Taken as a whole this means that the powerful antiarrhythmic effect of EDHPI can be realized in the absence of its negative chronotropic effect on muscarinic receptors, it is a primary effect of the compound on the myocardium, and it requires further study by methods of molecular pharmacology.

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# ROLE OF THE PROSTACYCLINE-THROMBOXANE SYSTEM IN MECHANISMS PREVENTING ARRHYTHMIAS INDUCED BY CORONARY OCCLUSION IN ADAPTED RATS


**L. N. Maslov, I. G. Khaliulin, G. Ya. Dvurechenskaya, N. A. Barabash, and Yu. V. Lishmanov**



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**KEY WORDS:** adaptation, arrhythmia, prostacycline, thromboxane.


The possibility of prevention of arrhythmias induced by acute myocardial ischemia (AMI) by preliminary adaptation to the periodic action of short periods of immobilization, physical exercises, and high-altitude hypoxia has been demonstrated [1-3]. According to Meerson's concept, an important role in the mechanism of the effect of adaptation is played by activation of the stress-limiting systems (SLS), to which it belongs and, in particular, to the prostaglandin system [2]. The opinion is held that, of all the prostaglandins, the most important role in the pathogenesis of cardiac arrhythmias in AMI is played by thromboxane $A_2$ ($T \times A_2$) [5, 8, 9]. Meanwhile, it has been shown that the physiologial antagonist of $TxA_2$ is prostaclcyline (PC), which dilates the coronary vessels and prevents the onset of arrhythmias after coronary artery occlusion [5]. $TxA_2$, on the other hand, induces coronary spasm and a disturbance of the cardiac rhythm [5, 9]. These observations have led many investigators [5, 8, 9] to consider that the PC/Tx ratio plays, if not the chief, then at least a leading role in the development of arrhythmias associated with AMI.

The aim of this investigation was to analyze the effect of different types of adaptation on the value of the PC/Tx ratio and the frequency of arrhythmias in experimental coronary arterial occlusion.

## EXPERIMENTAL METHOD

Experiments were carried out on 60 male Wistar rats weighing initially 200-250 g. The animals were divided arbitrarily into four groups: 1) intact, 2) adaptation to cold, 3) to physical exercise (swimming), 4) to a combination of exposure to cold and


Laboratory of Radionuclide Methods of Investigation, Research Institute of Cardiology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR.


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TABLE 1. Effect of Various Types of Adaptation on Cardiac Arrhythmias in Conscious Animals with Acute Ischemia

| Parameter | Group of animals | | | |
|---|---|---|---|---|
| | 1- ($n$=20) | 2- ($n$=15) | 3- ($n$=13) | 4- ($n$=20) |
| Extrasystoles | 13 | 9 | 6 | 10 |
| Ventricular tachycardia | 13 | 9 | 6 | |
| VF | 16 | 8 | 7 | 8* |
| Animals dying | 14 | 8 | 6 | 6* |
| Mean duration of VF calculated per animal of the group (M ± m, sec) | 81,6± ±15,6 | 78± ±14,6 | 54,8± ±12,1 | 37,9± ±11,6** |

**Legend.** Significance of differences from control: $*p < 0.05$ (by Wilcoxon—Mann—Whitney test), $**p < 0.05$ (by Student's test); $n$) number of experimental animals.

TABLE 2. Effect of Adaptation and AMI on $TxB_2$ and PC — PG-6-keto-$F_{1\alpha}$ Level ($M \pm m$)

| Group of animals | Zone of myocardium | Heart | | | Blood plasma | | |
|---|---|---|---|---|---|---|---|
| | | 6-keto-PG, ng/g | $TxB_2$, ng/g | PC/Tx | 6-keto-PG, ng/ml | $TxB_2$, ng/ml | PC/Tx |
| 1- | | 34,0±1,7 $n$=10 | 23,5±1,1 $n$=10 | 1,44±0,05 $n$=10 | 3,0±0,1 $n$=7 | 1,6±0,1 $n$=6 | 1,27±0,05 $n$=8 |
| 2- | | 32,0±3,7 $n$=10 | 24,8±0,4 $n$=10 | 1,45±0,10 $n$=10 | 1,5±0,2 $n$=5 | 1,5±0,2 $n$=5 | 1,34±0,15 $n$=5 |
| 3- | | 36,2±0,9 $n$=9 | 23,7±1,4 $n$=10 | 1,46±0,05 $n$=9 | 3,5±0,2 $n$=5 | 1,5±0,2 $n$=6 | 2,11±0,30* $n$=5 |
| 4- | | 41,1±1,1** $n$=5 | 23,2±1,8 $n$=8 | 1,66±0,05* $n$=5 | 3,9±0,2* $n$=5 | 1,7±0,2* $n$=6 | 1,64±0,12* $n$=5 |
| 5- (AMI control) | Nonischemic | 32,4±1,7 $n$=10 | 21,3±1,6 $n$=10 | 1,35±0,05 $n$=9 | 2,6±0,1** $n$=7 | 2,2±0,1* $n$=8 | 1,01±0,05* $n$=7 |
| | Ischemic | 23,3±1,8*** $n$=6 | 23,3±1,9 $n$=7 | 1,00±0,05*** $n$=5 | | | |
| 6- (adaptation to swimming and to cold + AMI) | Nonischemic | 30,4±2,5*4 $n$=8 | 19,5±1,6 $n$=8 | 1,43±0,12*4 $n$=8 | 2,4±0,2 $n$=8 | 2,0±0,2 $n$=6 | 1,38±0,09 $n$=6 |
| | Ischemic | 30,6±3,3*4 $n$=9 | 21,3±3,3 $n$=8 | 1,48±0,08 $n$=5 | | | |

**Legend.** $n$) number of animals; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared with control; $****p < 0.01$ compared with group 4.

physical exercise. Adaptation to cold was carried out by exposing the rats in a refrigerator at 1-2°C. On the 1st day the rats were kept in the refrigerator for 30 min, and each successive day the duration of exposure was increased by 30 min. On the 6th day and on all subsequent days, the duration of stay of the rats in the refrigerator was 3 h. Adaptation to physical exercise was carried out by making the animals swim in water at 32°C. The duration of swimming on the 1st day was 10 min, and it was increased by 10 min on each successive day. On the 6th and all subsequent days the duration of swimming was 1 h.

Rats of group 4 were subjected to cold and physical exercise of the same intensity and duration as during exposure to each factor separately. Exposure to cold alternated with swimming at intervals of 1 day, and as a result the rats were put in the refrigerator and exposed to physical exercise for 3 days during the week. All experimental procedures were repeated 6 times a week, and the total duration of adaptation was 60 days. At the end of the period of adaptation, AMI was induced in several animals of each group by occlusion of the left coronary artery without general anesthesia, and without thoracotomy, by the method in [7]. The ECG was observed and recorded continuously for 20 min, after which the animals were decapitated, and samples of blood and myocardial tissues were taken and quickly frozen in liquid nitrogen. To inhibit prostaglandin (PG) synthesis in vitro blood was collected into test tubes containing the sodium salt of EDTA in a final concentration of 20 $\mu$M and indomethacin 50 M. Immediately after the blood samples were taken they were centrifuged in a refrigeration centrifuge at 2000g

for 15 min. PG were extracted from the blood plasma and myocardial specimens by the usual methods [6]. The content of the stable PC metabolite PG-6-keto-$F_{1\alpha}$ and of the end product of $TxA_2$ metabolism, namely thromboxane $B_2$ ($TxB_2$) was determined with the aid of commercial RIA kits from the Isotope Institute, Hungary. The results were subjected to statistical analysis by Student's *t* test and the nonparametric Wilcoxon—Mann—Whitney test.

## EXPERIMENTAL RESULTS

It will be clear from Table 1 that adaptation to the combined periodic action of cold and swimming led to a significant reduction in the frequency and duration of ventricular fibrillation (VF) during the first 20 min after coronary arterial occlusion. In animals exposed to this combined form of adaptation, mortality was significantly lower after coronary occlusion, when it is known to be mainly due to irreversible VF. Adaptation of the rats by exposure to one of these factors (either cold or swimming) was not accompanied by a decrease of mortality and it did not affect the frequency or duration of arrhythmias in rats with coronary occlusion compared with the control. Thus of the types of adaptation which were chosen, it was only the use of combined periodic action of the two different stressors that was effective for prevention of ventricular arrhythmias in AMI. It follows from Meerson's concept [2] that it is in such animals that we must expect the strongest activation of SLS and, consequently, the greatest increase in the value of PC/Tx ratio.

It will be clear from Table 2 that it was only in animals with combined adaptation to the two stressors that the PC/Tx ratio for myocardial tissue was significantly higher than in intact animals. The same increase in the value of this parameter was observed in the peripheral blood of this group of animals. The increase in the PC/Tx ratio took place on account of an increase in PC synthesis, as shown by an increase in the plasma concentration of its stable metabolite PG-6-keto-$F_{1\alpha}$ by 33% compared with the group of intact rats (Table 2). This accumulation of PC in animals adapted to the combined action of two stressors could be both the result of intensification of PC synthesis and of a decrease in the catabolism of this compound, but our results do ot provide the answer to this question.

Of the other types of adaptation, only physical exercise led to a significant increase of 1.7 times in the PC/Tx ratio, but only in the peripheral blood (Table 2).

Under conditions of experimental coronary arterial occlusion a significant decrease in the value of the PC/Tx ratio was observed in the unadapted animals both in the blood plasma and in the zone of ischemia (Table 2). This decrease in the value of this ratio took place on account of an increase in the concentration of $TxB_2$ in the blood with a parallel decrease in the concentrations of PG-6-keto-$F_{1\alpha}$ in the blood plasma and myocardium (the zone of ischemia). The inhibition of PC synthesis which we found in the unadapted animals was evidently the result of excessive activation of the sympathicoadrenal system in AMI [2], for catecholamines are known to inhibit PC synthesis [4]. In the experimental animals adapted to a combination of cold and swimming the PC/Tx ratio after coronary arterial occlusion, although depressed compared with the adapted rats without AMI, did not differ significantly from the value of this ratio in intact animals (Table 2). It can be postulated that this was one factor leading to a decrease in the frequency of arrhythmias after coronary occlusion, because the greater resistance of animals with a high PC/Tx ratio to the arrhythmogenic action of AMI has been reported in the literature [5]. The increase in the plasma $TxB_2$ concentration of unadapted animals under conditions of ischemia is in agreement with data in the literature [8] and, in the opinion of the workers cited, it is the result of stress-induced activation of lipolysis and an increase in accessibility of the substrate (free arachidonic acid) for the process of $TxA_2$ formation.

Thus, adaptation to the combined action of cold and swimming is the most effective means of preventing arrhythmias and reducing mortality in experimental animals with AMI. In rats adapted to the isolated action of cold or swimming, resistance of the heart to the arrhythmogenic action of ischemia was not increased. The fact that the highest values of the PC/Tx ratio in the myocardium were observed only in rats trained by the combined method, and that it was only very slightly above the control level when only one of the adaptive factors was used, indicates the need for choice of the method of adaptation with optimal intensity, so as to activate SLS and, in particular, PC synthesis, on the one hand, while at the same time not causing stress-induced heart damage.

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# ROLE OF GANGLION NODOSUM NEURONS IN COMPENSATORY RESPIRATORY MECHANISMS IN ACUTE MYOCARDIAL ISCHEMIA

**S. D. Mikhailova, T. M. Semiushkina, and N. A. Bebyakova**

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In myocardial ischemia compensatory mechanisms not only of the cardiovascular, but also of the respiratory system are activated. The realization of these compensatory processes is usually linked with changes in the blood gas composition and hemodynamics and with stress reactions [4, 6-9]. However, it is not yet clear what role is played in these compensatory reactions by different components of the afferent apparatus of the cardiovascular and respiratory systems. It was shown previously under artificial respiration conditions that changes in spike discharges of the integrative neurons of the ganglion nodosum, receiving afferent information from the reflexogenic zones of the heart and lungs, arise immediately after restriction of the coronary blood flow [1].

The aim of this investigation was to study under natural breathing conditions the character of changes in spike activity of different kinds of ganglion nodosum neurons during the development of myocardial ischemia, complicated or not by ventricular fibrillation.

## EXPERIMENTAL METHOD

Experiments were carried out on 74 male and female cats weighing 3-4 kg, anesthetized with pentobarbital (40 mg/kg, intraperitoneally). Myocardial ischemia was induced by the method in [2]. The animals were switched to natural breathing by airtight closure of the chest wall, with careful suturing of the pericardium to the thoracic muscles. Activity of ganglion nodosum neurons was recorded extracellularly with glass microelectrodes by the method described previously [10]. The spike discharge, the ECG in standard lead II, the blood pressure in the femoral artery, and the pneumogram were recorded on an M-42 myograph ("Medicor"). These parameters were recorded on magnetic tape by SDR-41 tape recorder ("Nihon Kohden") and in a parallel procedure, on RF-3 70-mm film with an MR-4 photographic recorder ("Medicor"). Activity of 44 ganglion nodosum neurons was analyzed: 31 integrative neurons (late inspiratory, continuous with respiratory modulation, cardio-pulmonary, and inspiratory-expiratory), five complete inspiratory and eight cardiovascular neurons. The numerical results were subjected to statistical analysis by Student's, chi-square, and signs tests.

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