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The role of calcium in modulating the reactivity of the smooth muscle cells during ischemia/reperfusion. Part 2

Rola jonów wapnia w modulowaniu reaktywności mięśniówki gładkiej podczas niedokrwienia i reperfuzji. Część 2

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Summary

Background: Damage of transplanted organs during reperfusion is still a problem that prompts the search for new drugs able to diminish the risk of graft rejection. The aim of this study was to examine the influence of antioxidant system on the contraction of arteries induced by angiotensin II during ischemia/reperfusion and to determine the role of intracellular and extracellular calcium ions under these conditions.

Material/Methods: The experiments were performed on male Wistar rats' tail arteries. The effects of angiotensin II on vascular tone were examined after ischemia/reperfusion in the presence of catalase or aminotriazole. To determine the role of intracellular and extracellular Ca^{2+}, the experiments were performed in Ca^{2+}-free PSS and PSS.

Results: Angiotensin II increased perfusion pressure in both Ca^{2+}-free PSS and PSS. After ischemia, the reactions induced by angiotensin II were lower, while after reperfusion they were higher. In the presence of catalase the effects induced by angiotensin II were lower and in the presence of aminotriazole higher.

Conclusions: Ischemia inhibits and reperfusion augments the perfusion pressure induced by angiotensin II. The results confirm the vasoprotective effect of catalase and the destructive influence of aminotriazole in modulating the reactions of vascular smooth muscle cells to ANG II after ischemia/reperfusion. These results suggest that the antioxidant system plays a role in modulating the reactions induced by angiotensin II after ischemia/reperfusion and that reperfusion disturbs the balance between antioxidants and the production of reactive oxygen species.

Key words: **angiotensin II • ischemia • reperfusion • catalase • aminotriazole**

Streszczenie

Wstęp: Uszkodzenia przeszczepianych narządów, wyzwalane reperfuzją, stanowią poważny problem w transplantologii i są powodem poszukiwania leków, które zmniejszałyby ryzyko niepowodzeń, związanych z przywróceniem przepływu w przeszczepie. Celem pracy było określenie wpływu

układu antyoksydacyjnego na skurcz tętnic wyzwalany przez angiotensynę II po niedokrwieniu i reperfuzji, z uwzględnieniem udziału wewnątrzkomórkowej i zewnątrzkomórkowej puli jonów wapnia.

Materiał/Metody: Badania przeprowadzono na perfundowanych tętnicach ogonowych szczurów, samców szczepu Wistar. Badano skurcz wyzwalany przez angiotensynę II z udziałem wewnątrzkomórkowej i zewnątrzkomórkowej puli jonów wapnia po niedokrwieniu i reperfuzji oraz w obecności katalazy i aminotriazolu.

Wyniki: Angiotensyna II wyzwala wzrost ciśnienia perfuzyjnego z udziałem wewnątrz- i zewnątrzkomórkowej puli jonów wapnia. Po niedokrwieniu, w sposób zależny od czasu trwania, dochodzi do obniżenia reakcji mięśniówki gładkiej na angiotensynę II, a reperfuzja prowadzi do nasilenia skurczu. Obecność katalazy powoduje obniżenie, zaś aminotriazolu podwyższenie reakcji wyzwalanych przez angiotensynę II.

Wnioski: Niedokrwienie redukuje, natomiast reperfuzja wzmaga reakcję tętnic na ANG II. Wyniki potwierdzają wazoprotekcyjne działanie katalazy oraz destrukcyjny wpływ aminotriazolu w modulowaniu reakcji mięśniówki gładkiej naczyń na ANG II po niedokrwieniu/reperfuzji. Wyniki sugerują, że układ antyoksydacyjny moduluje reakcje wyzwalane przez ANG II, a reperfuzja zaburza równowagę pomiędzy antyoksydantami i produkcją reaktywnych form tlenu.

Słowa kluczowe: **angiotensyna II • niedokrwienie • reperfuzja • katalaza • aminotriazol**

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Introduction

The blood vessel wall is an active structure formed by cellular elements (endothelial cells, smooth muscle cells, and fibroblasts) and extracellular matrix. Its components undergo dynamic changes in shape, growth, and reorganization under the influence of stimulation by various physiological and pathological factors [8]. Smooth muscle performs a significant role in the normal function of blood vessels, accounting for their shrinkage, growth, remodeling, and repair. It also serves a role in the pathogenesis of vascular diseases such as atherosclerosis, hypertension, and restenosis [2,15,21].

The contractility of blood vessels is affected by their proper structure and the availability of calcium ions. Muscle action is regulated by a number of local and systemic factors, such as vasoactive peptides which contract vessels (e.g. angiotensin II, endothelin-1) and vasodilatory substances (e.g. nitric oxide, prostacyclin) [19,24,32]. Important for this process are also pathological processes, such as ischemia and reperfusion (I/R) and oxidative stress [18].

Angiotensin II (ANG II), one of the strongest factors shrinking blood vessels, is an important mediator stimulating the production of reactive oxygen species (ROS) and the activation of early mechanisms of inflammation, but it also activates, in response to, among others, I/R, the transcription of factors that promote inflammation and performs an important role in the pathophysiology of endothelial dysfunction, unstable angina pectoris, acute myocardial infarction, and heart failure. ANG II-dependent hypertension models indicate that peroxide anion production is increased as a result of the activation of vascular NADH/NADPH-oxidase [17,18,37]. ROS generated by NADH/NADPH-oxidase interact in vascular hypertrophy mediated by ANG II in hypertension. The inhibition of NADH/NADPH-oxidase prevents hypertrophy of vascular smooth muscle cells induced by ANG II, suggesting a potential role of ROS as stimulators of cell growth in hypertension [34,35]. The protective effect of antioxidant therapy against the action of hypertrophic ANG II was demonstrated.

Calcium ions perform an important role in regulating cell function. The level of free calcium ion in the cytoplasm is maintained within a very narrow range; ensuring its physiological function as a signaling system in the cell. Increased calcium ion concentration also occurs in pathological situations, such as hypoxia and ischemia, whereby cells reach a deficit of energy [28].

The conditions for I/R develop during surgical interventions such as bypass operations and the transplantation of organs and may be responsible for impaired function or graft rejection. Tissue damage caused by hypoxia depends on mechanisms associated with reactive forms of oxygen (ROS) and nitrogen (reactive nitrogen species, RNS) [4,16]. The damage triggered by reperfusion is determined by the increase in the mitochondrial production of persistent and diffusion oxidants, peroxides and the release of

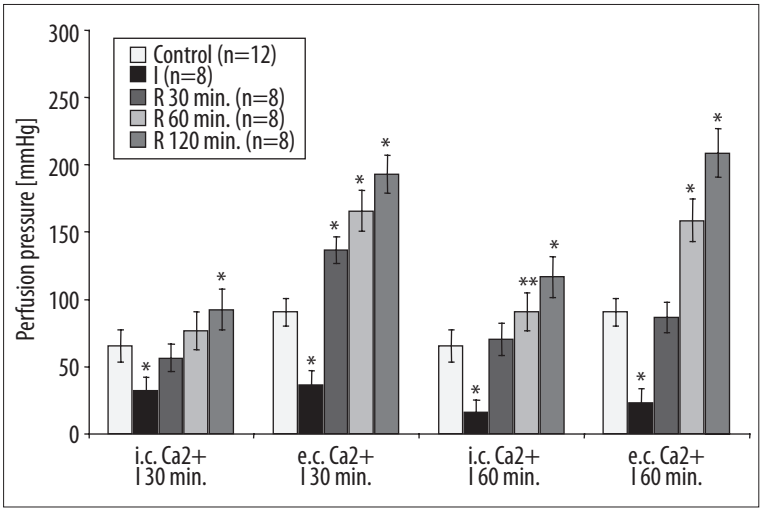

Fig. 1. Perfusion pressure induced by ANG II after ischemia (I)/reperfusion (R) mediated by intracellular (i.c. Ca^{2+}) and extracellular (e.c. Ca^{2+}) calcium ions (mean ±*SE*). *$p<0.0001$ *vs.* control, **$0.05>p>0.0001$ *vs.* control

H_2O_2, which activate a cell signaling system or react with proteins and lipids of cell membranes [11]. The activation of neutrophils significantly contributes to deepening the process of tissue damage associated with ischemia and reperfusion of blood vessels [11,25,36]. ROS and RNS activate mechanisms which, during hypoxia and reperfusion, lead to cell damage and trigger signals releasing mediators. These mediators then induce apoptosis or cell death [38].

In the first part of this study the role of calcium ions and the importance of G protein reactivity of arteries triggered by ANG II, phenylephrine, and Bay K8644 after I/R were evaluated. The aim of this part was to determine the influence of the antioxidant system on arterial contraction triggered by ANG II after I/R, including the involvement of intracellular and extracellular pools of calcium ions in the test reactions.

Material and Methods

The experiments were performed on isolated and perfused tail artery of Wistar rats weighing from 250 to 350 g and euthanized by urethane injected intraperitoneally at a dose of 120 mg/kg. The experiments of arterial contraction after I/R were performed using the same method and experimental arrangement as in Part 1.

To assess the contribution of the intracellular (i.c. Ca^{2+}) and extracellular (e.c. Ca^{2+}) pools of Ca^{2+} in the reactions triggered by ANG II, the experiment was carried out using two types of Krebs' fluid:

- FPSS: Ca^{2+}-EGTA-free Krebs' fluid;
- PSS: Ca^{2+}-EGTA Krebs' fluid (standard), after emptying the intracellular calcium pools.

Contraction was triggered using the AT1 receptor agonist ANG II. Dependencies for the test conditions in the presence of catalase (500 U/ml) and aminotriazole, an inhibitor of catalase (10 mM/l), and after ischemia (30 and 60 min.) and reperfusion (30, 60, and 120 min.) were determined. The results are presented as mean values and standard deviations. Statistical differences were assessed by Student's t test. Values were considered statistically significantly different with $p<0.05$. Calculations were performed with the program Statistica 6.0 PL.

Results

ANG II triggered an increase in perfusion pressure in FPSS and PSS, with higher values in PSS (Fig. 1). After 30 and 60 min. of ischemia, reductions in the response of arteries to ANG II in both types of experiments were observed and the effect depended on the duration of ischemia. In PSS after 30, 60, and 120 min. of reperfusion, perfusion pressur was increased, while in FPSS perfusion pressure after reperfusion was less pronounced. The perfusion pressure values triggered by ANG II after I/R in FPSS and PSS are presented in Figure 1.

In the presence of catalase a reduction of the contracting action of ANG II is observed. The values of perfusion pressure after reperfusion were lower than in the experiments without this enzyme. The effect of catalase on the contraction of blood vessels triggered by ANG II after I/R in FPSS and PSS is shown in Figure 2.

In the presence of aminotriazole, arterial reaction invoked by ANG II after ischemia and reperfusion is higher. Figure 3 shows the perfusion pressure triggered by ANG II after I/R in FPSS and PSS in the presence of aminotriazole.

Discussion

The process of replantation is always associated with a period of ischemia/hypoxia and reperfusion, triggering a local increase in vascular resistance and a reduction of the transplanted organ's perfusion [10,13]. Prolonged ischemia can also subsequently lead to the total diminishment of perfusion of the transplanted organ. The increase in tonic tension of smooth muscle after I/R, as demonstrated by studies, leads to an uncontrolled increase in Ca^{2+} ion concentration and to damage to endothelial cells and smooth muscle and, afterwards, disturbances in the balance between modulating factors and the triggering of vessel contraction and vasodilation factors [7,13,14].

In recent years, ROS were examined for their significance as part of the cell signaling system [9,23,27]. It was demonstrated that ROS can induce cell proliferation because it influences the intracellular mechanisms associated with activation of the cell cycle [3,22,33].

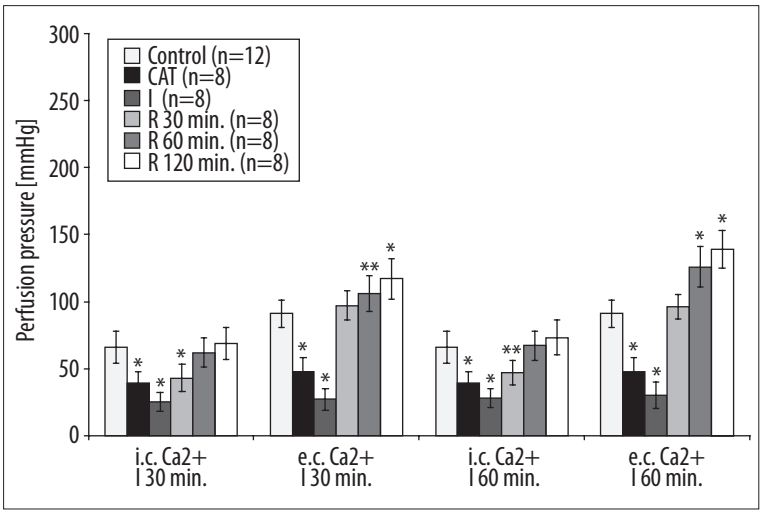

Fig. 2. Perfusion pressure induced by ANG II in the presence of catalase (CAT) after ischemia (I)/reperfusion (R) mediated by intracellular (i.c. Ca^{2+}) and extracellular (e.c. Ca^{2+}) calcium ions (mean ±*SE*). *$p<0.0001$ *vs.* control, **$0.05>p>0.0001$ *vs.* control

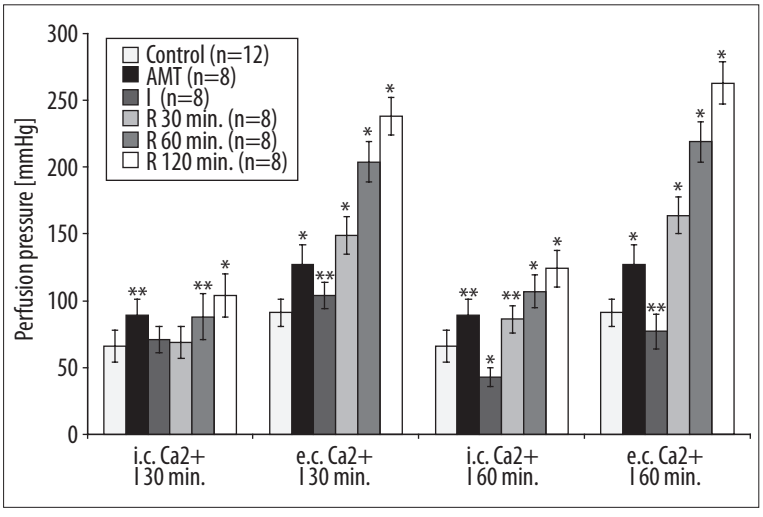

Fig. 3. Perfusion pressure induced by ANG II in the presence of aminotriazole (AMT) after ischemia (I)/reperfusion (R) mediated by intracellular (i.c. Ca^{2+}) and extracellular (e.c. Ca^{2+}) calcium ions (mean ±*SE*). *$p<0.0001$ *vs.* control, ** $0.05>p>0.0001$ *vs.* control

The present study analyzed the influence of catalase and its inhibitor, aminotriazole, on arterial reactions triggered by ANG II after I/R. To determine the importance of calcium ions (from intracellular stores and extracellular fluid), the experiments were carried out in a calcium ion-free liquid (to evaluate the importance of the intracellular pool) and in a standard Krebs' liquid after emptying the stores of cellular calcium (to assess the participation of the extracellular pool). In this study the artery contraction triggered by ANG II through the agency of the intracellular and extracellular pools of calcium ions and the reaction in PSS was more significant for research. The study provided data indicating a reduction of vascular reactions with ANG II after ischemia and a subsequent increase in the vascular reactions to this peptide during reperfusion.

It was demonstrated that the inhibitory effect on artery contraction is associated with the presence of endothelium, the synthesis of nitric oxide, and the activation of cGMP [30]. In the modulations of the arteries' responses to ANG II, ROS reacts in an antagonistic way to NO.

The present study indicated that artery contraction triggered by ANG II in the presence of catalase is reduced. Comparison of the effect of catalase with the influence of an inhibitor of this enzyme, aminotriazole, showed that inhibition of this enzyme causes a significant increase in the response to ANG II. The reported results as well as those of Aoshiba et al. [1] confirm the protective effect of catalase (as well as the destructive action of aminotriazole) and its role in preventing an excessive increase in ROS. Under ischemic conditions, catalase did not significantly affect the responses to ANG II. Catalase reduces the modulating reperfusion effect on the reactions triggered by ANG II, leading to a reduction of the maximum reactions obtained.

Aminotriazole diminishes the inhibitory effect of 30 and 60 min. of ischemia on the artery responses to ANG II. In reperfusion, in contrast, significant intensification of the reactions to ANG II was observed especially in the experiments with the participation of the extracellular pool of calcium ions. Similar observations come from studies of artery reactivity after I/R triggered by increasing concentrations of ANG II [31].

The presented results confirm the participation of catalase in modulating reactions during ischemia and reperfusion. Catalase as well as SOD is a metalloprotein which dimi-

nishes the toxic effects of ROS, but the use of these enzymes to reduce damage induced by I/R and ROS caused different effects [6,12,29]. However, low-molecular-weight substances imitating the action of SOD (SOD mimics, SODm) indicate experimental models of the potential anti-inflammatory effects limiting the damage triggered by I/R and prolonging the half time of NO, anticoagulant, and relaxing action. SODm can therefore be applied in the future to treat diseases such as inflammation, shock, and damage triggered by I/R [5,20,26].

Conclusions

Ischemia reduces while reperfusion enhances the response of arteries to ANG II. The results confirm the vasoprotective action of catalase and the destructive influence of aminotriazole in modulating the reaction of vascular smooth muscle to ANG II after I/R. The results suggest that the antioxidant system modulates the responses triggered by ANG II and reperfusion impairs the balance between antioxidants and the production of reactive oxygen species.

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