

# **Preconditioning by Hypoventilation Increases Ventricular Arrhythmia Threshold in Wistar Rats**

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## **Summary**

Hypoventilation, as one of ventilatory disorders, decreases the electrical stability of the heart similarly as ischemia. If preconditioning by short cycles of ischemia has a cardioprotective effect against harmful influences of a prolonged ischemic period, then preconditioning by hypoventilation (HPC) can also have a similar effect. Anesthetized rats (ketamine 100 mg/kg + xylazine 15 mg/kg i.m., open chest experiments) were subjected to 20 min of hypoventilation followed by 20 min of reoxygenation (control group). The preconditioning (PC) was induced by one (1PC), two (2PC) or three (3PC) cycles of 5-min hypoventilation followed by 5-min reoxygenation. The electrical stability of the heart was measured by a ventricular arrhythmia threshold (VAT) tested by electrical stimulation of the right ventricle. Twenty-minute hypoventilation significantly decreased the VAT in the control and 1PC groups ( $p < 0.05$ ) and non-significantly in 2PC vs. the initial values. Reoxygenation reversed the VAT values to the initial level only in the control group. In 3PC, the VAT was increased from  $2.32 \pm 0.69$  mA to  $4.25 \pm 1.31$  mA during hypoventilation ( $p < 0.001$ ) and to  $4.37 \pm 1.99$  mA during reoxygenation ( $p < 0.001$ ). It is concluded that cardioprotection against the hypoventilation/reoxygenation-induced decrease of VAT proved to be effective only after three cycles of HPC.

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## **Key words**

Hypoventilation • Cardioprotection • Preconditioning • Ventricular arrhythmia • Rats

## **Introduction**

Hypoxic states of the heart result from disproportion between the amount of oxygen supplied to the cardiac cell and the amount actually required by the cell. The degree of hypoxic injury does not depend only on the intensity and duration of the hypoxic stimulus, but also on the level of cardiac tolerance to oxygen deprivation. Such oxygen deprivation can result from systemic hypoxia or local ischemia with consequences of two different mechanisms of action at the cellular level. Systemic hypoxia is usually a generalized phenomenon diffusely involving the whole myocardium, whereas

ischemia is confined to the area supplied by the affected coronary artery. In ischemia, there is not only a drop in the supply of oxygen and other substrates, but also a significant reduction in the clearance of metabolites. In contrast, in ischemic hypoxia (often described as “cardiac hypoxia”) there is a combined action of both ischemia and hypoxia, while perfusion results in partial elimination of metabolites. Ischemic hypoxia is clinically manifested primarily in ischemic heart disease (coronary artery disease) and its acute form, myocardial infarction, whereas systemic hypoxia is associated with chronic cor pulmonale of various origin, cyanosis due to a hypoxemic congenital heart disease, ventilatory disorders and

exposure to low barometric pressure, e.g. at high altitudes (Ošťádal *et al.* 1999).

It is known that some ventilatory disorders are often accompanied by ventricular arrhythmias. This relation was investigated in both experimental (Otsuka and Watanabe 1990, Tomori *et al.* 1997) and clinical studies (Peter 1990, Tomori *et al.* 1999, Kujanik *et al.* 2000). Hypoventilation, similarly to ischemia, decreases the electrical stability of the heart and increases the vulnerability of the myocardium to ventricular arrhythmias. These changes were detected not only in the isolated perfused heart but also during *in vivo* experiments (Kujanik *et al.* 1984) and also in the circadian dependence (Švorc *et al.* 1997, 2000).

During the past decade, it has been demonstrated that brief periods of myocardial ischemia (ischemic preconditioning) followed by reperfusion provide endogenous myocyte protection against a subsequent ischemic insult (Reimer *et al.* 1981, Barber 1983, Murry *et al.* 1986). The effect of hypoxic preconditioning is relatively less studied. Shizukuda *et al.* (1993) suggest that preconditioning with local hypoxia offers more advantages than preconditioning with ischemia and reperfusion. One of the advantages is the maintenance of blood flow. The experiments with hypoxic preconditioning were performed in various animals, but the effect of such preconditioning was followed only during a prolonged period of ischemia. Using the guinea-pig papillary muscles Kasamaki *et al.* (1997) demonstrated that hypoxic preconditioning can significantly enhance post-hypoxic recovery of developed tension, protect the myocardium against reoxygenation arrhythmias and cause other significant electromechanical changes. For example, the action potential duration and overshoot decreased more quickly in preconditioned than in non-preconditioned muscles (Nojiri *et al.* 1999).

Therefore, it is important to know whether preconditioning by hypoventilation can also reduce the experimentally induced ventricular arrhythmias or increase the electrical stability of the heart against a subsequent prolonged period of hypoventilation and reoxygenation. Experiments with hypoxic preconditioning were mostly performed in isolated perfused hearts, whereas the responses to hypoxic preconditioning from *in vivo* experiments and mainly under the conditions of hypoventilation are less known. We hypothesized that if hypoventilation, as one of ventilatory disorders, decreases the electrical stability of the heart, preconditioning by hypoventilation could have a comparable effect as ischemic preconditioning in rats.

Therefore, the aim of our study was to obtain information concerning new aspects of myocardial preconditioning by hypoventilation and its cardioprotective effect in experiments *in vivo*.

## Methods

### *Experimental animals and conditions of adaptation*

The experiments were performed in female Wistar rats of a standard breed ( $261 \pm 25$  g body weight, 4-5 months old). The rats were adapted to a standard laboratory conditions (daily light-dark cycle of 12:12 h with the dark period from 18:00 to 06:00 h, relative moisture 40-60 %, temperature  $24^{\circ}\text{C}$ , the intensity of artificial illumination 800 Lux, 2 animals per cage for 4 weeks). The experiments were performed only during the light phase (sleeping time) in the course of a whole year, and the obtained results were averaged independently of the seasons.

### *Experimental methods*

The experiments were performed in anesthetized rats (ketamine 100 mg/kg, SPOFA Prague + xylazine 15 mg/kg, SPOFA Prague, i.m.) at rectal temperature ( $36.4 \pm 0.5^{\circ}\text{C}$ ) measured before the application of anesthetic agent. Rectal temperature was decreased after application of the anesthetic agent. Heating of anesthetized animals by an infrared lamp was used to prevent the hypothermia effect on the heart rate.

The animals were divided into four experimental groups. The control group ( $n=12$ ) was subjected to 20 min of hypoventilation and a subsequent period of 20 min of reoxygenation without preconditioning. Group 1PC ( $n=8$ ) was exposed to one 5-min cycle, group 2PC ( $n=10$ ) to two 5-min cycles and group 3PC ( $n=8$ ) to three 5-min cycles of hypoventilation alternating with 5-min cycles of reoxygenation. All three experimental groups with preconditioning were also exposed to 20 min of hypoventilation followed by 20 min of reoxygenation (Scheme 1). The experimental protocol with hypoxic preconditioning was similar to the ischemic preconditioning (Tani *et al.* 1996).

Hypoventilation (respiratory rate 20 breaths/min, tidal volume 0.5 ml/100 g b.w.) and reoxygenation (respiratory rate 40 breaths/min, tidal volume 1 ml/100 g b.w.) were achieved by a respirator (E. Zimmermann, Germany) through a tracheal cannula. The heart rate changes were recorded from the electrocardiogram at each minute of the experiment, as the mean value of four consecutive heart cycles. The cardiac stimulation,

triggered by the R wave at spontaneous heart rate, was performed directly in the open chest of artificially ventilated animals. The stimulating electrodes (diameter 1 mm and 5 mm interelectrode distance) were placed at the base of the right ventricle. Parameters of stimulation were as follows: rectangular pulses with a frequency 30 Hz, 10 ms duration, volleys of stimulation 400 ms. The current intensity was increased progressively stepwise by 0.3 mA until ventricular arrhythmia was obtained. The ventricular arrhythmia threshold (VAT) was estimated after 5, 10, 15 and 20 min of ventilation. Termination of various type of ventricular arrhythmias was followed by spontaneous resuscitation of the rats during 4-5 min. Animals with any spontaneous

arrhythmia or disorders of the heart cycle were eliminated from the experimental protocol. In the control group, 6 animals out of the total of 18 animal, 1PC 10 out of 18, 2PC 3 out of 13 and 3PC 4 out of 12 animals. The ventricular arrhythmias were the mixed type with spontaneous mutual transitions between ventricular fibrillations, ventricular tachycardia and flutter and they were comparable in the particular groups. The values of blood gases and the acid-base balance were screened by the ASTRUP method (COMPACT 2, Austria) from blood samples taken from the femoral artery at the end of the period of hypoventilation and reoxygenation as a control of the ventilatory effects.

**Scheme 1.** *Protocol of the experiments with hypoventilatory preconditioning.*

Control group



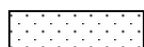
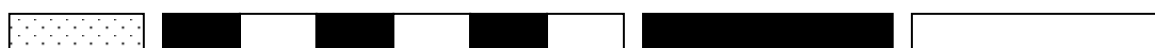
1PC group



2 PC group



3 PC group



the initial phase of the experiments with heating of animals to the rectal temperature measured before the application of anesthesia, tracheotomy, thoracotomy, period of stabilization and the first value of VAT (initial value)



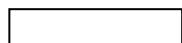
5 min hypoventilation



5 min reoxygenation



20 min hypoventilation

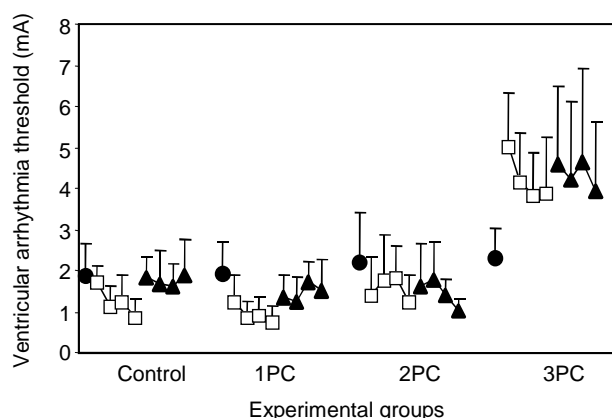


20 min reoxygenation



stimulation of the myocardium by electrical current for the estimation of ventricular arrhythmia threshold

The data are given as the arithmetical means  $\pm$  S.D. The statistical level  $p < 0.05$  was considered significant using non-paired parametrical t-test (testing of the differences from the whole period of hypoventilation and reoxygenation) and non-parametrical tests (testing of differences in separate time intervals during respective ventilation).  $\chi^2$  test was used for the confirmation of assumed experimental results of the cardioprotective effect of the preconditioning by hypoventilation.



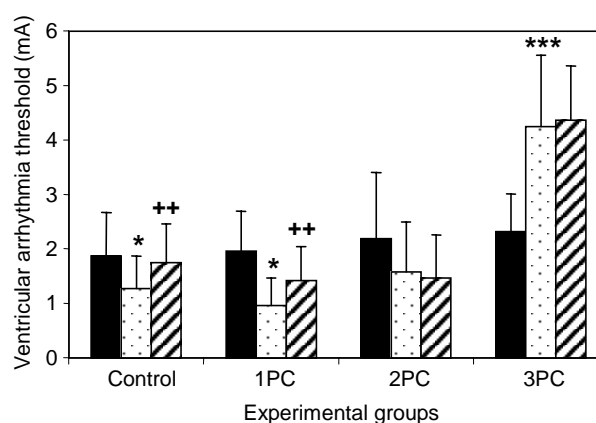
**Fig. 1.** The dynamics of the VAT changes in separate intervals of the measurement (5 min, 10 min, 15 min and 20 min) in the course of 20 min of hypoventilation (empty squares) and subsequent 20 min of reoxygenation (full triangles) in all experimental groups. The initial VAT values – full circles. Control – control groups without preconditioning by hypoventilation (HPC), 1PC – one 5-min period of the HPC, 2PC – two 5 min periods of the HPC, 3PC – three 5 min periods of the HPC). The values are means  $\pm$  S.D.

## Results

### Ventricular arrhythmia threshold

The initial VAT values varied inter- and intraindividually in a relatively wide range probably reflecting seasonal influences. No significant differences between initial VAT values from all groups were found. The VAT values after 5, 10, 15 and 20 min of hypoventilation and reoxygenation, regardless of the individual reactions of animals to the electrical stimulation (increase or decrease against the previous value) are shown in Figure 1. The average VAT value non-significantly decreased in the control, 1PC and 2PC groups already after 5 min of hypoventilation and

gradually declined further to the end of 20 min of hypoventilation. A significant increase of the VAT was found only in the 3PC group ( $p < 0.001$ ; initial value  $2.32 \pm 0.69$  mA vs. 5 min hypoventilation  $5.03 \pm 1.28$  mA). Five minutes of reoxygenation significantly increased the VAT values against the value from the 20 min of hypoventilation in the control group ( $p < 0.001$ , 20 min hypoventilation  $0.86 \pm 0.44$  mA vs. 5 min reoxygenation  $1.82 \pm 0.52$  mA) and in the 1PC group ( $p < 0.05$ , 20 min hypoventilation  $0.75 \pm 0.40$  mA vs. 5 min reoxygenation  $1.35 \pm 0.53$  mA). Practically, no changes were found in the 2PC and 3PC groups. The values fluctuated within a narrow range without statistically significant changes.



**Fig. 2.** The average VAT values from the whole period of hypoventilation (dotted column) and reoxygenation (hatched column) in all four groups compared to the initial values (full column). The values are means  $\pm$  S.D. \*\*\*  $p < 0.001$ , \*  $p < 0.05$  – significant differences between hypoventilation and initial value, \*\*  $p < 0.005$  significant differences between reoxygenation and hypoventilation.

The initial VAT and the VAT values found from the whole period of the 20 min hypoventilation and reoxygenation, respectively, were compared regardless of the individual reactions of animals to the electrical stimulation (decrease or increase during hypoventilation against the initial value or during reoxygenation against the value from the hypoventilatory period) (Fig. 2). A significant decrease was found during hypoventilation compared to initial values in both the control group ( $p < 0.05$ ;  $1.27 \pm 0.60$  mA vs.  $1.87 \pm 0.80$  mA) and the 1PC group ( $p < 0.05$ ,  $0.96 \pm 0.49$  mA vs.  $1.96 \pm 0.73$  mA). A similar decrease of the VAT, although non-significant, was also found in the 2PC group. The VAT significantly

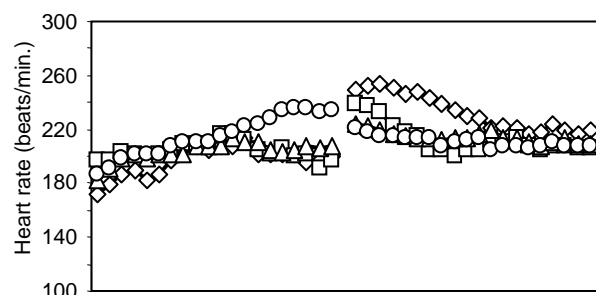
increased in the 3PC group ( $p < 0.001$ ;  $4.25 \pm 1.31$  mA vs.  $2.32 \pm 0.69$  mA). Reoxygenation reversed the VAT to the initial level only in the control and partly in the 1PC groups. A reverse situation was found in the 2PC group, where a further decrease against the hypoventilatory value was observed. In the 3PC group, reoxygenation increased non-significantly the VAT against the hypoventilatory value. This increase was significantly higher against the initial VAT value ( $p < 0.001$ ,  $2.32 \pm 0.69$  mA - initial value vs.  $4.37 \pm 1.99$  mA - reoxygenation).

The evaluation of individual reactions of animals indicated that the responses to direct stimulation by electrical current (decrease or increase of the VAT against the previous values) were different in the experimental groups. In the control group, 20 min of hypoventilation decreased the VAT in 10 of 12 rats (83.49 %), increased it in one rat (8.3 %) and did not affect it in another rat (8.3 %). In the 1PC group an decrease was found in all animals (100 %). In the 2PC group, a decrease was present in 8 out of 10 animals (80 %), and an increase in 2 rats (20 %). In the 3PC group, the VAT decreased only in one out of 8 animals (12.5 %) and increased in 7 animals (87.5 %). Reoxygenation increased the VAT values against hypoventilation in 10 out of 12 animals (83.3 %) in the control group, in 6 of 8 rats (75 %) in the 1PC group, in 4 out of 10 animals (40 %) in the 2PC group and in 5 out of 8 rats (75 %) in the 3PC group. Significant cardioprotection of the preconditioning by hypoventilation was confirmed by the  $\chi^2$  test ( $p = 0.003$ ) only during a prolonged period of hypoventilation but not during reoxygenation ( $p = 0.176$ ).

#### Heart rate

In the initial phase of the experiments (initial HR, tracheotomy, thoracotomy and period of stabilization) the HR behavior was the same in all groups. Thoracotomy significantly decreased the HR ( $p < 0.001$ ) compared to the state after tracheotomy. After 5 min of stabilization (with normal ventilation), the HR gradually increased, but did not reach the initial values. The HR increased in all four groups within 10-11 min from the start of hypoventilation. In the control, 1PC and 2PC groups the HR was stabilized till the end of hypoventilation, with the exception of the 3PC group, where a further increase was found. Reoxygenation increased the HR in the control, 1PC and 2PC groups, but decreased it in the 3PC group as compared to the preceding hypoventilation. A non-significant moderate decrease and stabilization of the HR was recorded at

approximately the same level till the end of reoxygenation in all four groups (Fig. 3). However, the HR changes did not correlate with the VAT in any experimental group.



**Fig. 3.** The dynamics of heart rate changes in the course of 20 min hypoventilation and subsequent 20 min reoxygenation in four separate groups. The values are expressed as arithmetic means at each minute of ventilation. Control group – rhombus, 1PC group – square, 2PC group – triangle, 3PC group – circle.

#### Blood gases and acid-base balance

The differences were minimal in the values of  $pH_a$ ,  $paO_2$  and  $paCO_2$  from the end of hypoventilation and reoxygenation in all experimental groups. Significant differences were found between hypoventilation and reoxygenation in the separate experimental groups (Table 1). The hypoxic state persisting after 20 min of reoxygenation probably results from the chest opening and artificial ventilation.

#### Discussion

The reason for our experiments was the fact that systemic hypoxia and ischemic hypoxia have different mechanisms of action on the myocardium. The main aim of our study was to gain information concerning new aspects of a phenomenon of cardioprotection by preconditioning (PC) and to evaluate the role of systemic hypoxia in PC. We hypothesized that if hypoventilation decreases the electrical stability of the heart, similarly as ischemia, thus the preconditioning by hypoventilation (HPC) could induce similar protection of the myocardium against ventricular arrhythmias during a prolonged period of hypoventilation, as ischemic preconditioning (IPC). Whereas the endpoints of preconditioning are not only the size of infarction, necrosis and contractile

dysfunctions (Tani *et al.* 1996), but also disorders of the heart cycle and ventricular arrhythmias (Lukas and Botsford 1997). Therefore, we studied mainly one sensitive parameter of the electrical stability of the heart, i.e. ventricular arrhythmia threshold. The electrical

stability of the heart, a determinant of the ventricular vulnerability to arrhythmias, is markedly changed by various ventilatory disorders (Otsuka and Watanabe 1990, Peter 1990, Kujaník *et al.* 2000, Švorc *et al.* 1997, 2000).

**Table 1.** Basic parameters of blood gases and acid-base balance (paO<sub>2</sub>, paCO<sub>2</sub> and pH<sub>a</sub>) obtained at the end of 20-min hypoventilation and reoxygenation periods.

	paO <sub>2</sub> (kPa)	paCO <sub>2</sub> (kPa)	pH <sub>a</sub>
<i>IPC group</i>			
<i>hypoventilation</i>	5.55 ± 0.88	7.57 ± 1.21	7.136 ± 0.05
<i>reoxygenation</i>	9.46 ± 1.85**	4.62 ± 0.71**	7.365 ± 0.06***
<i>2PC group</i>			
<i>hypoventilation</i>	6.6 ± 1.00	8.24 ± 1.27	7.074 ± 0.07
<i>reoxygenation</i>	9.85 ± 1.04***	3.41 ± 0.57***	7.444 ± 0.05***
<i>3PC group</i>			
<i>hypoventilation</i>	6.64 ± 1.80	8.19 ± 1.14	7.093 ± 0.07
<i>reoxygenation</i>	10.38 ± 1.46***	3.22 ± 0.30***	7.460 ± 0.11***

Data are means ± SD. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$  statistical significance of the differences between values from period of hypoventilation and reoxygenation in the same experimental group.

Our results indicate that the electrical stability of the heart exposed to 20 min of hypoventilation was only slightly changed by one and two cycles of preconditioning, but it was significantly increased after three cycles of HPC. Therefore, one or two cycles of HPC have practically no protective effect against hypoventilation-induced decrease of the electrical stability of the heart. In the rabbit IPC model, Lukas and Botsford (1997) found that a single IPC period completely protects the myocardium against ischemia-induced ventricular fibrillation. It seems that the IPC probably mobilizes the mechanisms of cardioprotection more rapidly and more effectively than the HPC and it may also depend on the species of experimental animals.

The cardioprotection against hypoventilation-induced VAT decrease proved to be effective only after 3 cycles of the HPC. The percentual increase in the number of animals reacting by VAT increase after 3 cycles of HPC (87.5 %) versus IPC (0 %) and 2PC (20 %) groups, also supports the cardioprotective effect of the HPC. The possibility that these changes partially result from simple prolongation of the experiment in the separate groups (control group - 40 min, 1PC group - 50 min, 2PC group - 60 min and 3PC group - 70 min)

could not be excluded. However, the time effect probably does not play a role in the adaptation of the heart, because corresponding VAT changes are not seen in the IPC and 2PC time dependence. Our results with three cycles of the HPC are comparable with the results of other authors working with IPC models in different species. For example, in the rat heart the IPC reduced the number of ventricular premature beats, abolished the incidence of ventricular tachycardia, ventricular fibrillation, irreversible ventricular fibrillation, QT interval and QT dispersion (parameters which are associated with a high incidence of ventricular arrhythmias induced by ischemia) (Lu *et al.* 1999).

Reoxygenation reversed the VAT values to the initial level in rat hearts without preconditioning. In groups with hypoxic preconditioning, reoxygenation does not change the myocardial vulnerability to ventricular arrhythmias as described in IPC models. In the isolated non-preconditioned rat hearts, all hearts showed ventricular fibrillation and a significant decrease of action potential duration (APD) as early as in the first minute of reperfusion (Perchenet and Kreher 1995, Tosaki *et al.* 1994). In a preconditioned group (two cycles of the IPC) during reperfusion increased APD which persisted at

values near those of the stabilization period (Allen *et al.* 1993). A decreased incidence of ventricular fibrillation and ventricular tachycardia was also recorded after preconditioning by four cycles of global ischemia (Tosaki *et al.* 1994). Our results from the 3PC group are similar to above mentioned findings, where the VAT was significantly increased in comparison to prehypoventilatory values.

It remains an open question, whether HR changes are only reflections of changes in the autonomous nervous system activity or whether they are the result of a whole complex of factors changing during hypoventilation and reoxygenation after preconditioning. Under the conditions of hypoventilation-induced systemic hypoxia, the HR was decreased in all experimental groups which may be the result of reduced tissue noradrenaline (Chanine *et al.* 1993). The gradual HR increase until 10-12 min of hypoventilation probably only reflects the adaptation to hypoxic conditions irrespective of preconditioning. The average HR value of the whole 20-min reoxygenation period was increased to initial levels in the control group only. The HR increase during reoxygenation to the initial values can be

accompanied by a massive noradrenaline release into the coronary circulation and by stimulation of  $\alpha$ -adrenoreceptors (Winslow *et al.* 1983, Merato *et al.* 1996, Zumino *et al.* 1997). Preconditioning by one, two and three cycles of hypoventilation probably reduces this process because prehypoventilatory values were not reached in these groups.

It is concluded that one or two cycles of the HPC, under our experimental conditions, have a negligible protective effect against a hypoventilation-induced decrease of the electrical stability of the heart. Cardioprotection against prolonged hypoventilation followed by reoxygenation proved to be effective only after three cycles of the HPC. Reoxygenation reversed the VAT values to the initial level only in the group without preconditioning and partly in the 1PC group. An important unanswered question remains whether the mechanisms responsible for cardioprotection after HPC are identical to the IPC rat heart model.

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**Reprint requests**

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