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HYPOXIC AND PHARMACOLOGICAL PRECONDITIONING PRESERVES VASOMOTOR RESPONSE OF PORCINE CORONARY ARTERY

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Vasomotor response of the coronary artery depends on both endothelial and smooth muscle cells. Response is altered by hypoxia-reoxygenation-induced damages. Hypoxic preconditioning and pharmacological preconditioning as well can prevent these alterations. We compared the effectiveness of both types of preconditioning against hypoxia-reoxygenation-induced changes in vasomotor response of the isolated artery. Porcine arterial rings (3–4 mm wide) were cut from the left anterior descending porcine coronary artery and placed in Krebs-Henseleit solution. In order to obtain control response of the arteries, we contracted arterial rings with 20 mM KCl before ("standard contraction") and after 60-min hypoxia and 30-min reoxygenation. In other groups, nitric oxide-synthase and cyclooxygenase were inhibited. Then, the rings were pre-contracted with U46619 and relaxed by cumulative addition of the substance P. Contractions and relaxations of non-preconditioned and hypoxically or pharmacologically preconditioned rings were compared. Hypoxic preconditioning was performed by two periods of 5-min hypoxia and 10-min reoxygenation. For pharmacological preconditioning, we used application of adenosine, adrenaline, acetylcholine and angiotensin II. Analysis was performed with one-way ANOVA, followed by Dunnett's Multiple Comparison Test. After hypoxia-reoxygenation, in non-preconditioned rings KCl-induced contractions were significantly increased compared to standard contraction. Relaxations of hypoxically and pharmacologically preconditioned rings (expressed as percentages of U46619-induced pre-contraction) were significantly decreased ($p < 0.01$) compared to hypoxic but not to normoxic rings. Hypoxic and pharmacological preconditioning may preserve contraction and endothelium-dependent relaxation of porcine coronary artery after long-lasting hypoxia-reoxygenation.

Key words: *preconditioning, porcine coronary artery, contraction, relaxation*

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Abbreviations: ATP – *adenosine triphosphate,* COX – *cyclooxygenase,* EDHF – *endothelium-derived hyperpolarizing factor,* L-NNA – *N(ω)-nitro-L-arginine,* NO – *nitric oxide,* NOS – *nitric-oxide synthase,* PGI_2 – *prostacyclin,* U46619 – *9,11-dideoxy-9a,11a-methanoepoxy prostaglandin F(2alpha)*

INTRODUCTION

An ischemic preconditioning, discovered in 1986 [16], induced by short periods of ischemia-reperfusion can preserve myocardial function of isolated hearts after longer lasting ischemia. A signal cascade of this preconditioning is partly identified in cardiomyocytes, but the end effector is still unknown [24]. During ischemic preconditioning receptor-dependent or receptor-independent mediators (e.g. free radicals, nitric oxide [NO]) are released. They can induce protective response in an isolated heart. Similar protective effect can be evoked by pharmacological agonists, too (e.g. adenosine, bradykinin, opioids, endothelin) [24]. Intracellular signal transduction may be similar in endothelial cells [23].

Most of the preconditioning studies were conducted on the isolated heart model or with isolated cardiomyocytes. Less attention was dedicated to studies of isolated coronary arteries or coronary artery endothelial cells. Although the signal cascade of hypoxic preconditioning in vascular cells of the coronary artery could be similar to the one occurring in the ischemic preconditioning in cardiomyocytes, some differences between the isolated heart and the coronary vessels were found. As it was shown with adenosine A_2 receptors, stimulation of the same subtype had protective effect on coronary endothelial cells, while the A_1 and A_3 receptor-mediated protective effects in cardiomyocytes were abolished [23]. In contrast to myocardium, only adenylate cyclase-coupled A_2 receptors are present in coronary endothelial cells, while there is no evidence of A_1 receptors in these cells. Agonists of these receptors cause vasodilatation by acting on both endothelium and vascular smooth muscle cells [19]. Protective effect of preconditioning after activation of A_2 receptors in endothelial cells is mediated *via* the protein kinase C pathway [31] that is implicated in endothelial preconditioning [12]. In cultured porcine coronary artery cells, activation of A_2, but not A_3 receptors induces production of endothelium-derived NO [18], which may also play a role in preconditioning [12].

Functional efficiency of coronary vasculature, especially of endothelial cells is essential for the appropriate perfusion of myocardium. Because of the essential importance of the appropriate perfusion, coronary arteries are potential target for anti-ischemic/hypoxic treatments and prevention, including preconditioning. However, studies on isolated hearts may provide only limited data on the role and mechanisms of vascular preconditioning. For further evaluation of the influence of activators on endothelium and on vascular smooth muscle cells, hypoxic model of isolated vessels can be used. That would allow us to determine the role of both tissues in preconditioning and the resulting outcome of the ischemic/hypoxic conditions in the heart.

While the process of preconditioning in the heart is based on triggers in cardiomyocytes, the response in coronary arteries might depend on vascular smooth muscle cells and on the endothelium-dependent release of biochemical mediators as well. ATP-sensitive K^+-channels, adenosine, bradykinin, heat shock proteins and free radicals are involved in vascular preconditioning, but its exact mechanism is not known yet [7, 21–23].

Ischemic and hypoxic injury may trigger apoptosis and necrosis of myocardial [5] and endothelial cells [30]. Endothelial cells of coronary artery are more susceptible to ischemia-reperfusion injury than cardiomyocytes themselves [14]. Ischemia/hypoxia followed by reperfusion/reoxygenation lead to loss of the endothelium-dependent vasodilatation in response to anti-aggregating and anti-adhesive effects in circulating platelets and leukocytes [11, 12, 23, 28, 29]. During reoxygenation, the contractile response of isolated porcine coronary artery to KCl is increased. On the other hand, relaxation, not mediated by NO and prostacyclin (PGI_2), is reduced [22]. If nitric oxide synthase (NOS) and cyclooxygenase (COX) are inhibited, only relaxation mediated by endothelium-derived hyperpolarization factor (EDHF) remains.

In our study, we compared possible beneficial effects of hypoxic and pharmacological preconditioning on preservation of contractile and relaxation response of the isolated coronary artery after hypoxia-reoxygenation. We limited our study to receptor-dependent triggers that have triggered preconditioning in myocardial cells. We compared

contractions and relaxations at the end of experiments during normoxia and after hypoxia-reoxygenation. Endothelium-mediated relaxation was used as an indicator of endothelium functional efficacy while contraction was used as an indicator of integrity of the whole vessel wall.

MATERIALS and METHODS

Chemicals

Acetylcholine, adenosine, adrenaline, angiotensin II, substance P, and N(ω)-nitro-L-arginine (L-NNA) (all from Sigma-Aldrich Chemie, Steinheim, Germany) were dissolved in distilled water. Indomethacin (Sigma-Aldrich Chemie, Steinheim, Germany) was dissolved in ethanol. The concentration of ethanol in organ baths was below 1% and did not affect the experimental results. Thromboxane analogue U46619 (Alexis, Lausen, Switzerland) was dissolved in dimethylsulfoxide. The concentration of dimethylsulfoxide in organ baths was below 1% and did not affect the experimental results [9]. All other chemicals were of analytical grade.

Preparation of arterial rings

Experiments were carried out on isolated porcine coronary arteries *in vitro*. Porcine hearts were obtained from the local slaughterhouse. They were transported and stored at a temperature not higher than 4°C in Krebs-Henseleit solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, and glucose 11. Arterial rings (3–4 mm in diameter) were prepared from the left anterior descending coronary artery. The endothelium was preserved by cautious dissection of the rings. In denuded rings, the endothelium was mechanically removed [6] by a wooden stick [22, 29]. Rings were placed in 10-ml organ baths, filled with warm (38°C) Krebs-Henseleit solution, aerated with a mixture of 95% O_2 and 5% CO_2 (oxygenated Krebs-Henseleit solution). Isometric contractile responses were measured with the mechano-electrical transducer (ITIS, Slovenia) and recorded through A/D conversion (A/D converter Adlink, Taiwan) on the hard disk of the personal computer by the Dewescope software (Dewesoft, Slovenia).

At the beginning of all experiments, the rings were equilibrated for 60 min with 50 mN resting tension (standard equilibration protocol used in our laboratory) [6]. In order to obtain stable contractions, rings were contracted two or three times with 60 mM KCl. Only rings which sufficiently responded to 60 mM KCl were used [3]. After that all rings were contracted two or three times with 20 mM KCl in order to obtain stable response. The last of these contractions was used as a standard contractile response ("standard contraction" in the subsequent text).

Hypoxia was performed by the aeration of organ baths with a mixture of 95% N_2 and 5% CO_2 after wash-out of the oxygenated Krebs-Henseleit solution. Hypoxia was stopped by washing-out the rings with oxygenated Krebs-Henseleit solution.

Protocols

In the pilot group of experiments (n = 5), we determined the contractile response of arterial rings to increasing concentrations of KCl (10, 15, 20, 30 and 60 mM). The contractile response to 20 mM KCl was used as standard contraction.

Control experiments

Normoxia. After the standard contraction with 20 mM KCl was determined, arterial rings were washed out and during normoxia at the end of experiments contractile response to 20 mM KCl was recorded (Fig. 1a). Relaxation responses were studied in rings incubated for 30 min with 0.36 mM L-NNA and 9.8 mM indomethacin used to inhibit NOS and COX. Then, after precontraction with 50 nM U46619, which is an agonist of thromboxane receptor, the relaxation was induced by cumulative addition of substance P at concentrations from 1 pmol/l to 0.1 μmol/l (Fig. 1e).

Hypoxia. After the standard contraction, rings were washed out and subjected to 40- or 60-min hypoxia, followed by 30-min reoxygenation. For the termination of hypoxia rings were washed out with fresh oxygenated Krebs-Henseleit solution. After 30-min of reoxygenation, rings in the contraction group were contracted with 20 mM KCl (Fig. 1b). Rings in the relaxation group were first incubated with L-NNA and indomethacin for 30 min, then precontracted with U46619 and relaxed with cumulative addition of substance P (Fig. 1f).

Preconditioning was induced by:

1. Hypoxia. After standard contraction, arterial rings were subjected to two periods of 5-min hypoxia followed by 10-min reoxygenation. The long-lasting hypoxia (60 min) was followed by

30-min reoxygenation and the final contraction was recorded at the end of this period in the contraction group (Fig. 1c). Rings in the relaxation group were incubated in L-NNA and indomethacin for 30 min, precontracted with U46619 and relaxed with cumulative addition of substance P (Fig. 1g).

2. Pharmacological agonists. After standard contraction, arterial rings were first washed out, and then an agonist (final concentrations: 0.1 μM acetycholine, 5 μM adrenaline, 10 μM adenosine, or 10 nM angiotensin II) was added. Contractions or relaxations in response to agonists were measured. Rings were washed out prior to 60-min hypoxia and 30-min reoxygenation. Final contraction to 20 mM KCl was registered at the end of this period in the contraction group (Fig. 1d). Rings in the relaxation group were incubated with L-NNA and indomethacin for 30 min. After that rings were first precontracted with U46619 and then relaxed with cumulative addition of substance P (Fig. 1h).

Statistical analysis

Contractile response to KCl, precontraction with U46619 and relaxation with substance P were registered and measured using Dewescope software (Dewetron, Slovenia). All statistical analyses were performed by GraphPad Prism 4.0 (GraphPad Software, USA) and Excel 2002 (Microsoft, USA) software.

Contractile responses to 20 mM KCl in rings without preconditioning before and after hypoxia-reoxygenation were compared. After hypoxia-reoxygenation contractile responses of the preconditioned rings were compared with the contractile response of the non-preconditioned rings. All contractions were expressed in the percent of the standard contraction induced by 20 mM KCl before hypoxia.

Concentration-relaxations curves and maximal relaxations of the rings during normoxia at the end of experiments and after hypoxia-reoxygenation were compared. Relaxations were expressed in the percent of the precontraction induced by 50 nM U46619.

One-way analysis of variance (ANOVA) was used to test statistical significance of differences between groups in contractions induced by 20 mM KCl, and pD_2 or maximum relaxations in relaxation groups. Dunnett's test was used as a post-test. Data were expressed as the mean ± SEM; $p < 0.05$ was considered significant.

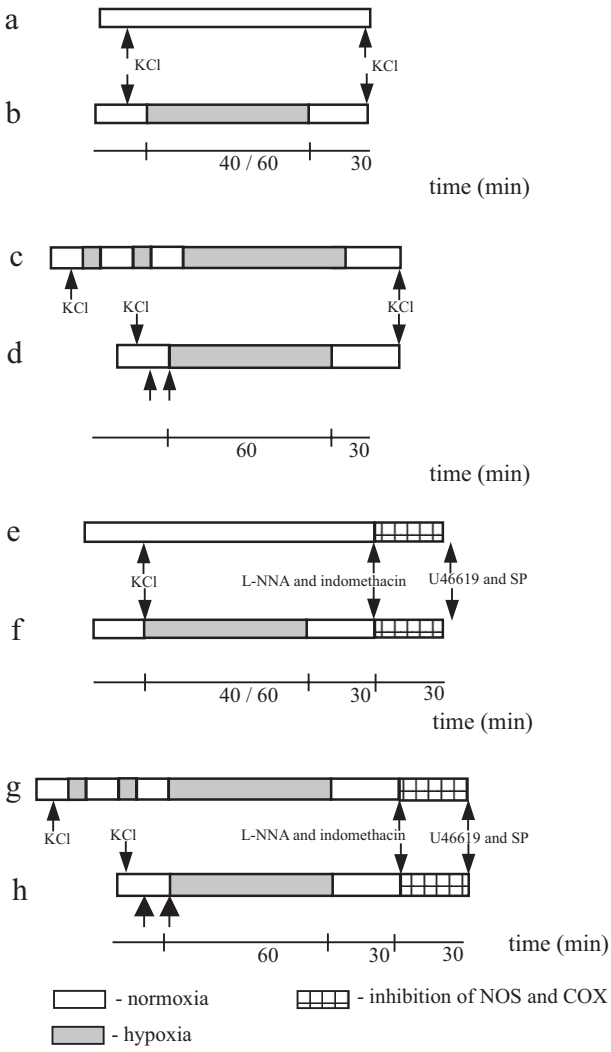

Fig. 1. Schematic diagrams of experimental protocols with normoxia alone and with hypoxia-reoxygenation. (a), (b), (c), (d) – groups with contraction induced by 20 mM KCl at the end of experiments; (e), (f), (g), (h) – groups with relaxation induced by cumulative addition of substance P (SP) at the end of experiments. Control experiments in contraction group were performed in normoxia (a) and in 40- or 60-min hypoxia, followed by 30-min reoxygenation (b). Hypoxic preconditioning was performed with two periods of 5-min hypoxia and 10-min reoxygenation (c), while pharmacological preconditioning was performed with agonists that were washed out before 60-min hypoxia (d). Control experiments in relaxation group were performed in normoxia (e) in with 40- and 60-min hypoxia, followed by 30-min reoxygenation (f). Hypoxic preconditioning was performed with two periods of 5-min hypoxia and 10-min reoxygenation (g), while pharmacological preconditioning was performed with agonists that were washed out before 60-min hypoxia (h). In all experiments with agonists, they were left in Krebs-Henseleit solution until the maximal response (contraction or relaxation) to the agonist was detected

RESULTS

Control groups

KCl (10-60 mM) induced contractions of coronary arteries in a concentration-dependent manner. Contractile response at the beginning and at the end of experiments with normoxia did not significantly differ in rings with endothelium (n = 6) from those in denuded (n = 6) rings (99.5 ± 6.92% *vs.* 103 ± 10.4%). Also, the contractile response after 40-min (n = 12) hypoxia followed by 30-min reoxygenation was similar (95.7 ± 3.29%) to the final contraction in normoxic group of intact rings. However, the contractile response after 60-min hypoxia and 30-min reoxygenation (134 ± 4.3%, n = 15) was significantly higher ($p < 0.01$). There was no significant difference between intact and denuded rings (130 ± 5.1%, $n = 14$) in this group (Fig. 2a).

Maximal relaxations induced by the substance P were not significantly decreased in rings after 40-min hypoxia and 30-min reoxygenation (50.5 ± 5.3%, n = 16) compared to normoxic rings (61.0 ± 3.69%, n = 15). However, relaxations were significantly ($p < 0.01$) impaired after 60-min (32.1 ± 3.54%, n = 19) hypoxia and 30-min reoxygenation (Fig. 2b and c).

Preconditioned arterial rings

After 60-min hypoxia and 30-min reoxygenation, the final contractile response was significantly higher ($p < 0.01$) in non-preconditioned control group with endothelium (134 ± 4.3%, n = 15) than in the hypoxically preconditioned group (105 ± 3.6%, n = 15) in which it was similar to the standard contraction (Fig. 3a)

Final contractile responses after hypoxia-reoxygenation of pharmacologically preconditioned rings were also similar to the standard contraction. They were significantly lower ($p < 0.01$) than the final contractile responses of non-preconditioned rings (134 ± 4.3%, n = 15). The contractile responses of preconditioned rings were as follows: adenosine group 85.2 ± 1.25% (n = 7); adrenaline group 102 ± 4.1% (n = 8); angiotensin II group 82.2 ± 2.87 (n = 8), and acetylcholine group 86.7 ± 4.99% (n = 7) (Fig. 3a).

Maximal relaxation of the hypoxically preconditioned group (67.9 ± 3.95%, $n = 8$) was similar to that of the normoxic group (61.0 ± 3.69%; $n = 15$), but significantly ($p < 0.01$) more expressed than in non-preconditioned rings (32.1 ± 3.54%, n = 19) (Fig. 3b and d).

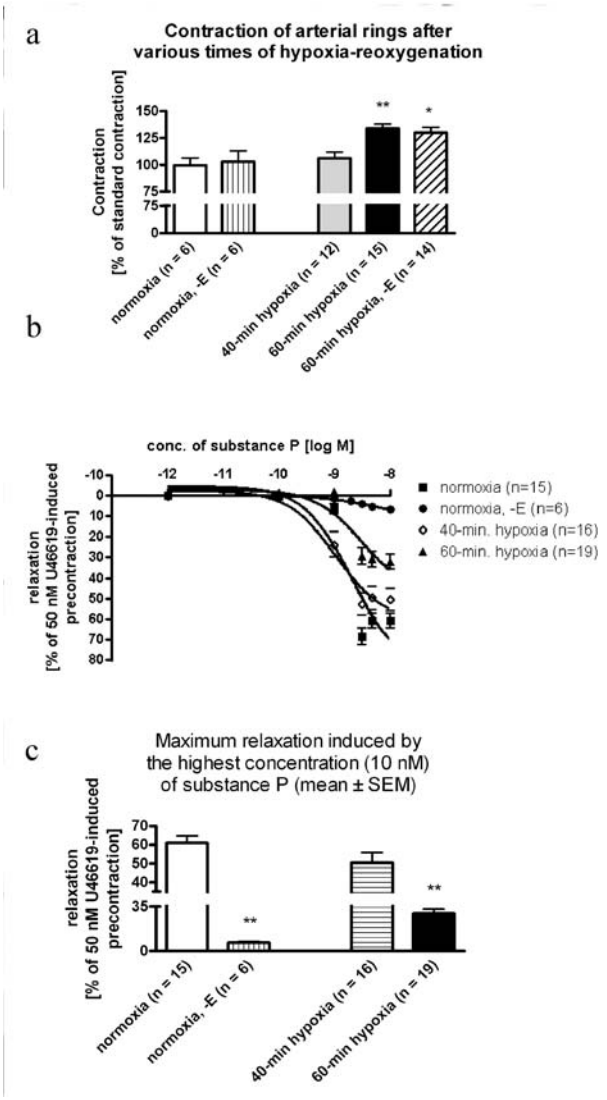

Fig. 2. Contraction and relaxation after hypoxia-reoxygenation. Contractile response to 20 mM KCl in normoxia and after 40- and 60-min hypoxia, followed by 30-min reoxygenation (a); substance P-induced relaxation of rings precontracted with U46619 in normoxia and after 40- and 60-min hypoxia, followed by 30-min reoxygenation (b); maximal relaxation induced by substance P in normoxia and after 40- and 60-min hypoxia, followed by 30-min reoxygenation (c). -E: rings without endothelium; * $p < 0.05$; ** $p < 0.01$ (mean ± SEM compared to normoxic rings with endothelium)

Maximal relaxations of pharmacologically preconditioned rings after hypoxia-reoxygenation were similar to maximal relaxations of rings in normoxic group (61.0 ± 3.69%, n = 15). Preconditioned rings relaxed significantly more ($p < 0.01$) than non-preconditioned rings (32.1 ± 3.54%, n = 19)

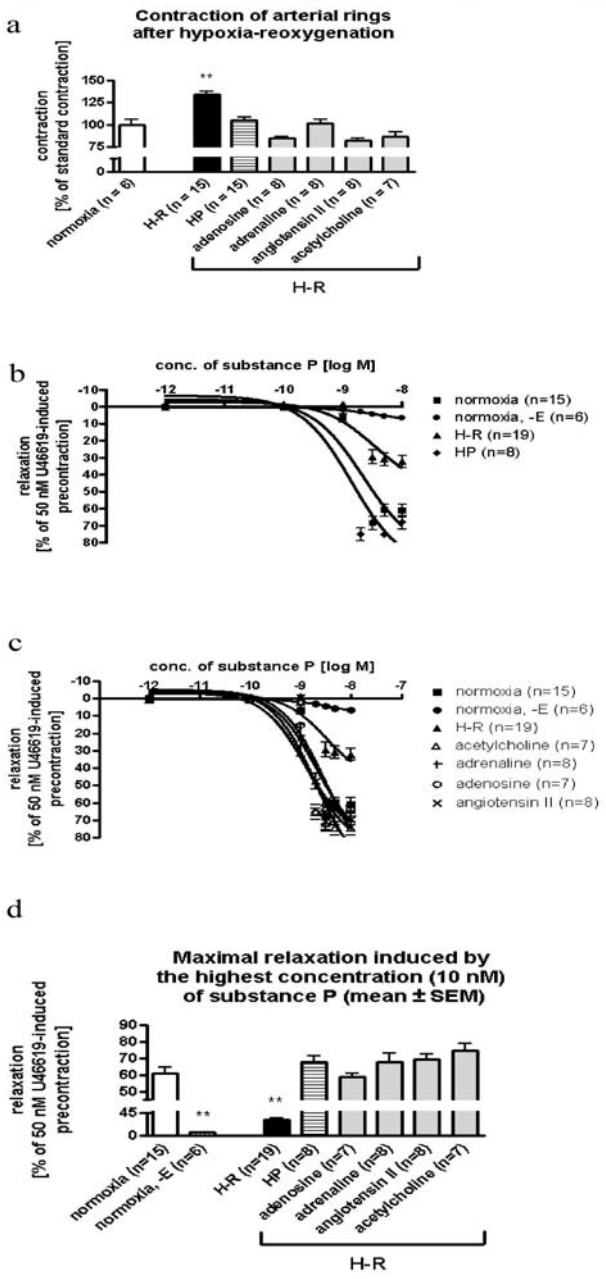

Fig. 3. Vasomotor reponses after hypoxia-reoxygenation in non-preconditioned and preconditioned artery rings. Contractile response to 20 mM KCl of non-preconditioned and preconditioned rings after hypoxia-reoxygenation (a); relaxation induced by substance P of non-preconditioned and hypoxically preconditioned rings (pre-contracted by U46619) after hypoxia-reoxygenation (b); relaxation induced by substance P of non-preconditioned and pharmacologically preconditioned rings (precontracted by U46619) after hypoxia-reoxygenation (c); maximal relaxation induced by substance P of non-preconditioned and preconditioned rings (precontracted by U46619) after hypoxia-reoxygenation (d). H-R: 60-min hypoxia followed by 30-min reoxygenation; HP: hypoxic preconditioning; -E: rings without endothelium; ** $p < 0.01$ (mean ± SEM compared to normoxic rings with endothelium)

after hypoxia-reoxygenation. Maximal relaxations of preconditioned rings were as follows: adenosine group 62.2 ± 1.41% (n = 7); adrenaline group 67.7 ± 5.33% (n = 8); angiotensin II group 76.6 ± 6.14% (n = 8) and acetylcholine group 74.8 ± 3.91% (n = 7) (Fig. 3c and d).

DISCUSSION

In our study, we compared the effects of hypoxic and pharmacological preconditioning on preservation of vasomotor response, contraction and relaxation of the isolated coronary artery after hypoxia-reoxygenation. Our main focal point was the preservation of non-NO- and non-PGI_2-mediated relaxation. Both types of preconditioning were found to be similarly effective.

We used two types of agonists. The first type (e.g. adenosine) is released at sufficient concentrations to induce signal cascade during ischemic/hypoxic preconditioning (endogenous triggers). Others may serve only as exogenous triggers, probably because their level during ischemic preconditioning is too low (e.g. adrenaline, angiotensin II) [24]. We studied whether the effectiveness of ischemic preconditioning used previously in studies on the isolated heart was comparable with the effectiveness of hypoxic and pharmacological preconditioning in the isolated coronary artery. Apart from endothelial cells, functional efficacy of smooth muscle cells in the vessel wall may be important to preservation of the contractile response after hypoxia-reoxygenation [15]. The response of the whole vessel wall was evaluated as contractile response, while the response of endothelium was studied as non-NO- and non-PGI_2-mediated relaxation.

Contraction and relaxation as indicators of vasomotor response of the coronary artery after hypoxia

So far, most of the studies of ischemic injuries and their prevention in the heart have been focused on the myocardium, although coronary arteries are probably even more susceptible to hypoxia than cardiomyocytes [23]. Bradykinin-induced relaxation, which is probably mediated by EDHF is impaired after long-lasting hypoxia [22]. On the other hand, adenosine [10] and prostaglandin E_1 [4] may amplify relaxation after hypoxia, if COX and NOS are not inhibited.

Vasomotor reactivity of the coronary artery and its impairment can be evaluated by measuring contraction and relaxation induced by combinations of various substances. High concentration of KCl is known to induce endothelium-independent contraction of the coronary artery [3, 22]; however, it can be used when contraction of the complete vessel wall is studied. It may be argued that KCl would inhibit the release of NO and other endothelium-derived factors responsible for protection and would, therefore, inhibit preconditioning. However, other contracting agents may induce preconditioning by themselves, what makes them less appropriate. In addition, KCl was washed-out before preconditioning and hypoxia. As the use of the lowest possible concentration still inducing sufficient contraction was preferred, we used 20 mM KCl. After hypoxia-reoxygenation, the fine regulation of contraction is impaired probably due to the damage of endothelial and smooth muscle cells induced by these events. We compared contractile response to 20 mM KCl after 40- and 60-min hypoxia, each of them followed by 30-min reoxygenation. Contraction of intact rings after 60-min hypoxia (134 ± 4.3% of the standard contraction) was significantly increased ($p < 0.01$) in comparison to contraction after 40-min hypoxia or after normoxia alone (Fig. 2a). Therefore, we decided to use 60-min hypoxia and 30-min reoxygenation in our further experiments. The experiments to obtain the complete concentration-response curve were not considered necessary, because of the significant difference in contraction present even when only one concentration (20 mM) of KCl was used. Because of the variations in diameter and width of arterial rings, the use of absolute values may cause higher differences in forces of contraction and relaxation compared to the use of relative values (i.e. comparing standard contraction of the same ring before and after hypoxia-reoxygenation).

The impairment of vasomotor response after 60-min hypoxia followed by 30-min reoxygenation was further supported by relaxation studies. As mentioned before [22], long-lasting hypoxia impairs EDHF-mediated relaxation. Different substances are used to evaluate this mechanism. We decided to use thromboxane analogue U46619 for precontraction and substance P for relaxation in the presence of NOS and COX inhibitors [22]. We performed a series of control experiments with different durations of hypoxia to determine an appropriate time of hypoxia to evoke injuries. After 40-min hypoxia, relaxation was similar to that in normoxic rings. The relaxation after 60-min hypoxia and 30-min reoxygenation was significantly reduced ($p < 0.01$) in comparison to normoxia (Fig. 2b and c). Therefore, we used 60-min hypoxia in subsequent experiments.

Preconditioning of the coronary artery

The prevention of ischemic injury by preconditioning of the heart was first described by Murry et al. [16]: the ischemic preconditioning consisted of several short ischemic periods that preceded the long-lasting terminal ischemia. Similar protective effect in the heart can be achieved with the administration of some pharmacologically active substances [24] triggering the pharmacological preconditioning. Preconditioning can be triggered by non-receptor triggers also. Although there is an extensive research on signaling transduction in preconditioning, the precise mechanism is still not fully understood [24]. Clinical studies are conducted with adenosine receptor agonists [27]. Opiate receptor agonists and mitochondrial K^+-channel openers are also promising candidates for clinical protocols [17].

Most of the studies concerning preconditioning have been focused on the isolated heart or myocardial cells and only few on coronary arteries. Data concerning arteries are very limited, although it would be important to know, what happens in coronary arteries, because of their greater susceptibility to hypoxia [23]. Also, some segments of the preconditioning signal pathway in coronary arteries might be similar to that in the myocardium. The precise role of receptor subtypes on endothelial and smooth muscle cells and probable differences in preconditioning process between endothelial and smooth muscle cells remain to be explained. That is also true for the end-effector of the preconditioning signal cascade. Hypoxic preconditioning may preserve the contractile response of the porcine coronary artery after long lasting hypoxia-reoxygenation [22]. Our study was performed to compare the effectiveness of acute hypoxic and pharmacological preconditioning of the whole coronary artery including intact endothelium.

Sufficiently strong signal is necessary for ischemic or hypoxic preconditioning to induce a protective effect in the heart or vessels [1, 22, 24, 25]. Thus, several cycles of short hypoxia-reoxyge-

nation periods are needed before long-lasting hypoxia. Too short period of hypoxia during preconditioning does not induce hypoxic preconditioning and if the duration of hypoxia is too long, it can induce hypoxic injuries. We performed hypoxic preconditioning by two cycles of 5-min hypoxia followed by 10-min reoxygenation that was similar to some described procedures [22, 25]. In our experiments, the hypoxic preconditioning performed according to this protocol successfully preserved the contractile response of arterial rings as well as EDHF-mediated relaxation (Fig. 3).

Sufficiently strong signal for pharmacological preconditioning of the heart against ischemia-reperfusion injuries is achieved with an appropriate concentration of the preconditioning agent [1, 17, 24]. Agonists of some receptors physiologically present in the heart (e.g. adenosine, angiontensin, endothelin) can also be used for this purpose [1, 17, 24]. Pharmacological protection can be achieved also by L-type calcium channel blockers [20], ACE inhibitors [17] or substances that are involved in signal transduction of ischemic preconditioning. Similar to the hypoxic preconditioning, the signal for induction of pharmacological preconditioning should not be too intense (high concentration), so that it would not induce injuries. We used some of the agonists known to induce preconditioning in the myocardium and, at the same time, their physiological function in coronary artery has also been confirmed [2, 8, 13, 17, 24–26]. Some of these substances are released in the myocardial tissue during ischemic or hypoxic preconditioning (e.g. adenosine). Drugs were used at concentrations which induced noticeable responses of arterial rings [2, 8, 13, 17, 24–26] and were effective in the lower range of EC_{50}; meaning that sufficiently strong signal was achieved. Agonists were left in the organ bath only long enough to induce contraction or relaxation of rings and then were immediately washed-out. Consequently, none or negligible concentration of the substance was present in the bath during hypoxia-reoxygenation and results could be attributed to preconditioning. The contractile response after hypoxia-reoxygenation was preserved with all agonists used and was significantly lower ($p < 0.01$) than in non-preconditioned rings. Also, the non-NO- and non-PGI_2-mediated relaxation was preserved by both types of preconditioning (Fig. 3). Therefore, we confirmed the hypothesis that hypoxic as well as pharmacological preconditioning with several different agonists of receptors present in endothelial and smooth muscle cells can preserve physiological vasomotor response of the porcine coronary artery after hypoxia-reoxygenation. Agonists used in our study are known to trigger preconditioning in the heart. Therefore, these processes in the coronary artery might be at least in some part similar to those occurring in the heart.

In our experiments, different agonists successfully induced preconditioning; so this protective mechanism may be preserved in, different types of cells allowing them to survive detrimental impact of hypoxia.

In conclusion, we observed the remarkable increase in contractile responses of the porcine coronary artery in the reoxygenation period which depended on the duration of hypoxia. At the corresponding times of experiments, the non-NO- and non-PGI_2-mediated (EDHF-mediated) relaxation induced by substance P was reduced. Both effects were prevented by short periods of preemptive hypoxia or by pharmacological preconditioning with agonists, physiologically present in different types of vascular cells. In our experiments, the protection of artery wall from hypoxia was achieved by the same agonists that protected myocardium from ischemic injury. Non-selective agonists that were used in our study activate receptor subtypes present in cardiomyocytes as well as in endothelial and vascular smooth muscle cells. Because of that we assume that the same agonists that trigger preconditioning in isolated heart may trigger preconditioning in isolated coronary artery as well.

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