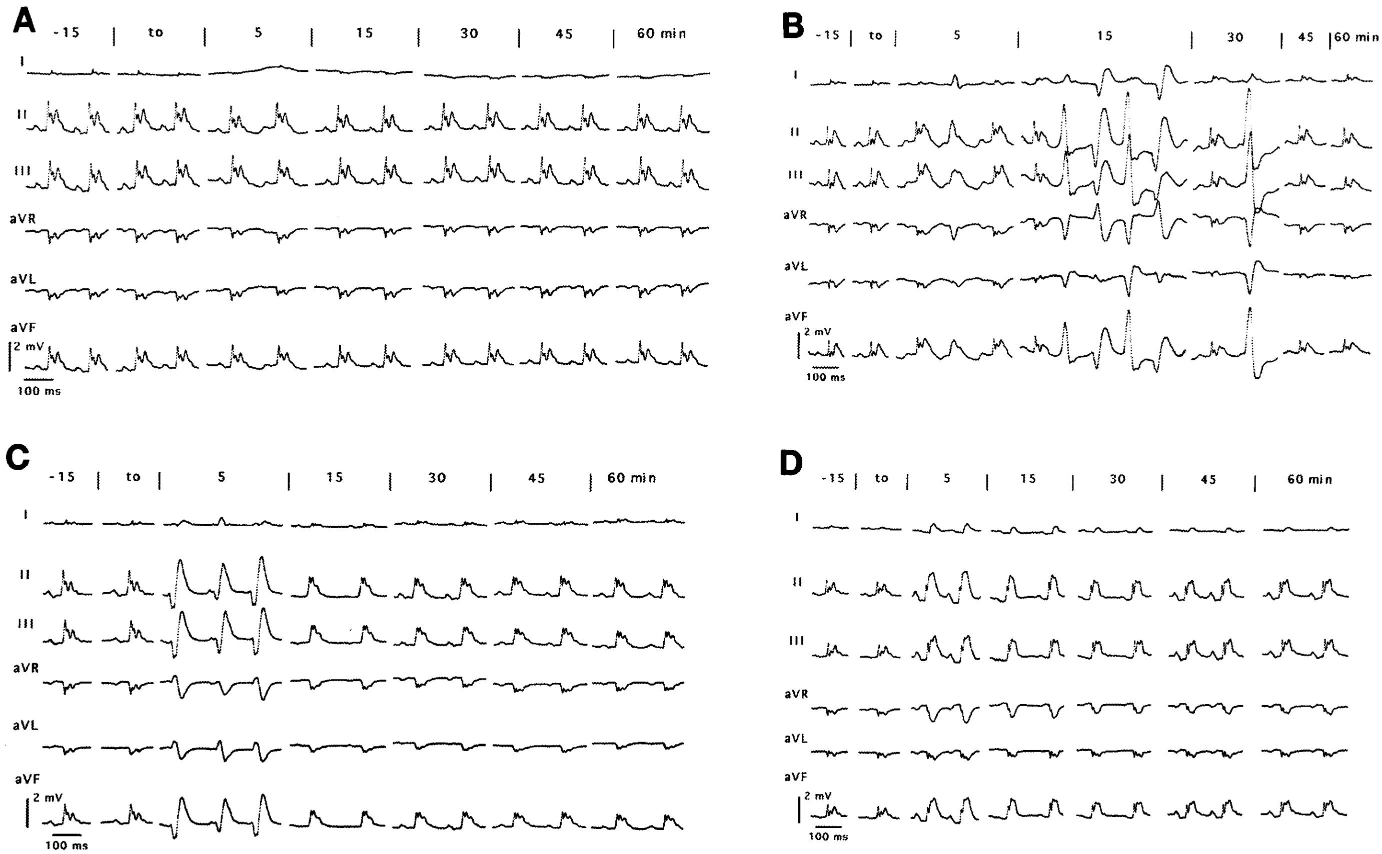

Fig. 6. Representative 6-lead electrocardiograms of animals in *group 4* (*A*, Vent 30% O_2) vs. *group 2* (*B–D*, Spont RA). Ischemic signs include ventricular arrythmias (*B* and *C*) and S-T segment elevation (*B-D*). Note recovery of S-T segment elevation in *B* until 45–60 min and persistent S-T segment elevation in *C* and *D*.

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 Pa_{O_2} 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 Pa_{O_2} levels in contrast to *group 3*. These alterations could be entirely prevented by the application of 30–40% O_2 (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 CO_2 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*

		Time After Thoracotomy, min							
		5		15		30		45	60
Group	*n*	S-T segment elevation	Ventricular arrhythmias	S-T segment elevation	Ventricular arrhythmias	S-T segment elevation	Ventricular arrhythmias	S-T segment elevation	S-T segment elevation
2	4	4	0	4	1	4	1	3	3
3	4	0	0	0	0	0	0	0	0
4	4	0	0	0	0	0	0	0	0
5	4	0	0	0	0	0	0	0	0
6	4	0	0	0	0	0	0	0	0
7	5	5	2	4	0	3	0	2	1

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 O_2 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 Pa_{O_2} 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 O_2 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, O_2, CO_2, 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, O_2 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). Hyperoxygenation with 90% O_2 results in high arterial O_2 tensions and could affect reperfusion (31, 35), i.e., through the excessive production of O_2 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% O_2 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, underlining 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% O_2. 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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