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# Influence of bradykinin on the course of ischemia-reperfusion syndrome

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Summary

**Acute ischemia and the subsequent reperfusion caused by temporary closure of blood flow in the main arteries, which activate generalized inflammatory response, lead to endothelial injury. The aim of the study was to examine the injury of tissues in the ischemia reperfusion syndrome. Additionally, we tried to estimate the role of B1 and B2 bradykinin-receptor antagonists. In our study we histologically assessed specimens of lungs, kidney, liver, small and large intestine and skeletal muscle from the thigh. We compared the samples obtained from the groups of animals that were exposed to 4 hours of complete ischemia and 120 minutes of reperfusion. We divided animals into 4 groups. Rats in the first group were the control group, animals from the second received bradykinin. In the third and fourth group respectively bradykinin along with B2 and B1 bradykinin-receptor antagonist were administered.**

**The results of microscopic examination revealed that bradykinin exerts a protective effect on the function and structure of distant organs as well as the skeletal muscle which was subjected to ischemia and reperfusion. The most visible effects of bradykinin were found in the samples of the lung, skeletal muscle, and the large and small intestines.**

**Administration of bradykinin receptor antagonists, especially B2 receptor blocker, reduces the advantageous effects of bradykinin. The conclusion of our study is that administration of bradykinin may be beneficial in diseases accompanied by limb ischemia where tissue blood flow and oxygen metabolism are dependent upon kinin release, which in turn will condition tissue repair.**

**Keywords: ischemia, bradykinin**

Ischemia and reperfusion induced by temporary closure of blood flow in the main arteries, which activate a generalized inflammatory response, lead to endothelial injury. It causes an increase in its permeability which is the key factor in reperfusion injury of distal organs. Counteracting neutrophil activation, mitigating regional blood flow derangements and blocking immunological response induction, all during ischemia-reperfusion injury, can improve the prognosis in this syndrome (5). Studies on the alleviation of oxidative stress in animals reported beneficial effects of insulin administration (16, 18). For that reason we decided to determine the protective effects of bradykinin on ischemic skeletal muscle and distant organs in rats during experimental ischemia-reperfusion syndrome. Conflicting reports in the literature led us to evaluate the modification of bradykinin effect by administration of specific B1 and B2 bradykinin receptor antagonists (10, 15).

## Material and methods

Male, Wistar rats, average weight 250 ± 30 g, fed on a normalized diet (granulated LSM rodent chow) and provided with water *ad libitum* were used in the study. Veterinary care was provided throughout the whole study period. Acute hind leg ischemia was induced by the application of a silicone tourniquet, from Mar-Med. Co (USA), to a thigh of an anesthetized rat in the area of inguinal ligament. The tourniquet was applied for 4 hrs and then the garter was released and reperfusion induced for 30, 60 or 120 minu-

tes. Before tourniquet placement the animals were intraperitoneally administered ketamine (90 mg/kg body mass), xylazine (10 mg/kg body mass), and during the experiment (should the need arise) ketamine and xylazine were administered at a dosage equaling 1/3 of the injected initial amount. The animals were assigned to the following groups: group I with normal limb blood supply (control), group II – subjected to limb ischemia for 4 hours, group III – ischemia for 4 hours and then reperfusion for 30 or 60 or 120 minutes. Each group was further divided into the following subgroups: 1.1. – control subgroup. 60 minutes before sample collection animals received 1 ml of 0.9% NaCl subcutaneously; 1.2. – 60 minutes before sample collection animals received bradykinin subcutaneously (320 µg/kg body mass in 1 ml 0.9% NaCl); 1.3. – 60 minutes before sample collection animals received bradykinin intraperitoneally and B2 - HOE 140, bradykinin receptor antagonist (200 µg/kg body mass intraperitoneally); 1.4. – 60 minutes before sample collection animals received bradykinin intraperitoneally and B1 - desArg$^9$ [Leu$^8$] – bradykinin, bradykinin receptor antagonist (200 µg/kg body mass intraperitoneally).

The study was approved by the Bioethical Committee on Animal Research, K. Marcinkowski University of Medical Sciences in Poznań (opinion no. 99/2001 and 44/2005).

Samples for morphological studies were collected from anesthetized rats after 4 hours of ischemia and 30, 60 or 120 minutes of reperfusion. Samples of skeletal muscle of the thigh, lung, kidney, liver, small intestine and large intestine were gathered, with special attention paid to retaining the same site of puncture. Samples were immediately immersed in the fixing agent at pH 7.4 and placed on ice for 4 hours. Next the samples were rinsed in 0.1 M phosphatic buffer and divided into 1 mm$^3$ pieces and further fixed with osmium tetroxide ($OsO_4$) at pH 7.4 for 2 hours at + 4°C. They were subsequently dehydrated through a series of graded concentrations of ethanol and immersed in eponic mixture Epon 812. After polymerization, semithin sections (1-2 µm) were cut, later dyed with toluidine blue in an alkaline environment and evaluated under a light microscope (with a magnification of 400-1200 ×). After the light microscope evaluation samples were preformed for electron microscope and ultra-thin sections were cut (about 400 nm). The light microscope evaluation served sample positioning before electron microscope slab preparation. Ultra-thin slabs were contrasted with uranyl acetate $(C_6H_5O_7)_2\ Pb_3 \times 3\ H_2O$ and lead citrate $(CH_3COO)_2\ UO_2 \times H_2O$ and studied with a Opton-Zeiss EM 900 electron microscope (with a magnification from 6000 to 20 000 ×). Specimens for histologic evaluation in the light microscope were fixed in a 10.0% formalin buffered solution. Routine preparation and dyeing H + E were applied.

## Results and discussion

After 4 hours of ischemia, the endothelia of all arteries were swollen. It was accompanied by platelet adhesion. Endothelial vacuoles and intermittent disruptions of ependymal continuity were also found. There were mitochondria with light matrix and shortened cristae.

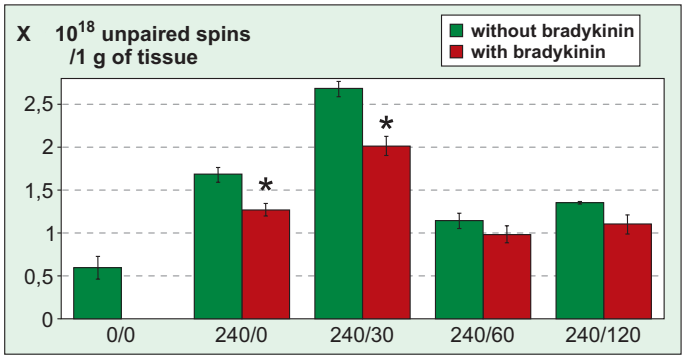


**Fig. 1. The level of free radicals in a rat's thigh skeletal muscle after 4 hrs of ischemia and reperfusion of the hind limb**

Explanations: Data are means ± SE; n = 8 rats for each group (controls, ischemia, ischemia + reperfusion (30 min or 60 min or 120 min); *statistically significant difference compared without bradykinin ($p < 0,05$)

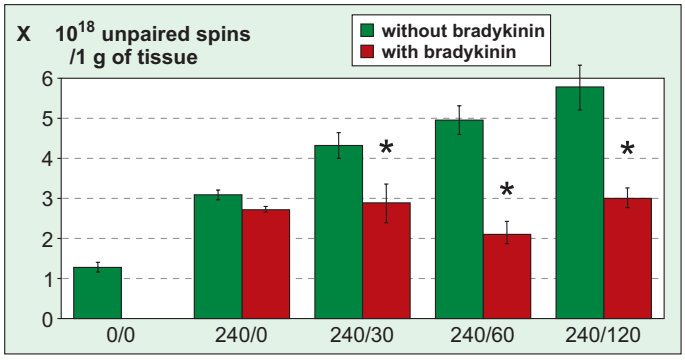


**Fig. 2. The level of free radicals in a rat's lung**

Explanations: as in fig. 1.

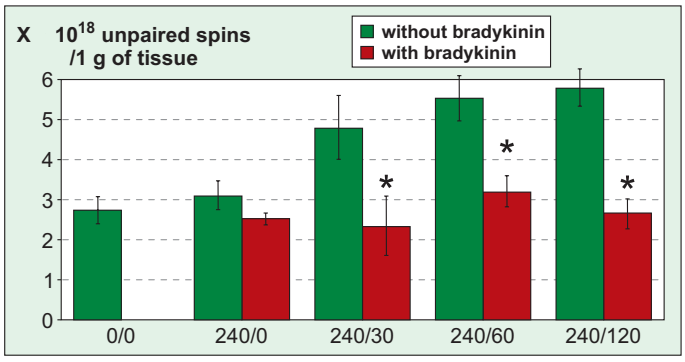


**Fig. 3. The level of free radicals in a rat's small intestine (jejunum)**

Explanations: as in fig. 1.

The histological evaluation of the skeletal muscle control and placebo group animals after 4 hours of ischemia revealed depleted glycogen stores, enlarged segments of mitochondria and significantly light cytoplasm. In skeletal muscle, muscle fibers were presented with damaged basal membranes. Many nuclei had obvious chromatin density reduction and regions of clumping of chromatin beneath nuclear membrane. Some sarcomeres in single fibers lacked myofibrils within the A band. Most mitochondria were swollen and presented with shortened cristae, lighter matrix and

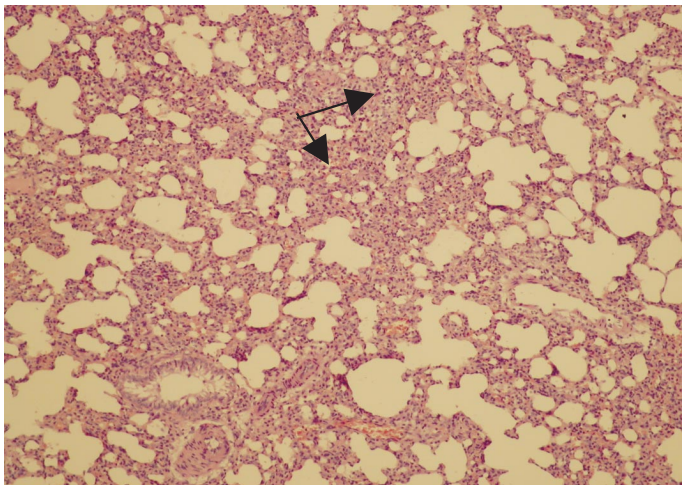

Fig. 4. The lung of a rat after 4 hours of ischemia and 120 minutes of reperfusion of the hind limb. Diffuse lymphocytic (→) infiltration in alveolar septa. H&E changes

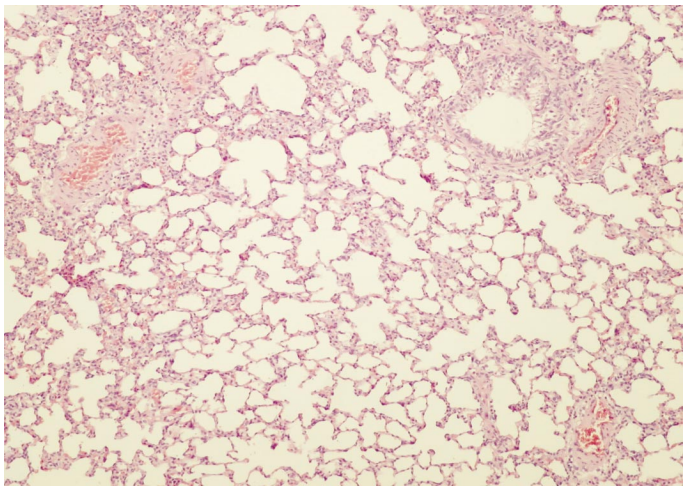

Fig. 5. The lung of a rat after 4 hours of ischemia and 120 minutes of reperfusion of the hind limb and after bradykinin staining administration. The lung without any microscopic magnification 100×. H&E staining, magnification 100×

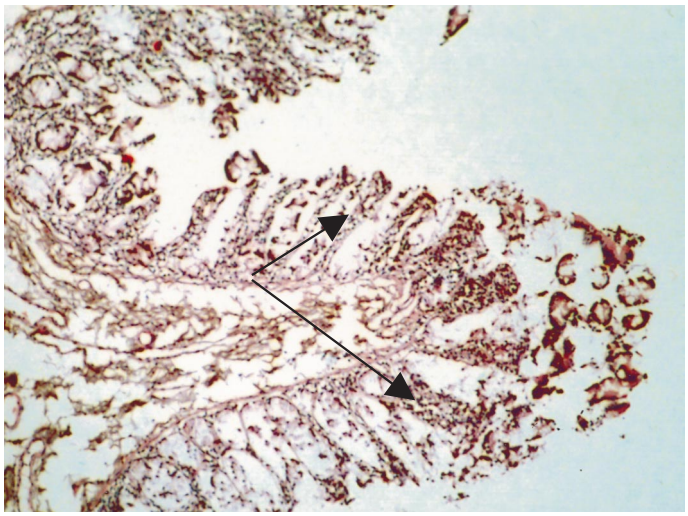

Fig. 6. Large intestine of a rat after 4 hours of ischemia and 120 minutes of reperfusion of the hind limb. Inflammatory infiltration with numerous (→) lymphocytes. H&E staining, magnification 50×

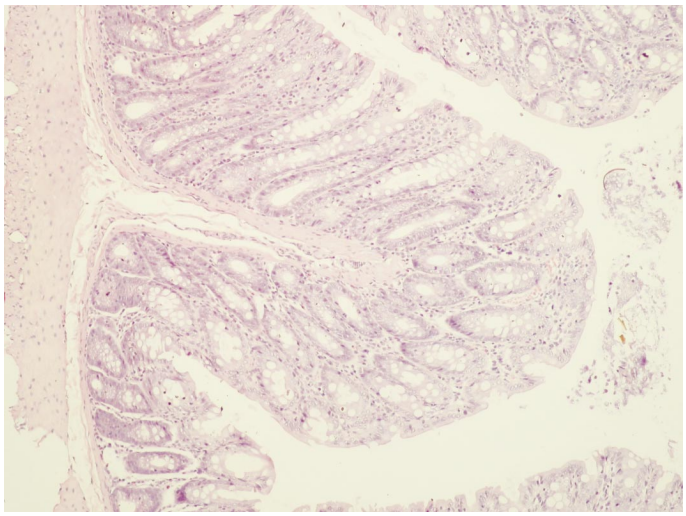

Fig. 7. Large intestine of a rat after 4 hours of ischemia and 120 minutes of reperfusion of the hind limb after bradykinin administration. The large intestine without any microscopic changes. H&E staining, magnification 50×

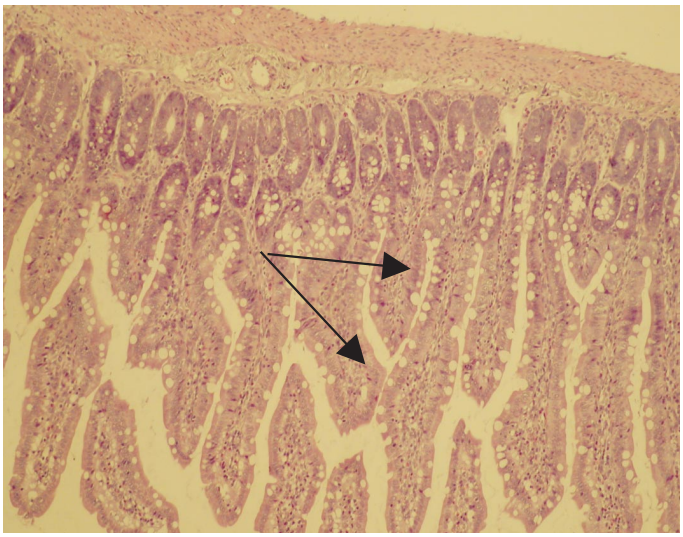

Fig. 8. Small intestine of a rat after 4 hours of ischemia and 120 minutes of reperfusion. Inflammatory infiltration with Lymphocytes (→) and quite numerous eosinophils. H&E staining, magnification 50×

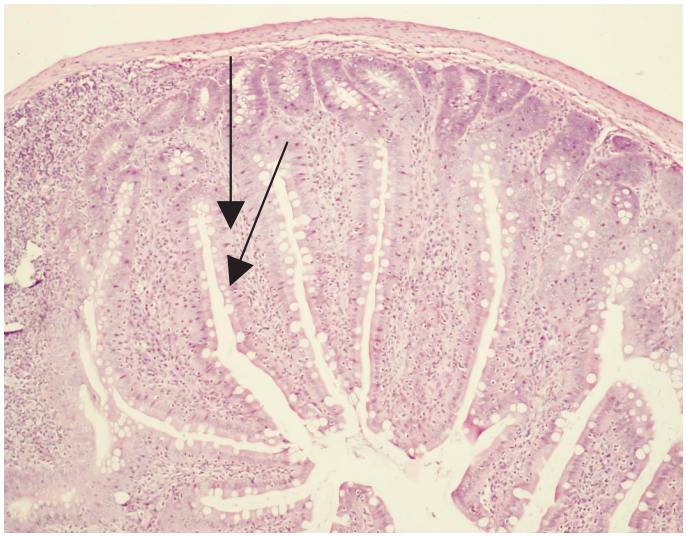

Fig. 9. Small intestine of a rat after 4 hours of ischemia and 120 minutes of reperfusion with bradykinin administration. Lymphocytes and quite numerous eosinophils. H&E staining, magnification 50×

lacked Pallad granules. Substantial changes were observed in the electron microscope after four hours of ischemia and two hours of reperfusion. Strains of mitochondria showed matrix density reduction and numerous cristae. Muscle fibers contained the smallest amount of glycogen. Histological analysis of organs distant from ischemia sites in controls and placebo animals (with light microscope) after 4 hours of ischemia and 2 hours of reperfusion yielded a marked increase in the lymphocyte count in the small intestine, lung samples presented with dilatation of the capillary bed in alveolar septae and the presence of numerous lymphocytes within alveolar septae (fig. 4). Similar lymphocyte infiltration was observed in the large intestine (fig. 6). These animals displayed parenchymatous degeneration in the kidneys and no changes in the livers. The results obtained for rats with ischemia-reperfusion syndrome show that most significant structural changes in distant organs affected the lungs and large intestine.

After bradykinin and bradykinin with B1 bradykinin receptor antagonist administration small vessels i.e. arterioles and capillaries were present with abnormal structure, i.e. very wide lumen. There were quite numerous erythrocytes, foci of platelets with traits of adhesion. Endothelial cytoplasm contained, just like the former group, many pinocytic transport vesicles. No evidence of damage to the endothelium was found, even in those vessels in which platelets adjacent to endothelium were present. Basement membranes were not unaffected either. Many fragments contained extravasated blood cells. All evaluated muscle fibers were with well visible bands, normal spaces between sarcomeres and the normal framework of structures beneath sarcolemma. All studied samples had normal mitochondria, T and L systems, endoplasmic reticulum and nuclei. The only feature differentiating these cells from normal cells was the depletion of glycogen granules between sarcomeres (1, 2). After bradykinin and bradykinin with B1 bradykinin receptor antagonist administration there were no microscopic changes in the lungs (fig. 5) and large intestine (fig. 7). Mucous membranes in small intestine samples were infiltrated with few lymphocytes and many eosinophils. Skeletal muscle and distant organ analysis in animals that were administered bradykinin and bradykinin with B2 receptor-antagonist led to the partial reversal of changes incurred by bradykinin itself, microscopic changes resembled those in control and placebo groups animals.

In conclusion, rats with ischemia-reperfusion syndrome after bradykinin and bradykinin with B1 bradykinin receptor antagonist present had fewer structural changes in vessels and skeletal muscles, which was manifested mainly in the lack of significant traits of nuclear damage, fewer damaged mitochondria and an increase in glycogen storage. In animals receiving bradykinin with B2 bradykinin receptor antagonist, there was lymphocytic and eosinophilic infiltration both in small and large intestine and in the lungs there was lymphocytic infiltration adjacent to bronchioles. In order to verify histopathologic changes, free radical counts were assessed in skeletal muscles and distant organs. The levels of free radicals in tissues of animals which were administered bradykinin were markedly decreased in comparison with the remaining animals (fig. 1, 2).

A few studies (6, 9) reported that moderate amounts of bradykinin can mitigate damage occurring during ischemia-reperfusion syndrome. Bradykinin acts mainly via 2 types of receptors: B1 and B2 (8). It was shown that B2 receptors are permanently present on the surface of many cell types. B1 receptor expression takes place several dozen minutes after the inflammatory stimulus triggering their activation (4, 11, 12). An activating factor stimulating B1 receptor expression is also B2 receptor activation (14). Bradykinin acting on the B2 receptor exerts a protective influence through vasodilation and decreased synthesis of free radicals and in turn diminishing the overall damage to local tissues and tissues distant from the site of ischemia (17). B1 receptor activation leads to detrimental effects, including increased vascular permeability and neutrophil accumulation. Data available in the literature also mention the influence of bradykinin and its receptor antagonist dosage on the final blockade of the receptors (7).

These studies aimed at answering the question whether bradykinin itself and specific receptor blockers can influence the course of ischemia-reperfusion injury. Results obtained from histopathologic evaluation show that bradykinin administered to animals subjected to experimental ischemia-reperfusion leads to a decrease in tissue damage – both in the ischemic area – skeletal muscle of the hind limb and in distant organs – lungs, small and large intestines. The simultaneous administration of bradykinin and B2 receptor antagonist diminished beneficial effects achieved after bradykinin administration. B1 receptor blockade did not cause any significant changes in the microscopic picture compared with animals receiving bradykinin only. Bradykinin use led to a depletion in free radical levels which play a significant role in tissue damage during ischemia and subsequent reperfusion (3).

The obtained results, together with available data from literature (19-21), make it possible to claim that increased levels of free radicals are the main cause of complications observed in ischemia-reperfusion syndrome. An important finding in the current study is directly indicating the increase in free radical levels in tissues, while in the majority of other studies (13) it is attained indirectly by assessing levels of peroxides and malonic dialdehyde. Our study shows an ischemia-induced increase in free radical levels in skeletal muscles and small and large intestines. An increase in free radical levels in distant organs was incurred by

active granulocytes collecting in these organs, which further trigger a cascade of harmful phenomena observed in ischemia-reperfusion. Bradykinin and B2 receptor blocker administration reversed the changes induced by bradykinin itself. Early studies showed that exogenous bradykinin stimulates isocitrate and glucose-6-phosphate dehydrogenases while causing vasodilation. This action allows for proper oxygen use and prevents its reduction, which is crucial for endothelium and myocyte protection.

## Conclusions

Bradykinin exerts a protective effect on the structure and function of distant organs such as lungs and large intestines and skeletal muscles that were subjected to ischemia and reperfusion. Administration of bradykinin receptor antagonists, especially B2 receptor blocker, mitigates beneficial effects of bradykinin. Kinin synthesis inhibitor administration seems to be beneficial in diseases accompanied by limb ischemia when tissue blood flow and oxygen metabolism are dependent upon kinin release, which in turn will condition tissue repair.

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