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Wpływ dowieńcowego podania diphenyliodonium oraz L-argininy w buforze o kwasowym odczynie na wielkość blizny pozawałowej i występowanie zaburzeń rytmu serca u szczurów z niedokrwieniem i reperfuzją mięśnia sercowego

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

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Streszczenie w języku angielskim

Effect of intracoronary administration of diphenyliodonium and L-arginine in an acidic buffer on the size of the post-infarction scar and occurrence of cardiac arrhythmias in ischemic rats undergoing myocardial reperfusion.

Introduction

The principal cause of acute coronary syndrome is significant stenosis or even total occlusion of coronary artery, therefore the method of treatment of this disease is coronary angioplasty procedure (PCI). However, the sudden restoration of blood flow to the ischemic area causes reperfusion injury, which is responsible for increasing the final myocardial infarct size and the risk of life-threatening arrhythmias.

Work assumptions

The basic pathophysiological mechanisms of post-reperfusion injury are: reperfusion contracture, persistent opening of mitochondrial permeability transition pores, oxidative stress, inflammation and no-reflow phenomenon. It is impossible to determine which of them predominates, most likely they co-occur with each other. The hypothesis assumes that only an intervention that simultaneously interferes with different pathways of post-reperfusion injury may be effective in reducing the final size of myocardial infarction and reducing the incidence of life-threatening arrhythmias.

Aim of the work

The main purpose of the doctoral dissertation is to investigate whether intracoronary administration of diphenyliodonium and L-arginine in an acidic buffer reduces the likelihood

of ventricular fibrillation and has a positive effect on the size on the size of post-infarction cardiac necrosis in rats.

Methodology

The experiments were carried out on rat hearts *in vitro*.

Sixty-seven 12-week-old male Sprague-Dawley rats (SPRD/Clzd) were divided into eight groups. After connecting the heart to the Langendorff system, the first 10 minutes of the experiment constituted a stabilization period in all groups. Then, the buffer flow in the system was turned off for 15 minutes, which resulted in complete myocardial ischemia. During the first three minutes of reperfusion, the buffer was administered via separate access, for the next 42 minutes the buffer was returned to the original source.

In the first group, Krebs-Henseleit buffer was administered during reperfusion without any modifications (control group). The composition of the buffer in groups 2, 6, 7, 8 was modified by adding diphenyliodonium (20 μmol/l), in groups 3, 5, 7, 8 - L-arginine (3mmol/l) was used, and in groups 4, 5, 6, 8 - an acidic buffer, pH 6.8, was used during reperfusion.

The following parameters were assessed:

- diastolic pressure in the left ventricle of the heart during stabilization;
- time from the beginning of reperfusion to the restoration of the physiological rhythm of the heart;
- diastolic pressure in the left ventricle during reperfusion in the 15th, 30th and 45th minute of its duration, respectively;
- number of ventricular fibrillation episodes;
- the ratio of the infarcted area to the entire heart area expressed as a percentage;
- protein content - caspase-3 in the apex of the heart (Western blot);
- protein content - phosphatidylinositol 3-kinase in the apex of the heart (Western blot);

Results:

- There were no statistically significant differences in diastolic pressure in the left ventricle during stabilization between the groups.

- A comparative statistical analysis of the number of ventricular fibrillation episodes between the individual study groups and the control group was not performed due to too few ventricular fibrillation episodes.

- The mean time to recovery of physiological heart rhythm in the study groups was not statistically significantly different from the control group. There were also no statistically significant differences for this parameter between the individual study groups.

- The mean diastolic "deltas" (differences between diastolic pressures at certain moments - 15., 30. and 45. minute of reperfusion and diastolic pressures at stabilization phase) for study groups D and BKO + D + ARG were significantly lower than for the control group. In addition, the mean diastolic deltas at 30. and 45. minute of reperfusion for the ARG and BKO + ARG study groups were significantly lower than for the control group.

- The average percentage area of cardiac necrosis for the study groups: D, BKO + ARG, ARG + D and BKO + D + ARG was significantly lower than in the control group. There were no statistically significant differences in the mean percentage of cardiac necrosis between the individual study groups.

- The average ratio of caspase-3 protein content in the apex of the heart to the reference protein (β-actin) for the study groups: BKO and BKO + ARG was significantly lower than in the control group. In addition, it was shown that the average ratio of caspase-3 content to the reference protein (β-actin) for some study groups was significantly lower than in the others: BKO + ARG vs BKO + D, ARG + D vs BKO + D, BKO + ARG vs

ARG, D vs BKO + D, BKO vs ARG, BKO vs BKO + D, BKO + ARG vs BKO + D + ARG.

- The average ratio of the phosphatidylinositol 3-kinase protein content in the apex of the heart to the reference protein (β-actin) for the BKO + ARG study group was significantly lower than in the control group. In addition, it was shown that the average ratio of phosphatidylinositol 3-kinase content in the apex of the heart to the reference protein (β-actin) for some study groups was significantly lower than in the others: BKO + ARG vs ARG, BKO + ARG vs BKO, BKO + ARG vs ARG + D, BKO + D + ARG vs ARG.

Conclusions:

1. The use of additional substances - diphenyliodonium and a combination of an acidic buffer, L-arginine and diphenyliodonium in the Krebs-Henseleit buffer in the *ex vivo* myocardial infarction model using the Langendorff system significantly reduced the delta parameter of diastolic pressure (indicating the strength of reperfusion contracture) after 15., 30. and 45. minutes of reperfusion. The addition of L-arginine and the combination of L-arginine and an acidic buffer in this model significantly reduced the "delta" parameter of diastolic pressure after 30. and 45. minutes of reperfusion.

2. The use of additional substances - diphenyliodonium and the following combinations: 3 substances (acidic buffer, L-arginine and diphenyliodonium), 2 substances (L-arginine and diphenyliodonium) and other 2 substances (acidic buffer and L-arginine) in Krebs-Henseleit buffer in an *ex vivo* myocardial infarction model using the Langendorff system significantly reduces the size of cardiac necrosis.

3. The use of additional substances (an acidic buffer and a combination of an acidic buffer with L-arginine) in the Krebs-Henseleit buffer in the *ex vivo* myocardial infarction model using the Langendorff system reduces the content of caspase-3 protein (apoptosis marker) and kinase 3 -phosphatidylinositide (protective factor) at the apex of the heart.

Summary

The conducted studies confirmed the cardioprotective effect of administration of an acidic buffer (pH 6.8), L-arginine and diphenyliodonium during reperfusion. Simultaneous combination of substances, e.g., intracoronary administration of diphenyliodonium and L-arginine in an acidic buffer, was associated with a statistically significant reduction in the diastolic pressure delta in the left ventricle and the percentage of cardiac necrosis compared to the control group. The exact mechanism of action of these substances and the possibility of using these mechanisms requires further research.