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Ischemic preconditioning and myocardial tolerance to ischemia in experimental insulin-dependent diabetes mellitus.

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Introduction

It has been proven by a number of clinico-epidemiological studies that patients with insulin-dependent diabetes mellitus (type-1 diabetes mellitus, T1DM) have higher rate of myocardial infarction (MI) with more severe injuries, accompanied with intensive cardiac rhythm disorders and increased rate of such complications as postinfarction angina and heart failure in comparison with patients without diabetes [6, 11, 15]. It is considered that the greater risk of MI occurrence and its complications in patients with T1DM is associated with active atherosclerotic process underlying diabetic macroangiopathy, and also with more severe left ventricular diastolic dysfunction [11, 15]. At the same time, the results of experimental studies on myocardial tolerance to ischemia in the animals with T1DM are controversial [3, 8]. Some studies are parallel to the clinical data and show decreased myocardial tolerance to ischemia in the animals with diabetes mellitus that is confirmed by larger MI size [4], more serious rhythm disturbances [2] and a smaller degree of restoration of left ventricular pressure in the model of isolated heart [12]. Meanwhile, some studies have shown that a myocardium of animals with T1DM is paradoxically more tolerant to the ischemia than the myocardium of healthy animals that is manifested, in particular, in smaller infarct size [3, 5]. These data have given the basis to consider the increased tolerance of myocardium to ischemia in experimental T1DM as one of poorly studied forms of the cardioprotective

response similar to the condition of ischemic preconditioning of a myocardium.

Ischemic preconditioning (IPC), for the first time described by Murry et al. in 1986, results in significant increase in myocardial tolerance to prolonged ischemia after one or several short-term bouts of an ischemia-reperfusion [7]. IPC mechanisms for the last two decades were an object of numerous studies [14]. However the overwhelming majority of these works was carried out on healthy animals without any pathology. The last circumstance essentially complicates an extrapolation of the received results on a clinical practice in which the myocardial ischemia arises generally on a background of chronic associated diseases, e.g. diabetes mellitus. To date, little is known about the characteristics of myocardial IPC in animals with T1DM.

The aim of this work was to elucidate a degree of myocardial tolerance to ischemia estimated by an infarct size and an intensity of ischemic arrhythmias in rats with T1DM. Another aim of this work was to study the infarct-limiting effects of IPC induced by a single short-term bout of myocardial ischemia-reperfusion in animals with T1DM.

Materials and Methods

All experiments were performed in accordance with the «Guide for the Care and Use of Laboratory Animals» (publication of National Institute of Health, U.S.A. No. 85-23) and were approved by the institutional ethical committee of the St.-Petersburg I.P. Pavlov Federal Medical University.

Model of an insulin-dependent diabetes mellitus. The chemical model of T1DM was used. Fasting Wistar male rats (200-250 g body wt) (Rappolovo nursery, The Russian Science Academy) were subjected to subcutaneous administration of alloxan monohydrate (Lancaster, UK) in a dosage of 125 mg/kg. Blood glucose levels were registered with glucometer Elite 3904M (Switzerland) twice: on 3rd week after an injection of alloxan and immediately before modeling of a myocardial infarction. Experimental animals were selected by a level of glucose measured on 3rd week that was not less than 18 mM/L. A myocardial infarction and IPC were reproduced 6 weeks after (42±3 days) injection of alloxan.

Model of a myocardial ischemia in vivo. Experiments were carried out on 36 rats anaesthetized by pentobarbital (60 mg/kg i.p. administration and subsequent maintaining i.v. infusion) under mechanical ventilation through a tracheostome (rate ~ 50 min-1, tidal volume ~ 3 ml/100 g body weight). Blood pressure (BP) was monitored by a transducer (“Baxter”, U.S.A.) connected to a catheter introduced through the common carotid artery into aorta, and then was processed on a computer by the software “PhysExp™ 2.0 for Windows” (SPMU, Russia). Also, an electrocardiogram in standard leads was continuously monitored in animals during experiment in order to estimate a heart rate (HR) and heart rhythm disturbances. Access to heart was made by left thoracotomy through the fourth intercostal space. After exposure of a pericardium we determined a localization of the prominent branch of the left coronary artery (LCA) and 6-0 thin polypropylene thread was passed around it. In order to create a reversible myocardial ischemia an occluder was formed.

Experimental protocol

The protocol of experiment consisted of 4 groups of animals:

1. Control (infarct) (n=12): 30-min myocardial ischemia and subsequent 60-min reperfusion without additional interventions in healthy rats.

2. Control + IPC (n=10): ischemic preconditioning in the form of single 5-min myocardial ischemia and subsequent 5-min reperfusion immediately before 30-min ischemia in healthy rats.

3. T1DM + infarct (n=8): diabetic animals with the same procedures as in group 1.

4. T1DM + IPC (n=6): diabetic animals with IPC in the same manner as in group 2.

Blood pressure and heart rate were monitored at initial conditions, i.e. 15 min before LCA occlusion, immediately before LCA occlusion, at 15 and 30 min of ischemia, and also at the end of experiment, i.e. at 60 min of reperfusion.

Assessment of an infarct size. Assessment of an infarct size was done by macroscopic "double" staining with Evans blue solution and triphenyltetrazolium chloride (TTC) (ICN Pharm., U.S.A.). At the end of the reperfusion the LCA was reoccluded followed by the intravenous administration of 5% Evans blue solution for identification of the anatomic area at risk (AR) that resulted in identification and easy delineation of the area by the absence of blue dye. The rat was then sacrificed by KCl injection while under deep anesthesia, and the heart was quickly excised and cut into four slices (2.5 mm) parallel to the atrioventricular groove. The basal surface of each slice was photographed using digital camera Olympus C-2020. The images of the basal surface of the slices were digitized using the software VideoTest 4.0 (ISTA Ltd., Russia) for the calculation of the anatomic area at risk (Evans-negative sites) and non-ischemic myocardium (Evans-positive sites). The total volume of AR for the given heart was calculated by summation of multiplications of the Evans-negative site area of each slice by its thickness.

Then samples were then placed in 1% solution of TTC at 37ºC for 15 min. After incubation with TTC, samples were photographed again and a total volume of an infarct zone was calculated by the described above technique. Data were represented as the ratio of the total volume of an anatomic zone at risk to the total volume of heart, and also as the ratio of the total volume of an infarct zone to the total volume of AR.

The analysis of rhythm disturbances. We hemodynamically analyzed significant rhythm disturbances that have arised during 30 min ischemia. Because of a variety of transitive gradations and difficulties of differentiation between a ventricular tachycardia and a ventricular fibrillation (VF) when using this animal model, rhythm disturbances of the two types specified were categorized as ventricular tachyarrhythmias (VT) and were estimated in accordance with the Lambeth Conventions [13] by following criteria:

1. A number of animals with at least one VT episode in a group;

2. A number of VT episodes in recalculation on one animal (from those having these rhythm disturbances);

3. An average duration of one VT episode in recalculation on one animal (in seconds);

4. A number of animals with developed persistent VT that led to death of an animal.

Statistical significance of the differences in hemodynamic data at each time point, and also the plasma concentrations of glucose and the volumes of an anatomic zone at risk and an infarct zone were estimated by software ANOVA Statistica for Windows (Scheffe test). Data by category, in particular, occurrence of VF and VT, were compared by Fisher's exact test. All data are presented as means+/-SD, and a p value < 0.05 was considered significant.

Results

Blood glucose levels in experimental groups. In group 1 (Control) venous glucose level before experiment was 5.6+/-0.8 mM/L, and in group 2 (Control + IPC) it was 5.3+/-0.6 mM/L. In groups 3 and 4 (T1DM + infarct and T1DM + IPC, respectively) glucose levels did not differ and were 21.3+/-5.5 and 23.1+/-4.8 mM/L, respectively, that was significantly

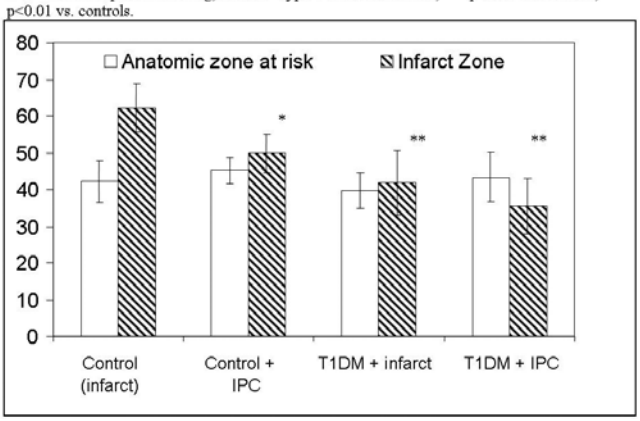
higher than in controls (p<0.01).

Table 1. Hemodynamic data in experimental animal groups. Data were represent as "mean+/-standard deviation". BP – blood pressure, HR – heart rate, IPC – ischemic preconditioning, T1DM – type 1 diabetes mellitus, * – p<0.05 vs. controls, ** – p<0.01 vs. controls.

Hemodynamic data	Control (infarct) (n=10)	Control + IPC (n=10)	T1DM + infarct (n=8)	T1DM + IPC (n=6)
Baseline:				
BP	116 ± 9	110 ±15	91 ± 8*	89 ± 10*
HR	386 ± 14	406 ± 18	338 ± 15*	342 ± 23*
Before occlusion:				
BP	105 ± 10	107 ± 12	82 ± 7*	78 ± 11*
HR	394 ± 22	387 ± 19	330 ± 20**	322 ± 26**
15 min after ischemia:				
BP	98 ± 8	92 ± 10	72 ± 12*	75 ± 8*
HR	385 ± 18	398 ± 27	333 ± 19*	320 ± 18**
30 min after ischemia:				
BP	102 ± 11	110 ± 9	68 ± 6**	71 ± 9*
HR	372 ± 25	375 ± 21	326 ± 21**	315 ± 13**
60 min after reperfusion:				
BP	95 ± 12	100 ± 11	65 ± 8**	69 ± 11*
HR	369 ± 22	368 ± 14	309 ± 19**	312 ± 15**

Hemodynamic data. Hemodynamic data from experiments in all groups are summarized in Table 1. Diabetic animals in groups 3 and 4 at initial conditions had significantly smaller values of an average BP and the HR than control animals. The specified tendency was lasted during all experiment.

Figure 1. The volumes of anatomic zone at risk and infarct zone in experimental animal groups. IPC – ischemic preconditioning, T1DM – type 1 diabetes mellitus, * – p<0.05 vs. controls, ** – p<0.01 vs. controls.



Anatomic zone at risk and infarct zone. The volumes of an anatomic zone at risk did not differ significantly between all groups (Figure 1) and were 42.2+/-5.8, 45.3+/-5.6, 39.8+/-4.9 and 43.4+/-6.8 % in groups 1, 2, 3 and 4, respectively. Thus, the volume of ischemic myocardium in all four groups was identical. The infarct size in control group was 62.3+/-6.6 % from the size of the zone at risk (Figure 1). IPC led to significant decrease of the infarct size (49.9+/-5.2 %, p<0.05 in comparison with controls). The infarct size in T1DM rats was 39.8+/-8.8 %, that also was significantly smaller than in controls (p<0.01). IPC in T1DM animals did not lead to the further limitation of the infarct size (35.5+/-7.5 % vs. 39.8+/-8.8 % in T1DM, p>0.05).

Ischemic arrhythmias. The major endpoint of myocardial tolerance to ischemia and IPC efficiency in this work was infarct size. However, incidence and duration of ischemic VT were used as additional criterion.

Table 2. Occurrence and characteristics of ischemic ventricular tachyarrhythmias (VT) in experimental animal groups. T1DM – type 1 diabetes mellitus, IPC – ischemic preconditioning, * – p<0.05 vs. controls.

Parameters	Control (infarct) (n=12)	Control + IPC (n=10)	T1DM + infarct (n=8)	T1DM + IPC (n=6)
Number of animals with VT	12	3*	2*	0*
Number of VT bouts	2,3±0,8	1,3±0,4	1,5±0,5	-
Mean duration of VT, s	45±22	12±8*	35±10	-
Number of animals with lethal forms of VT	2	0	0	0

The data obtained from analysis of ischemic VT (Table 2) revealed that all animals in control group had VT episodes during ischemia, and two cases were lethal. Accordingly, these hearts have been excluded from estimation of the infarct size in control group. VT occurrence and duration in healthy animals (group 2) were significantly lower after IPC than in controls, and lethal arrhythmias did not develop (Table 2). Also ventricular tachyarrhythmias were less often in T1DM animals than in controls. It is necessary to note, however, that frequency of paired and grouped polytopic premature beats in diabetics was higher than in control animals. Ventricular tachyarrhythmias did not arise in T1DM animals with IPC.

Discussion

These results suggest that: firstly, the myocardium of T1DM animals after 6 weeks of disease is more tolerant to ischemic damage than a myocardium of healthy animals, and, secondly, an ischemic preconditioning in T1DM animals is less effective in limitation of the infarct size than in control animals.

Hadour et al. [5] showed smaller myocardial infarct size in rabbits with alloxan-induced T1DM as compared to controls that allowed the authors to introduce the concept of metabolic preconditioning of myocardium for the first time. According to this concept, molecular and cellular characteristics of cardiomyocyte phenotype which occur in experimental T1DM might be responsible for enhanced myocardial tolerance to ischemia. Thus, the intracellular mechanisms that are involved in the cardioprotective response in experimental T1DM and IPC may actually share certain extent of similarity. In particular, there are some data that experimental T1DM is associated with chronic activation of cardiomyocyte ATP-sensitive potassium channels which are believed to be one of most probable final IPC effectors [1]. It is necessary to emphasize, that, apparently, the «pure» metabolic myocardial preconditioning in T1DM exists only as the experimental phenomenon simulated in animals that genetically are not predisposed to atherosclerosis. In the clinical setting, putative cardioprotective effects of metabolic preconditioning in the patients with T1DM are most likely diminished because of the accompanying advanced atherosclerotic lesions of coronary arteries. Nevertheless, studying of the mechanisms of metabolic preconditioning is very interesting as it can be a basis for development of new methods in pharmacotherapy for limitation of ischemic myocardial injury.

Some authors considered a decreased rate of glycolysis associated with a smaller degree of intracellular acidosis in ischemia and changes in sarcolemmal ion channels as possible mechanisms of the increased myocardial tolerance to ischemia in T1DM [3]. From these mechanisms more attention is paid to decreased activity of sarcolemmal Na+/H+ exchanger in T1DM. This, in turn, may lead to limitation of Ca2+ inward current through Na+/Ca2+ exchanger in cardiomyocytes, and therefore may retard the irreversible ischemic injury [3].

On the other hand, observed increased myocardial tolerance to ischemia in T1DM rats may be explained by the difference in hemodynamic parameters between control and diabetic animals. Significantly lower values of blood pressure and heart rate in diabetic rats in comparison with controls may result from hypovolemia, myocardial contractile dysfunction, and diabetic autonomic dysfunction. It is known that lower values of blood pressure and heart rate are associated with smaller cardiac performance and, hence, smaller myocardial oxygen demand. Therefore, it can not be excluded that the increased myocardial tolerance to ischemia in the animals with diabetes mellitus can be attributed to hypotension and bradycardia observed in this group. At the same time, the effect of metabolic preconditioning in T1DM is confirmed by other researchers also in the lack of difference in a hemodynamics between healthy and diabetic animals [5].

Analysis of the current available experimental data on myocardial tolerance to ischemia in T1DM revealed an important role of disease duration. There are two stages of experimental T1DM: acute (1-3 weeks) and chronic (6-9 weeks) [9]. Most researchers acknowledge that an infarct-limiting and antiarrhytmic effects of metabolic preconditioning are most intensive in acute T1DM stage and then gradually decrease in a chronic stage [9]. In our experiments, the significant infarct-limiting and antiarrhytmic effects of metabolic preconditioning were observed at 6 weeks of T1DM, i.e. in the beginning of a chronic stage.

The question of a capability of realization of myocardial IPC in animals with T1DM now remains open. Ravingerova et al. [10] have demonstrated on the isolated hearts of diabetic rats that antiarrhytmic IPC effects occurred only at the chronic stage of diabetes mellitus (9 weeks) whereas at the acute stage IPC did not lead to lower incidence of ischemic arrhythmias. Our experiments have demon-

strated that IPC in animals with chronic stage of T1DM does not lead to the further limitation of the infarct size, i.e. IPC does not potentiate the effects of metabolic preconditioning.

Thus, obtained data suggest the existence of metabolic myocardial preconditioning in experimental T1DM, but do not support the idea that myocardial tolerance to ischemia can be enhanced by ischemic preconditioning in diabetic animalsa.

Summary

The data on myocardial tolerance to ischemia in the animals with experimental diabetes are controversial. In our study, myocardial sensitivity to ischemia and infarct-limiting effect of ischemic preconditioning have been investigated in the in vivo rat model of myocardial infarct in alloxan-induced insulin-dependent diabetes mellitus. It has been shown that 6 weeks after alloxan injection in the diabetic rats infarct size determined by TTC staining was significantly smaller than in healthy controls (39,8±8,8 and 62,3±6,6%, respectively, P<0.01). Also, occurrence of ischemic tachyarrhythmias was smaller in diabetic rats than in controls. Single episode of ischemic preconditioning in diabetics showed much less protection against infarction than in controls. Therefore, the data obtained support the existence of endogenous protective myocardial phenotype in diabetes, although the effectiveness of ischemic preconditioning in diabetes is reduced.

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Editor's Column

San Diego, here we come!

It is nearing time for the 40th annual Society for Neuroscience meeting. It is always a pleasure to meet in San Diego. What a wonderful town and I am sure it will be a wonderful time for all those attending. As many of you prepare to go to San Diego, some of us can remember the first meeting in Washington, DC in 1971. We could not imagine then what a large and diverse group the Society for Neuroscience would become. What we did know was that the discipline of physiological psychology needed a home. It seemed to many of us that the study of the brain and spinal cord as they related to behavior were being abandoned by other psychological societies. I am sure that there were other disciplines that were feeling the same way as they began to delve more deeply into the relationship between their areas and behavior. As we now know, the fledgling society blossomed with a speed and dynamic that none of us who were original members could have foreseen. Now we look forward to what could be the largest and most dynamic meeting yet. In fact some decry the sheer size of the meeting. However, it seems to me that the size is one of the very strengths of the meeting; if you are interested in expanding your research or contacts into another area of the neurosciences, you can find someone there to help.

The David Kopf Lecture on Neuroethics, now in its sixth year, is a very special event at the meeting., The lecture this year will be presented by Henry T. Greely, JD of Stanford University. His topic will be "The Neuroscience Revolution and Society". The lecture will be on Monday, November 15 from 10-11:10 am. The society leadership has been very successful in picking outstanding lecturers for this series, and this year is certainly no exception. We all need to be more aware of how our work and efforts affect society, as more secrets of the nervous system and its control of behavior are uncovered. We also have to be aware that there are those who feel that the science of brain and behavior is a threat and serves to undermine human dignity. We have to find better ways to counter this thinking and to advance public knowledge of what our science is doing. We encourage you to attend this outstanding lecture.

The present Carrier article is written by three of our Russian colleagues and presents some of their work on cardiac ischemia and diabetes. We thank them for sharing their work with us. As you know, David Kopf Instruments has been publishing the Carrier since 1973, which makes it the oldest such scientific newsletter in the field. All issues are available online at the company website, www.kopfinstruments.com/

If you are coming to the meeting, please stop by the Kopf booth (1213) to say hi and to look at the great line of stereotaxic instruments and accessories. The company is adding new items regularly and making improvements based on your feedback regularly. If you have a suggestion (or even a complaint), need advice on how to use an item, or just want to renew friendships with some of the company people, we would really like to see you. Remember, the Kopf line of stereotaxic products is the oldest and largest in the world and continues to serve the neuroscience community with the best, most reliable stereotaxic products available.

Here in Florida, we have had a very active hurricane season thus far, but fortunately, none have come close to us. The last one, Nicole, threatened us, but then went by well out to sea before really dumping a lot of rain on North Carolina and on up to New York. We hope that the prevailing winds blowing these storms up into the Atlantic where they do no harm continue for two more months. In any

event, we are having a stretch of beautiful weather and hope it is like this in San Diego. Hope to see you there.

Should anyone reading this want to write an article for the Carrier, please contact me or the Kopf Company for any help. We will publish articles on various topics, including history of neuroscience, neuroethics, neuroscience techniques as well as interesting data based articles. The Kopf Instrument Company has generously supported the Carrier for over 36 years and continues to make the articles available to the entire community through their web site. All of us in the Neuroscience community are grateful for this support.

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