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HYPOVENTILATION PRIOR TO ISCHEMIA PRECONDITIONS RAT MYOCARDIUM

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ABSTRACT

Repetitive episodes of brief regional ischemia have been shown to reduce the severity of life-threatening ventricular arrhythmias and infarct size that occur during prolonged occlusion of the coronary artery. This phenomenon is known as ischemic preconditioning. During studies in anesthetized, open chest Wistar rats, we accidentally observed that poorly ventilated animals had a reduced severity of ischemic arrhythmias. Therefore, we wished to (1) find out whether acute hypoventilation prior to coronary ligation affords protection to the ischemic rat hearts, and (2) to evaluate the effect of recovery period of normoventilation between hypoventilation and prolonged ischemia on the degree of protection. Male Wistar rats were anesthetized and prepared for left coronary artery ligation. Following a left thoracotomy, artificial respiration was immediately started with room air of 1.5 mL/100g and 54 strokes/min. Analysis of ventricular arrhythmias during 30 min occlusion was performed. In some experiments 30 min. sustained ischemia followed by 2 hours reperfusion and then the percentage of infarct size was measured. The reduction of volume of ventilation to 1.1 and 0.7 mL/100g only for 10 min immediately prior to coronary artery occlusion resulted in a marked decrease ($p<0.01$) in the total number of ventricular ectopic beats from 1336 ± 100 in control to 485 ± 75 and 328 ± 51, respectively, mainly by reduction of beats occurring as ventricular tachycardia (VT). Also, the time spent in VT and in reversible ventricular fibrillation was reduced significantly ($p<0.01$ and $p<0.001$, respectively). There was no mortality in animals subjected to hypoventilation, while 33% of control rats died due to irreversible VF. Hypoventilation also limited the infarct size very considerably ($p<0.01$) from $43.4 \pm 2.9\%$ in control animals to $12.1 \pm 1\%$ and $9.1 \pm 2.9\%$ in hypoventilated rats. The presence of an intervening period of normoventilation between hypoventilation and prolonged ischemia did not affect the antiarrhythmic effect of hypoventilation but abolished the protective effects of hypoventilated preconditioning against the infarction. These results may suggest that the heart can be preconditioned by hypoventilation prior to prolonged ischemia despite the lack of an intervening period of normoventilation.

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INTRODUCTION

A growing body of evidence indicates that brief episodes of ischemia followed by a short time of reperfusion can increase the heart's resistance to injury stemming from lethal sustained ischemia. This phenomenon is known as ischemic preconditioning. Ischemic preconditioning has been shown to reduce the severity of life-threatening ventricular arrhythmias[1] and infarct size[2,3] that occur during prolonged occlusion of the coronary artery. In addition to brief episodes of total coronary occlusion there are several stimuli such as hypoxia, stretch, pharmacological agents and technical complications[4] that provide the same protection. The mechanisms for this interesting phenomenon are not well known. Shizukuda and colleagues have shown that five minutes of hypoxic blood perfusion followed by 10 min of reperfusion produces the same extent of protection as provided by episodes of five minutes of coronary artery occlusion.[5] They also indicated that the protective effect was not due to the suppression of myocardial energy demand. In the study of Shizukuda it is not clear whether the presence of a reperfusion period after the hypoxic blood perfusion is necessary to produce the protection. In rats subjected to three weeks of hypoxia (chronic hypoxia), despite the lack of a period of reoxygenation, the increased myocardial tolerance to ischemia has been reported.[6] Thus, it seems that hypoxemia alone is as effective as brief periods of ischemia and reperfusion in inducing preconditioning. During studies in anesthetized, open chest Wistar rats, we accidentally observed that poorly ventilated animals had a reduced severity of ischemic arrhythmias. Therefore, we wished to find out whether acute hypoventilation prior to coronary artery ligation affords protection to the ischemic rat hearts. We also examined the effect of the presence or absence of a recovery period of normoventilation between hypoventilation and sustained ischemia on the degree of protection.

MATERIALS AND METHODS

The use of animals in this study was conducted in accordance with Guidance on the Operation of the Animal

Table I. Effect of hypoventilation on arterial blood gases and pH.

Group	Normoventilation VV= 1.5 mL/100g	Hypoventilation VV= 1.1 mL/100g	Hypoventilation VV= 0.7 mL/100g
pH	7.37±0.02	7.29±0.04	7.29±0.02
pO_2 (mmHg)	101±3	81±5*	66±1**
pCO_2 (mmHg)	35±0.8	58±4**	62±3**

* $p<0.05$ and ** $p<0.01$ as compared with controls.

Table II. Effects of hypoventilation on ischemic arrhythmias.

Arrhythmia	Group I (Control) (n=18)	Group II (n=12)	Group III (n=12)	Group IV (n=12)	Group V (n=11)
Arrhythmia Counts					
Single VEBs	353±41	264±42	197±39*	204±27*	224±26
Salvos	150±17	52±9*	63±23*	48±8**	72±14
VT	832±148	169±55**	67±26***	120±27**	323±67*
Total VEBs	1336±100	485±75**	328±51**	373±50**	620±90*
Duration (sec)					
VT	122±21	28±9**	10±3***	19±4***	57±16**
Reversible VF	54±9	12±4***	4±2***	6±2***	14±4***
Incidence (%)					
VT	100	83	70	100	100
Reversible VF	100	58	30	54.5	66.6
Irreversible VF	33	0	0	9	16.6
Total VF	100	58	30	63.5	83.3

Total VEBs is sum of arrhythmias occurring as single extrasystoles, salvos, and VT. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ as compared with controls.

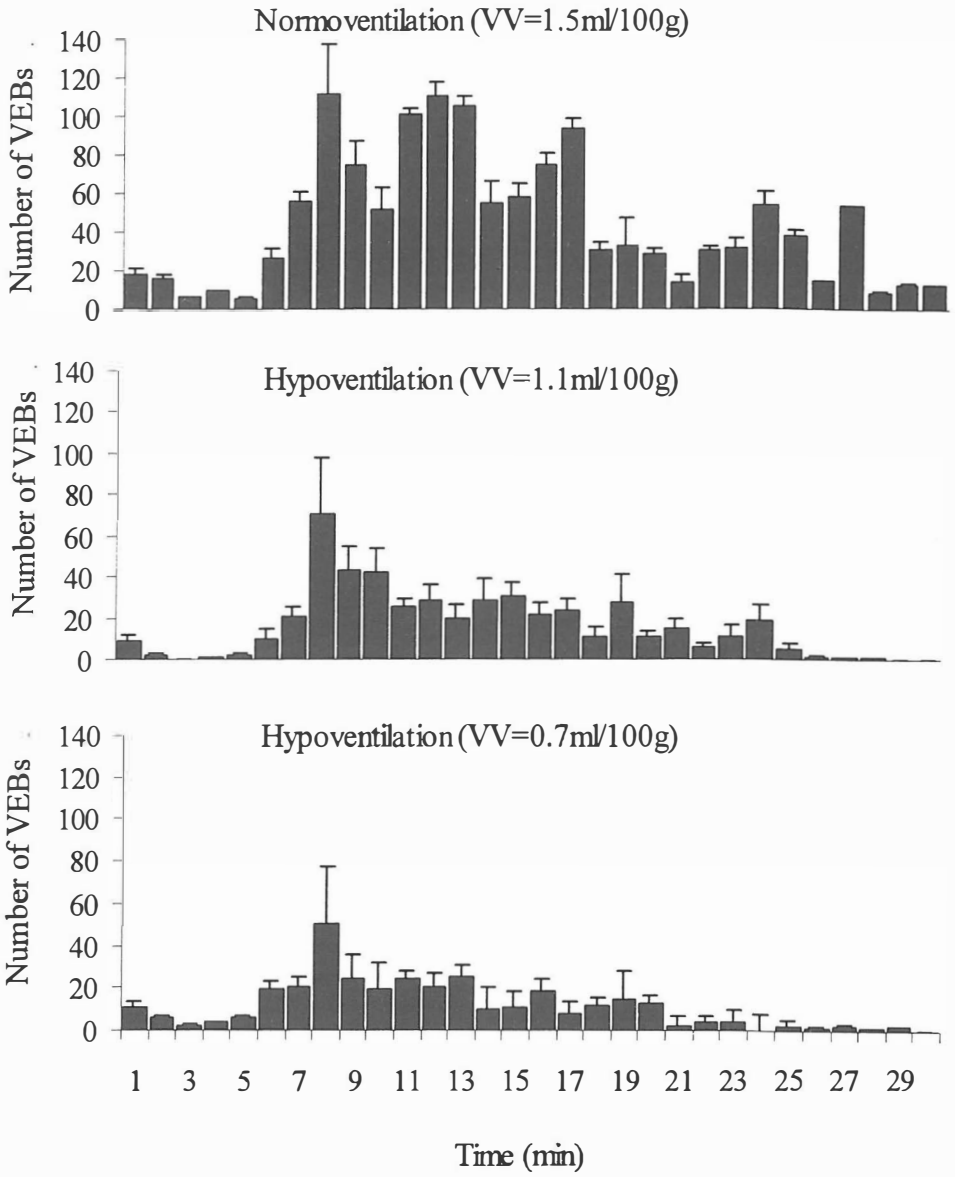

Fig. 1. Distribution of ventricular ectopic beats (VEBs) over 1 min intervals during 30 min occlusion of the left coronary artery of normoventilated (control, upper trace), hypoventilated (II, VV=1.1 mL/100g, middle trace), and hypoventilated (III, VV=0.7 mL/100g, lower trace) rats. Total number of ventricular ectopic beats (VEBs) was markedly decreased in hypoventilated rats.

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Surgical procedure

Male Wistar rats (280-300g) were anesthetized with sodium pentobarbitone (60 mg/kg intraperitoneally, I.P.). The trachea was cannulated for artificial respiration and systemic arterial bloodpressure was recorded from a catheter inserted into the left carotid artery. The right jugular vein was cannulated for administration of anesthetic as required. The ECG was continuously monitored over the experimental period via a standard limb lead I. The chest was opened using a left thoracotomy at a point ~2 mm to the left of the sternum; ribs 4 and 5 were then sectioned. Artificial respiration

1.5 mL/100g, rate 54 strokes/min).[7] After the pericardium was incised, the heart was exteriorized by gentle pressure on the ribcage and a 6/0 braided silk suture was placed around the left coronary artery as described previously.[8] The heart was replaced in the chest, the animal was allowed to stabilize for 15 min and then subjected to 30 min coronary artery occlusion (CAO). Any animal with mean arterial blood pressure (MAP) <70 mmHg was discarded.

Parameters measured and arrhythmia analysis

Systolic and diastolic blood pressure (BP) and mean arterial blood pressure (MAP) were measured from the arterial BP trace. Heart rate was calculated from the ECG. Both the ECG and BP were continuously recorded on a Narke (MK-III-S) physiograph. The index of oxygen consumption, indirectly, was calculated as: (HR×MAP)/ 1000. Ventricular arrhythmias were analyzed according to the guidelines of the Lambeth Conventions for Determination of experimental arrhythmias,[9] which were identified as single ventricular ectopic beats (VEBs), salvos (couplets and triplets), and ventricular tachycardia (VT, defined as a run of four or more consecutive ectopic beats). The total number of ventricular arrhythmias was calculated as the sum of these three types of arrhythmia. The incidence of VT and reversible and irreversible ventricular fibrillation (VF) was determined for each group.

Experimental protocols

Rats were allocated to one of 6 groups: I) normoventilated group (control; n=18); these animals were ventilated at a normal volume of 1.5 mL/100g during the period of the experiment; II, III) hypoventilated groups (n=12), in these groups the volume of ventilation (VV) was reduced to 1.1 and 0.7 mL/100g, respectively, only for 10 min prior to CAO; IV, V) in these groups (n=11-12) 10 min. of hypoventilation (VV=0.7 mL/100g) was followed by 5 and 10 min of normoventilation, respectively, prior to CAO; VI) animals in this group (n=5) were subjected to three 10 min episodes of hypo- (VV=0.7 mL/100g) and 5 min of normoventilation before CAO. In all experiments the rate of respiration remained constant (54 strokes/ min).

In 6 rats from groups I-V 30 min. of sustained ischemia was followed by 2 hours reperfusion, after which the hearts were removed for measurement of infarct size as a percentage of area at risk, as reported previously.[10] During the period of CAO (30 min) and reperfusion (2 h), VV was kept in normal range as control.

In another series of experiments (n=6) arterial blood was obtained from a cannula inserted into the right carotid, immediately before and at the end of hypoventilation. pO_2, pCO_2 and pH were measured with an automated gas analyzer.

Statistics

Except for the incidence of VT and VF, all results are expressed as mean ±SEM. Mann-Whitney nonparametric U test was used to compare the number of VEBs, duration of arrhythmias and infarct size between groups. Fisher-Irwin test (chi square with Yates correction) was used to compare the incidence of VT and VF. MAP, HR, pO_2 and pCO_2 data were assessed by one-way ANOVA and significant differences were examined by Newman-Keuls range test. Differences between groups were considered significant at $p<0.05$.

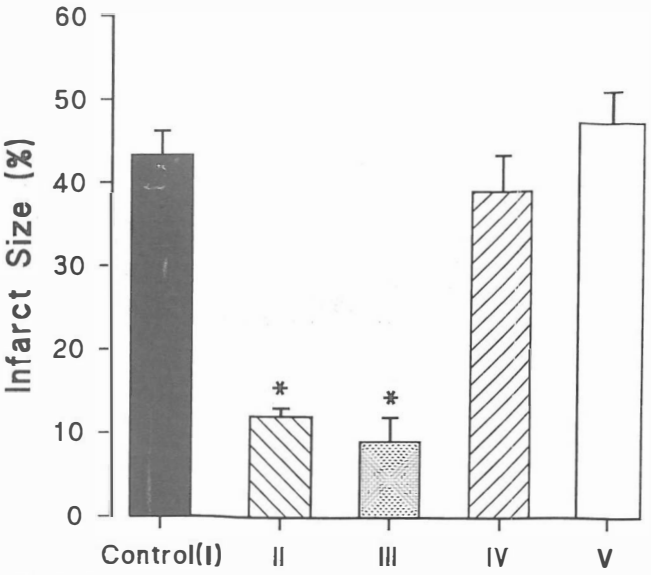

Fig. 2. The effect of hypoventilation on myocardial infarct size expressed as percent of the area at risk after 30 min coronary artery occlusion and 2 hours reperfusion. The infarct/risk zone percentage was limited markedly (*$p<0.01$) by hypoventilation in groups II and III.

RESULTS

Effect of hypoventilation on arterial blood gases and pH

Changes in pO_2, pCO_2 and pH of arterial blood before (when VV was normal) and at the end of hypoventilation (when VV was reduced) are shown in Table I. Artificial respiration of 1.5 mL/100g and 54 strokes/min was sufficient to maintain pCO_2, pO_2, and pH within normal limits (Table I). pH was 7.37 ±0.02 in the control group and 7.29 ±0.04 and 7.29 ±0.02 in groups II and III, respectively. Therefore, pH was not affected by volume of ventilation ($p>0.05$). Reduction of VV from 1.5 mL/100g in controls to 1.1 mL/ 100g in group II and to 0.7 mL/100g in group III produced a significant ($p<0.05$ and $p<0.01$) decrease in pO_2 (by 20 ±5.2 and 34.7 ±2.8 mmHg, respectively) and a significant ($p<0.01$) rise in pCO_2 (by 22.7 ±3.8 and 26.3 ±0.8 mmHg, respectively).

Effects of hypoventilation preconditioning on ischemic arrhythmias

Acute occlusion of the left main coronary artery in both control and hypoventilated anesthetized rats resulted in immediate ST-segment changes and in arrhythmias with a characteristic temporal pattern of distribution (Fig.1). Note the marked suppression of the total number of VEBs in hypoventilated-preconditioned rats. In most animals, ectopic beats began ~2-3 min after occlusion, reached a peak activity at ~5-15 min, and then declined by 20-25 min after occlusion. Table II summarizes the effects of hypoventilation on total arrhythmia count after coronary artery occlusion (CAO).

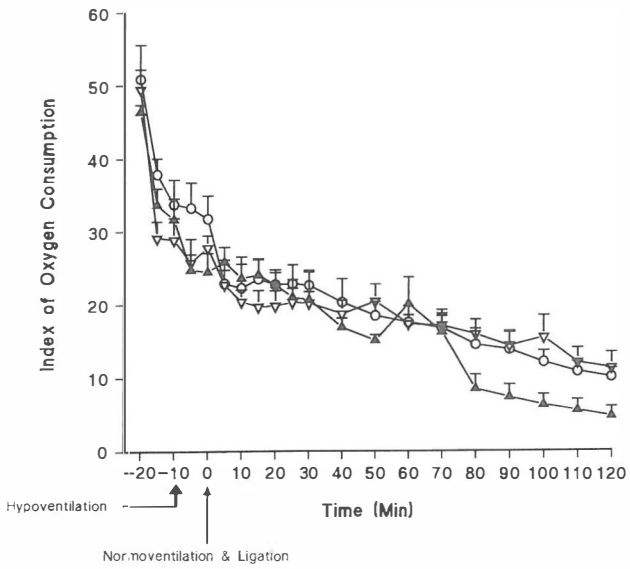

Fig. 3. The index of oxygen consumption (calculated as: (HR ×MAP)/1000) from immediately before thoracotomy and during the hypoventilation and 30 min period of ischemia to the end of the 2 hours reperfusion were measured. The thoracotomy in control (group I) and hypoventilated rats (groups II & III, started at time-15 min) caused a sharp decline in the index of oxygen consumption due to opening the chest and decline of MAP. In the hypoventilated groups reduction in the volume of ventilation was commenced 10 min before CAO (performed at time 0 min). The volume of ventilation had returned to the normal range at time 0.

Compared to normoventilated rats (control), reduction of volume of ventilation from the normal range (1.5 mL/100g) to 1.1 and 0.7 mL/100g in groups II and III, only for 10 min immediately prior to CAO, attenuated the total number of VEB's significantly ($p<0.01$ and $p<0.001$, respectively) as a result of decreasing all kinds of arrhythmias. The time spent in VT was also reduced very considerably ($p<0.01$ and $p<0.001$) in both groups. Although reduction in the incidence of VF did not attain statistical significance, the duration of the episodes of reversible fibrillation was reduced very markedly ($p<0.001$) by hypoventilation. There was no mortality due to irreversible VF in the preconditioned rats.

Similar to groups II and III, in groups IV and V in which there were 5 and 10 min recovery periods of normoventilation between the hypoventilation episodes and CAO, ischemic arrhythmias, time spent for VT, and reversible VF were suppressed significantly (Table II). However, the number of arrhythmias tended to be numerically higher in the hypo- and normoventilated preconditioned groups (IV and V) than in the only hypoventilated preconditioned group (III). Repeat of hypo- and normoventilation episodes for three times prior to CAO in group VI caused a sharp decline in BP and a high mortality (4 out of 5 animals) soon after occlusion. Therefore, data for this group are not available.

Effects of hypoventilation preconditioning on infarct size following sustained ischemia and reperfusion

Figure 2 shows myocardial infarct size expressed as a percentage (by weight) of the area at risk in controls and hypoventilated preconditioned rats after 30 minutes of CAO and 2 hours reperfusion. Area at risk was similar in all groups. The absolute infarct area in controls (266±48 mg) and hypoventilated preconditioned rats (group II and III; 108±23 and 75±34 mg, respectively) was significantly different ($p<0.05$). The infarct/risk zone percentage following a subsequent 30 minutes of occlusion and 2 hours of reperfusion was limited markedly ($p<0.01$) by hypoventilation immediately prior to CAO from 43.4±2.9% in control animals to 12.1±1% and 9.1±2.9% in hypoventilated rats (Fig. 2). However, separation of hypoventilation period from coronary artery ligation by a recovery period of normoventilation did not affect neither area at risk (292±60 and 245±75 mg in groups IV and V, respectively, compared to 266±48 mg in controls) nor infarct size (39.2±4.4% and 47.5±2.8% in groups IV and V, respectively, compared to 43.4±2.8% in controls).

Effects of hypoventilation preconditioning on hemodynamic variables

Mean arterial blood pressure (MAP) and heart rate (HR) from immediately before thoracotomy and during the hypoventilation to the end of the 30 min period of ischemia and, in the case of reperfusion, during the reperfusion time, were measured. The thoracotomy in both control and hypoventilated rats caused a sharp decline in MAP due to opening the chest. Figure 3 shows index of oxygen consumption changes (n=6), from 5 min before thoracotomy to the end of the reperfusion, in the three groups. There were no significant differences between groups in HR and MAP, as well as in the index of oxygen consumption at any time of the experiments. The hypoventilation induced a slight but not significant ($p<0.05$) decrease in MAP. There was a reduction in MAP 5 min after CAO in all groups due to the appearance of arrhythmias at this time. There was a slight decrease in HR during the pre- and post-occlusion period and during reperfusion time in all groups.

DISCUSSION

This study revealed that a short period of hypoventilation immediately prior to sustained ischemia was protective against ischemic arrhythmias and could limit considerably the size of an evolving myocardial infarct in rats. Also, the present study showed that the presence or absence of a recovery period of normoventilation between hypoventilation and sustained ischemia not only did not affect the degree of antiarrhythmic effect of hypoventilated preconditioning but also abolished the protective effect of hypoventilation against infarction. The effects of acute

preconditioning with ischemia or hypoxia have been extensively investigated. To our knowledge this is the first report showing the effects of reduced volume of respiration on the myocardial responses to ischemia and reperfusion. However, it is not clear whether the mechanism(s) may differ between this type of preconditioning and others. Shisukuda et al.[12] reported that hypoxic preconditioning reduced infarct size in canine myocardium. Neely and Grotyophann[13] were the first to report hypoxic preconditioning. They hypothesized that improvement of post-ischemic function in isolated perfused rat hearts subjected to hypoxic perfusion and reoxygenation before the sustained ischemia was due to reduced myocardial lactate accumulation as a result of hypoxia-induced glycogen depletion. Murry et al.[14] demonstrated that ischemic preconditioning similarly reduces glycogen breakdown and lactate accumulation. However, in the study of Tajima et al.[6] it was shown that ATP and creatine phosphate decreased to a similar and quite low level in both chronically hypoxic rats and normoxic controls. In addition they found that the lactate production was identical in both groups.

In the present study the significant decrease of pO_2 following reduction of the volume of ventilation in hypoventilated rats confirms that the reduced volume of ventilation was enough to produce hypoxemia. Hypoxemia might reduce the basal myocardial energy demand and thereby decrease ischemic arrhythmia and myocytic injury during ischemia/reperfusion. Several studies have shown[5,11] that the reduction of myocardial energy demand could not be a mechanism of preconditioning. In this study the mechanism(s) by which hypoventilation can produce protection were not directly examined. However, insignificant changes in indirectly measured index of oxygen consumption indicate that the protective mechanism(s) involved in this phenomena possibly is not mediated through the suppression of myocardial oxygen consumption, although the index of oxygen consumption declined during coronary artery occlusion and reperfusion periods in all groups compared to pre-occlusion time.

Hypoxemia results in vasodilatation[15] and therefore causes a high rate of blood flow through the organs of the body, as well as the heart, whereas ischemia is a condition in which inadequate O_2 delivery to the myocardium occurs because of low blood flow. In addition, the low blood flow fails to wash out accumulating metabolic products which can lead to further impairment of metabolism. Therefore, in ischemic preconditioning reflow between episodes of short time ischemia and sustained ischemia is essential. [2,16,17] Walsh et al.[18] have indicated that hypoxia prior to ischemia without an intervening period of reoxygenation reduces infarction. In the present study, to produce a recovery period of normoventilation between the hypoventilation period and coronary artery occlusion, similar to the standard method of inducing preconditioning, hypoventilation was separated by 5 and 10 minutes of normoventilation from sustained ischemia. Interestingly, the intervening period of normoventilation did not affect the antiarrhythmic effect of hypoventilation. Moreover, the normoventilation period between hypoventilation and prolonged ischemia decreased the protective effects of hypoventilated preconditioning against infarction significantly. This may indicate that different mechanisms are involved in the production of ischemic arrhythmias and ischemic/reperfusion injuries. The remarkable antiarrhythmic and infarct size limiting effect of hypoventilation immediately prior to ischemia indicates that reflow, rather than reoxygenation, is the critical feature of the reperfusion period in ischemic preconditioning.

In conclusion, the results of this study show that the heart can be preconditioned by hypoventilation immediately prior to prolonged ischemia, and the protective effects of the preconditioning are not affected by an intervening period of normoventilation between the hypoventilation period and coronary artery occlusion.

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