Chronobiological Aspects of Preconditioning by Systemic Asphyxia – Endogenous Cardioprotection?

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Abstract

There is evidence that repeating brief periods of myocardial ischemia and reperfusion may provide protection against electrical instability of the heart evoked by subsequent ischemia/reperfusion injury. Similar cardioprotective effectshave been obtained after pre-treatment with repetitive episodes of hypoxia. We focused to fact whether there are differences in the conditions and dynamics of the developing protective effects of myocardial preconditioning applied during the light (nonactive) and dark (active) parts of the day regimen of rats, and to obtain an understanding concerning chronophysiological aspects of this phenomenon in in vivo rat experiments.The experiments were performed in anaesthetized (ketamine/xylazine anaesthesia, 100 mg/kg + 15 mg/kg, i.m., open chest experiments)after adaptation to a LD cycle of 12h : 12h, with the dark part of day from 06:00 to 18:00hfor 4 weeks. The ventricular arrhythmia threshold (VAT) was estimated by direct electrical stimulation of the heart. Animals were artificially ventilated by humified room air at the parameters of the initial ventilation and reoxygenation: respiratory rate, 40 breaths/min; and tidal volume, 1 ml/100g b.w. During experimental hypoventilatory asphyxia, the respiratory rate and tidal volume were reduced to 20 breaths/min and 0.5 ml/100g b.w., respectively. One cycle of preconditioning by asphyxia is too weak of a stimulus for the production of cardioprotection in both light parts of the day.The cardioprotection probably starts after two cycles of preconditioning by asphyxia in both light parts, and the cardioprotective effect of preconditioning by asphyxia depends on the numbers of the preconditioning cycles and in the dependence on LD cycle - it is highlight by three cycles of preconditioning by asphyxia. It is concluded that there are different reactions of the rat myocardium for the asphyxic preconditioning in the dependence on the LD cycle.

Keywords

Chronobiology; Preconditioning; Systemic Asphyxia; Electrical Stability of the Heart; Rat

Introduction

There is ample evidence that repeating brief periods of myocardial ischemia and reperfusion may provide protection against electrical instability of the heart evoked by subsequent ischemia/reperfusion injury. This mechanism, known as ischemic preconditioning, was first suggested by Reimer et al. (1981) and later elaborated on by Murry et al. (1986).

Similar cardioprotective effects, although of variable intensity, have been obtained after pre-treatment with repetitive episodes of hypoxia, which may provide clinical benefit over ischaemia in that systemic blood flow into critical organs remains stable (Shizukuda et al., 1993). Most of the available information on hypoxic preconditioning has come from *in vitro* studies on isolated perfused hearts using transient local hypoxia. The duration of the arrhythmic activity is significantly lower (Kamasaki et al., 1997), the duration of the action potential and the effective refractory period is shortened more rapidly and is significantly shorter (Ravingerova et al., 1998) in hypoxia preconditioned isolated guinea pig papillary muscle. Thus, these results refer to the fact that hypoxic preconditioning can significantly attenuate arrhythmic activity. These include our recent results demonstrating the effects of hypoxic preconditioning on the onset and development of ventricular arrhythmias during prolonged asphyxia in rats(Svorc and Bracokova, 2003).

Unfortunately, there are no consistent data regarding the daytime dependence of preconditioning effects using the synchronization of animals to the light-dark cycle (LD cycle, 12 : 12h) as most of the *in vivo* experiments in rats have been done routinely in their non-active (i.e., light) part of the day. It is generally known that cardiac functions show a marked circadian rhythmicity and that the LD cycle is the strongest circadian synchronizator of the endogenous rhythms of animals.Many papers reporting on the factors

responsible for the onset and development of ventricular arrhythmias have mainly focused on the temporally current mechanical and metabolic changes in the myocardial cells, often irrespective of the circadian dependence.

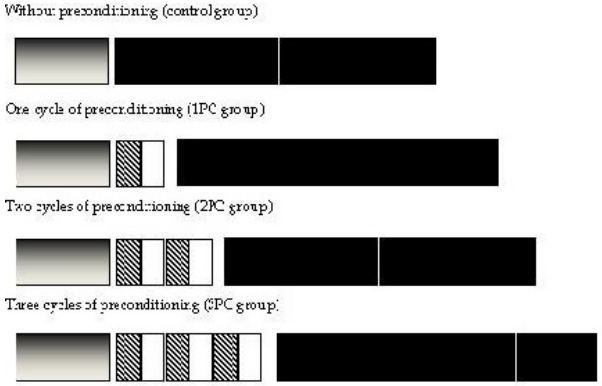
Therefore, it is important to know whether preconditioning by hypoventilation can also reduce the experimentally induced ventricular arrhythmias or increase the electrical stability of the heart against a effect of subsequent prolonged period of hypoventilation and reoxygenation. We hypothetized that 1. if hypoventilation, as ischemia, decreases the electrical stability of the heart, preconditioning by hypoventilation could have a comparable effects as ischemic preconditioning. Moreover, we focused to fact whether there are differences in the conditions and dynamics of the developing protective effects of myocardial preconditioning applied during the light (nonactive) and dark (active) parts of the day regimen of rats, and to obtain an understanding concerning chronophysiological aspects of this phenomenon in in vivo rat experiments.

Methods

The experiments were performed in anaesthetized (ketamine/xylazine anaesthesia, ketamine 100 mg/kg Narkamon Prague + xylazine 15 mg/kg Rometar Prague, i.m.) (weight, 300 ± 15 g; 3-4 months of age). The rats after adaptation to a LD cycle of 12h : 12h, with the dark part of day from 06:00 to 18:00hfor 4 weeks were divided into 4 groups. During the experiments, all animals were subjected to 20 min of artificial hypoventilation-induced asphyxia, followed by a 20 min recovery period (reoxygenation).

The first group of animals was without preconditioning (n = 19) and the other three experimental groups were pre-conditioned by one (1PC group; n = 9), two (2PC group; n = 15), and three (3PC group n = 11) 5 min cycles of hypoventilation (5 min), each separated by 5 min cycles of reoxygenation (scheme 1).

The control records of VAT were done after surgical interventions and a 5 min period of artificial ventilation with the parameters of the normal pulmonary ventilation. Values of VAT were measured in the 5^{th}, 10^{th}, 15^{th}, and 20^{th} min of hypoventilation and in the same intervals during ventilatory recovery. This parameter (VAT) was introduced because the ventricular arrhythmias were the mixed type, including the spontaneous mutual transitions between ventricular fibrillation, ventricular tachycardia, and flutter.



Scheme 1. Protocol of the experiments with preconditioning by systemic asphyxia. The black-white columns – initial phase of experiments with heating of animals to the rectal temperature measured before the application of the anaesthetic agent, tracheotomy, thoracotomy, 5 min. period of stabilization (normal artificial ventilation at the parameters of the artificial ventilation V_T 1 ml/100 g of b.w. and respiratory rate 50 breaths/min.), hatched columns – 5 min. cycles of preconditioning by systemic asphyxia, empty columns – 5 min. cycles of reoxygenation, black columns – 20 min. hypoventilation.

Animals were artificially ventilated by humified room air at the parameters of the initial ventilation and reoxygenation: respiratory rate, 40 breaths/min; and tidal volume, 1 ml/100g b.w. During experimental hypoventilatory asphyxia, the respiratory rate and tidal volume were reduced to 20 breaths/min and 0.5 ml/100g b.w., respectively. The respiratory effect of the ventilation was monitored by the analysis of the pH, pO_2, pCO_2, and O_2 saturation from blood samples taken from the femoral artery. The average values for dark part of the day were as follows: 1) after the surgery and 5 min of artificial ventilation (pH_a, 7.44 ± 0.11; paO_2, 10.8 ± 1.7 kPa; $paCO_2$, 3.22 ± 1.3 kPa; and O_2 saturation, 91.6 ± 9.9%), 2) at the end of 20 min of hypoventilation (pH_a, 7.15 ± 0.07; paO_2, 7.3 ± 1.7 kPa; and $paCO_2$, 7.3 ± 1.3 kPa; and O_2 saturation, 68.2 ± 13.7%);and 3) 20 min reoxygenation (pH_a, 7.37 ± 0.06; paO_2, 9.46 ± 1.85 kPa; $paCO_2$, 4.62 ± 0.71 kPa; and O_2 saturation, 93.3 ± 3.6%), respectively.

The chest was opened by parasternal thoracotomy and after gentle mediastinal preparation, the heart was exposed. The ventricular arrhythmia threshold (VAT) was estimated as the minimal amount of the electrical current (mA) needed for elicitation of the ventricular

arrhythmias by direct electrical stimulation of the heart (400 ms series of rectangular pulses; frequency, 30 Hz; and 10 ms impulse lengths). Stimuli were triggered by the onset of the R wave in the II. lead of ECG and the current intensity was increased progressively by steps of 0.2 mA until ventricular arrhythmias were obtained. Recovery of the sinus rhythm was spontaneous.

Results

The control values of VATs in the experimental groups did not show any significant difference, although systematically higher values were found during the dark part of the day compared to the light part of the day (control light, 1.87 ± 0.80 mA vs. control dark, 2.12 ± 0.93 mA; 1PC light, 1.96 ± 0.73 mA vs. 1PC dark, 2.44 ± 0.68; 2PC light, 2.19 ± 1.21 vs. 2PC dark, 2.48 ± 1.20 mA; and 3PC light, 2.32 ± 0.69 mA vs. 3PC dark, 1.85 ± 0.69 mA) (fig. 1).

In the dark part of the day, hypoventilation non-significantly decreased the VAT in the group without preconditioning (2.12 ± 0.93 mA [control] vs. 2.05 ± 0.85 mA [hypo]); in 1 PC (2.44 ± 0.68 mA [control] vs. 1.68 ± 0.87 mA [hypo]); and in 2 PC (2.48 ± 1.2 mA [control] vs. 1.87 ± 0.60 mA [hypo]). In the 3 PC group, the VAT was not changed and remained at the level of the prehypoventilatory value (1.85 ± 0.69 mA [control] vs. 1.87 ± 0.76 [hypo]). During the light part of the day, similar but significant VAT decreases were found in the group without preconditioning, in the 1 PC group and non-significant in the 2 PC group, except the 3 PC group, where the VAT was markedly ($p < 0,001$) increased above the control value. Significant LD differences were seen in all groups, with higher values in the dark part of the day, excepthe 3 PC group with a higher VAT in the light part of the day.

In the dark part of the day, reoxygenation after one and two cycles of the HPC did not change and recovery the VAT to control values and values from period of hypoventilation (1 PC group, 2.44 ± 0.68 mA [control] vs. 1.68 ± 0.87 mA [hypo] vs. 1.53 ± 0.58 mA [reoxy]), (2 PC group, 2.48 ± 1.2 mA [control] vs. 1.87 ± 0.60 mA [hypo] vs. 1.93 ± 0.57 mA [reoxy]). In the group without preconditioning (2.12 ± 0.93 mA [control] vs. 2.05 ± 0.85 mA [hypo] vs. 2.00 ± 0.86 mA [reoxy]) and in the 3 PC group (1.85 ± 0.69 mA [control] vs. 1.87 ± 0.76 mA [hypo] vs. 1.91 ± 0.69 mA [reoxy]) the VAT was not changed and remained on the pre- and hypoventilatory levels. In the light part of the day, similar VAT changes were seen in all groups, except the 3 PC group, where the VAT was markedly ($p < 0,001$) increased against control and hypoventilatory values. The higher VAT values were found in all groups, with higher values in the dark part of the day,

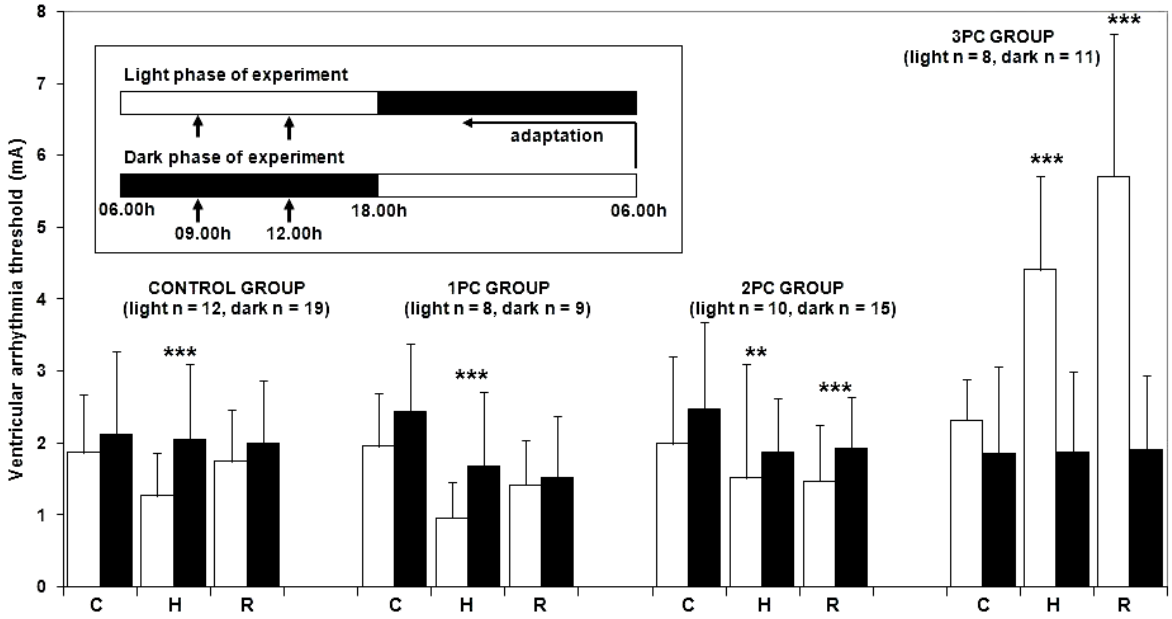


FIG. 1. MEAN ± SD VALUES OF THE VENTRICULAR ARRHYTHMIA THRESHOLD IMMEDIATELY BEFORE PRECONDITIONING (C), DURING 20 MIN. HYPOVENTILATORY ASPHYXIA (H) FOLLOWED 20 MIN. REOXYGENATION (R) IN THE CONTROL ANIMALS (CONTROL GROUP) AND GROUPS PRECONDITIONED BY 1 (1PC GROUP), 2 (2PC GROUP) AND 3 (3PC GROUP) SHORT CYCLES OF HYPOVENTILATION-INDUCED SYSTEMIC HYPOXIA, HYPERCAPNIA AND ACIDOSIS. EMPTY AND BLACK COLUMNS REFER TO LIGHT AND DARK PARTS OF THE DAY, RESPECTIVELY. EMBEDDED SCHEME SHOWS THE TIMINIG OF TRIALS (ARROWS) IN ANIMALS ADAPTED TO THE LIGHT-DARK CYCLE. *** $P< 0.001$, ** $P < 0.01$.

except the 3 PC group, in which a higher VAT occurred in the light part of the day. A significant effect of the preconditioning by the HPC was not confirmed by the χ^2 test nor for hypoventilation ($p < 0.09$) or for reoxygenation ($p < 0.64$) in the dark part of the day. In the light part, such significance was confirmed only for a prolonged period of hypoventilation ($p< 0.001$), but not during reoxygenation ($p < 0.39$).

Discussion

The main aim of this study was to gain information concerning the chronophysiologic aspect of cardioprotection by the hypoventilation-induced asphyxia preconditioning in *in vivo* rat experiments. A considerable intraindividual variability of results is a problem concerning mainly *in vivo* studies, which was confirmed also in our experimental groups. Such variability can be explained by production of spontaneous unpredictible alterations in the electrical stability of the heart induced by anaesthesia or hormonal and homeostatic reflexes operating only in intact animals (Lubbe et al., 1975).

The significant hypoventilatory LD differences in the thresholds show the different LD effects of hypoventilation–induced systemic hypoxia, hypercapnia, and acidosis on the electrical stability of the rat heart. The higher values in the dark part of the day in all experimental groups, except the 3PC group, are probably the result of the changing myocardial sensitivity to the systemic asphyxia in the LD dependence, although there are more papers refering to the depressive effect of hypoxia on the circadian rhythms in rats (Fenelon et al., 2000; Mortola and Seifert, 2000), in golden hamsters (Jarsky and Stephenson, 2000), or in humans (Bosco et al., 2003).

LD differences in the VATs are probably a reflection of the changes in electrophysiologic properties of the myocardium. These changes after preconditioning also have a place in the background of our observations. Possible mechanisms of protection might involve a faster shortening of the action potential (Ravingerova et al., 1998; Tan et al., 1993), also reflected as a shortening of refractoriness (Grover et al., 1994) during hypoxia after preconditioning. Also, the duration of the arrhythmic activity was significantly lower in quinea pig papillary muscles from hearts after preconditioning by hypoxia (Kasamaki et al., 1997), which refers to the fact that hypoxic preconditioning can significantly attenuate the arrhythmic activity. Unfortunately, these experiments were not performed in the LD dependence and no evidence about the effect of the hypoxic preconditioning by this manner can be determined. Thus, the question remains whether the effects of these electrophysiological changes protecting the myocardium depends also on LD cycle. Our results indirectly confirm the fact that the above described electrophysiological changes resulting from preconditioning are probably more effective mainly in the light (nonactive) part of the rat regime day.

The effect of preconditioning depends also on the balance between the intensity of the first stimulus and the duration and severity of the prolonged stress. Following the VAT changes during hypoventilation/ reoxygenation, one cycle of preconditioning by asphyxia had an identical proarrhythmogenic effect in the both light parts of the day, but with significantly higher values in the dark part of the day. However, the LD discrepancies in the VAT changes occurred during reoxygenation. In the light part of the day, reoxygenation partly recovered the VAT (antiarrhythmogenic effect), but in the dark part of the day, it was followed by the further VAT decrease (proarrhythmogenic effect). In the both light parts of the day, although hypoventilation/ reoxygenation still decreased the VATs in the 2PC group, the decrease was not significant, values were higher than in the 1PC group, but with the preservation of LD differences. Reoxygenation was without effect. The three cycles of preconditioning by asphyxia stabilized the VAT in the dark part of the day, but a marked and significant cardioprotection against the hypoventilation/reoxygenation decrease of the electrical stability of the heart was detected in the light one, meaning that there are different reactions of the rat myocardium for the HPC in the dependence on the LD cycle.

Although the average hypoventilatory VAT value was dropped in the 1PC group against hypoventilatory VAT value from the control group (without preconditioning) in both light parts of the day, the VAT increased gradually in the dependence on the number of cycles of preconditioning by asphyxia. It seems that 1) one cycle of preconditioning by asphyxia is too weak of a stimulus for the production of cardioprotection in both light parts of the day, 2) the cardioprotection probably starts after two cycles of preconditioning by asphyxia in both light parts, and 3) the effect of preconditioning by asphyxia depends on the numbers of the preconditioning cycles and in the dependence on LD cycle - it is highlight by three cycles

of preconditioning by asphyxia.

The dependence of the cardioprotection on the number of the preconditioning hypoxic cycles has been affirmed by others. Testoni et al. (2000) and Cerruti et al. (2002), in isolated rat hearts, showed that as far as the animals were exposed only to hypoxia (60 minutes) and reoxygenation (60 minutes), without the hypoxic preconditioning, the higher atrial and right ventricle contractile disorders and less posthypoxic recovery (others endpoints of preconditioning) were found. Whereas hypoxic preconditioning by one 5 min. cycle of hypoxia and subsequent 10 min reoxygenation had the small effect, preconditioning by two cycles of hypoxia exacerbated the contractile changes. O'Connor and Merrill (1995) refered to the fact that initial exposure to hypoxia can protect myocardium in *in vivo* conditions against arrhythmias during the second hypoxic period (significant percententual decrease of ectopy incidence). Similarly, blockade of cardiac β-adrenoceptors attenuates the incidence of arrhythmia in the second hypoxic period, demonstrating the possible role of catecholamines in the course of the HPC. Myocardial ischaemia, as well as non-ischaemic hypoxia, stimulate efferent adrenergic nervous endings (Daly and Scott, 1963, 1964; Herrmann and Feigl, 1992), the assumptionbeing that the ventricular arrhythmias induced by systemic hypoxia depend on the intact adrenergic inervation (O'Connor and Merrill, 1993); this was shown in our experiments also. These interventions deliver possible protection by preconditioning against electrogenic and mechanical effects of the prolonged ischaemic period of the myocardium (Lasely et al., 1993). The differences in the number of the cycles of hypoxia necessary for the mobilization of the cardioprotective mechanism in present work and previous studies performend *in vitro* and *in vivo*, could be explained by different experimental procedures. Low-oxygen perfusion of isolated heart *in vitro* (Testoni et al., 2000; Cerruti et al., 2002) may provide much faster entrance of cardioprotection a compared to in *in vivo* condition. The anaesthesia in *in vivo* experiments is an important variable, as is the animal species in use, e.g., ketamine anaesthesia inhibites preconditioning by anoxia in rats (Ko et al., 1997), in rabbits (Han et al., 2002), and our results, or α-chloralose in beagles (O'Connor and Merrill, 1993).

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