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CARDIAC cAMP AND cGMP DYNAMICS IN RATS WITH TRANSIENT CORONARY INSUFFICIENCY

L. F. Litvitskii, L. I. Vinnitskii, I. L. Zhidkov, M. B. Zuev, and N. T. Vorob'eva

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The principles and role of changes in the content of cyclic nucleotides (CN) — cAMP and cGMP — in the myocardium during ischemia and hypoxia of that structure have been studied intensively in recent years [1, 11-15]. However, no publications could be found relating to the study of the dynamics of CN concentrations in the heart in coronary ischemia and during the period of subsequent restoration of the coronary blood flow. Yet such information is of great interest not only to experimental, but also to clinical cardiology, for in most cases ischemic heart disease is manifested as transient coronary insufficiency (TCI), characterized by resumption of coronary perfusion after a period of myocardial ischemia (MI) of a certain duration. TCI in man occurs in three principal forms: angina, an intermediate coronary syndrome, and states after surgical (aortocoronary bypass) or drug-induced (thrombolysis) myocardial revascularization in the acute stage of infarction [2, 6, 10]. It will be recalled that disturbances of regulation of the contractile function and rhythm of the heart in animals and in man are observed both in the period of MI and in the early stage of postischemic reperfusion (PIR) [2, 3, 6].

Previously the writers discovered that the dynamics of heart function in TCI depends essentially not only on the degree and scale of myocardial damage, but also on the character of extra- and intracardial regulatory influences on it [5, 6, 8]. At the cardiomyocyte level the realization of these controlling influences is effected largely through cAMP and cGMP. This applies most of all to extracardial adrenergic and cholinergic mechanisms of regulation, whose activity regularly changes in TCI [5, 8].

Department of Pathophysiology, I. M. Sechenov First Moscow Medical Institute. Laboratory of Hormones, All-Union Scientific Center for Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Malinovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 1, pp. 37-39, January, 1984. Original article submitted January 18, 1983.

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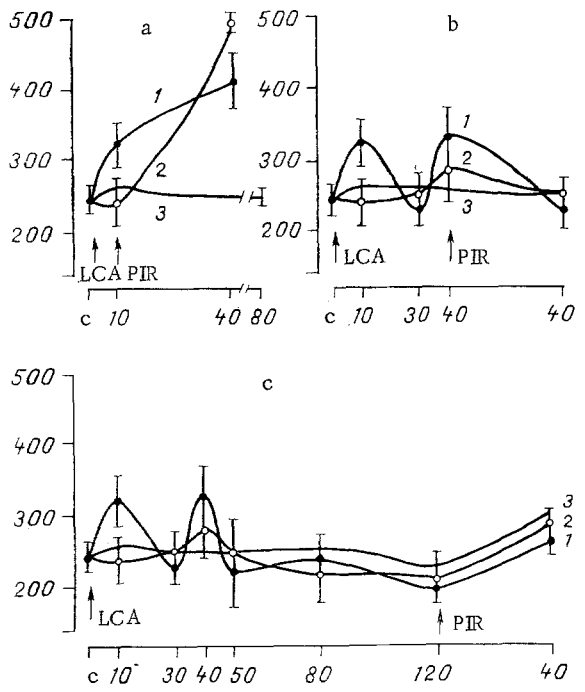

Fig. 1

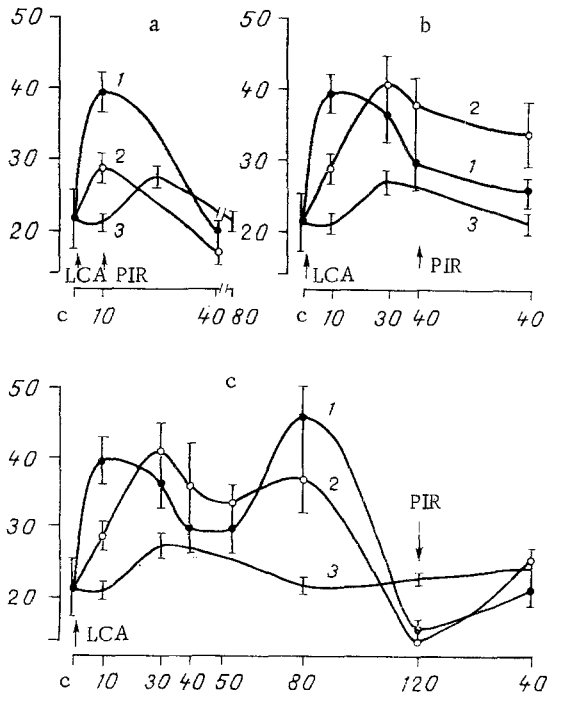

Fig. 2

Fig. 1. Dynamics of cAMP concentration in heart during TCI (M ± m). Abscissa, duration of periods of MI and PIR (in min); ordinate, cAMP concentration in myocardium (in pmoles/g tissue). a) TCI with duration of MI 10 min, b) 40 min, c) 120 min. 1) cAMP concentration in zone of MI followed by PIR, 2) in areas of heart distant from zone of MI and PIR, 3) in rats undergoing mock operations. LCA) Ligation of coronary artery, PIR) beginning of period of PIR.

Fig. 2. Dynamics of cGMP concentration in heart in TCI (M ± m). Ordinate, cGMP concentration in myocardium (in pmoles/g tissue). Remainder of legend as to Fig. 1.

In connection with the facts described above, the aim of the present investigation was to study the dynamics of the cAMP and cGMP content in the heart during local ischemia of varied duration, of coronary genesis, and in the subsequent reperfusion period.

EXPERIMENTAL METHOD

Experiments were carried out on 127 noninbred male albino rats weighing 200 ± 10 g. TCI was induced by temporary occlusion of the coronary artery by a ligature as described previously [2, 7], under urethane anesthesia (1200 mg/kg) and with artificial ventilation of the lungs with atmospheric air. In animals undergoing the mock operation the ligature was passed beneath the coronary artery but was not drawn tight. Myocardial tissue was excised with cold scissors from the center of the zone of MI and RIP, and also from distant areas of the heart, and quickly placed in a vessel containing liquid nitrogen. The CN content was determined after 10, 20, 30, 40, 50, 60, 80, 120, and 160 min of MI and also after 40 min of RIP by radioimmunoassay using a kit of reagents from Amersham Corporation (England). At each time point of the experiment material was taken from 8-12 animals.

EXPERIMENTAL RESULTS

The time course of the CN concentration in the heart in TCI revealed a series of regular stages. In the zone of MI biphasic changes were observed in the cAMP and cGMP concentrations: an increase in the CN content during the first 30-40 min of MI followed by a decrease (toward the 50th-80th minutes). Two states of affairs were discovered. First, in the early stage of MI (the first 50-80 min) the dynamics of the cGMP content was more mobile in character than that of cAMP. This was shown, in particular, by the fact that the cGMP concentration rose more rapidly and by a greater degree. For instance, toward the 10th minute of MI the cGMP concentration was increased by 82% compared with the background, and the cAMP level was raised by 33%. Second, in the late stages of MI (120-160 min) the cGMP concentration in the

heart fell much more than the cAMP level. For instance, by the 160th minute of MI the cGMP concentration was 20% higher, but the cAMP concentration was 29% lower than the background level. These facts suggest greater mobility and resistance of the guanylate cyclase system of the cardiomyocytes than of the adenylate cyclase system under MI conditions. This is in agreement with our own observations showing the greater resistance of the cholinergic than of the adrenergic mechanisms in the regulation of cardiac function in TCI [3, 6].

In areas of the heart distant from the zone of MI the CN content also rose during the first 30-40 min and then fell. The cAMP concentration had fallen below its initial value by the 80th minute, but returned to it again by the 120th-160th minute. The cGMP content remained 1.5-2 times higher than the initial level.

The dynamics of the CN concentration in the heart during PIR differed depending on the duration of preceding MI. The cAMP concentration during reperfusion after MI lasting 10 min (Fig. 1) continued to rise, and by the 40th minute of PIR it exceeded the ischemic level by 17.3%. This evidently reflects to some degree stimulation of the adenylate cyclase complex of the cardiomyocytes by adrenalin, which accumulates, as the writers showed previously [2, 5], in large quantities in the zone of PIR. Reperfusion after a longer period (40 min) of MI was accompanied by a fall in the cAMP concentration by 31.7% below that recorded at the 40th minute of MI. This fact can be largely explained by loss of cAMP by the myocardial cells as a result of a considerable reperfusion alteration. This is due to several factors. The most important of them, it must be accepted, are injury to the membranes and an increase in their "flowability" under the influence of activated phosphorylases, products of lipid peroxidation, which is considerably intensified in this type of TCI; and also the introduction of free fatty acids and lysophospholipids into the lipid bilayer of the membranes and destruction of a large number of reperfused myocardial cells as a result of their "osmotic" swelling and rupture, due to the accumulation of sodium and calcium ions and, consequently, of fluid in the cardiomyocytes [4, 6, 9].

Resumption of the coronary blood flow after prolonged (120 min) MI was characterized by an increase of 33.9% in the cAMP concentration compared with the period of MI. This fact can be largely attributed to the development of the phenomenon of nonrecovery of the blood flow. It is characterized by a marked decrease in the microcirculation of blood in the previously ischemic myocardium (mainly in its central areas) during PIR [12], which may prevent the flushing out of metabolites, including cAMP from it. Meanwhile during the period of PIR after prolonged (120 min) MI the adrenalin concentration in the heart rises [2, 5], and this evidently also stimulates cAMP formation in the heart.

The time course of the cGMP concentration (Fig. 2) during PIR after 40 and 120 min of MI changed similarly and coincided in direction with changes in the acetylcholine concentration in the heart [3, 6, 8]. PIR after 10 min of MI was accompanied by a fall in the cGMP concentration to close to the background level. This last effect was evidently due to predominance of adrenergic influences on the heart in this form of TCI, as the writers showed previously [2, 3, 5]. In areas of the heart remote from the zone of PIR the concentrations of both cAMP and cGMP follows the same time course as in the PIR zone, and differed only quantitatively (Figs. 1 and 2).

On the whole the results of the investigations revealed a regular pattern of time course of the CN content in the heart during local ischemia and subsequent PIR under TCI conditions. During MI biphasic changes were observed in the CN concentration: An initial considerable increase was followed by a decrease. The dynamics of the cGMP concentration was more mobile than that of cAMP. Postischemic restoration of the coronary blood flow as a rule was not accompanied by restoration of the normal CN content in the heart in the early stage of the PIR period (the first 40 min) and depended on the duration of preceding MI. The character of the change in CN concentration in the heart during TCI coincides to a large extent with the dynamics of activity of adrenergic and cholinergic mechanisms of regulation of cardiac activity described by the writers previously [5, 6, 8] and also with the contractile function of the heart and its rhythm under TCI conditions [2, 3].

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CHANGES IN MITOCHONDRIAL CYTOCHROME COMPOSITION AND FUNCTION IN THE ISCHEMIC HEART

R. V. Balaševičius, A. I. Toleikis, and A. K. Praškevičius

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A decrease in the level of cytochromes $a + a_3$ and c (or $c + c_1$) has been observed in mitochondria isolated from the ischemic heart [9] and liver [8]. However, no change in cytochrome oxidase activity was found during ischemia [1, 8, 10]. The contradictory nature of functional and quantitative data may be attributable to differences in the experimental conditions, due mainly to the variety of methods of oxidation and reduction of cytochromes and incomplete separation of the peaks of individual cytochromes at room temperature.

In this investigation the effect of ischemia on the quantitative composition of cytochromes in isolated cardiac mitochondria was studied by differential spectrophotometry at the temperature of liquid nitrogen; derivative spectra of the fourth degree also were recorded, which give much better separation of the cytochrome peaks with overlapping absorption bands [5]. Dependence of oxidation of succinate on exogenous cytochrome c was studied in a parallel investigation.

EXPERIMENTAL METHODS

Rabbits weighing 2.5-3.5 kg were used. The composition of the cytochromes and activity of succinate oxidation were studied in mitochondria isolated from the heart. Ischemia was produced by autolysis of heart tissue. The heart was washed in ice-cold (0°C) 0.9% NaCl solution and divided into two parts: One part acted as the control (mitochondria were isolated immediately after washing), whereas the other part, wahsed in warm (37°C) 0.9% NaCl solution, was subjected to autolysis for 2 h at 37°C in a humid chamber [4]. Homogenization was carried out in a glass-Teflon homogenizer. Mitochondria were isolated by differential centrifugation in medium containing 0.18 M KCl, 2 mM EDTA, pH 7.0, at 0-4°C [7] and suspended in the same medium. Differential spectra of the cytochromes were recorded at the temperature of

Laboratory of Metabolism, Research Institute of Physiology and Pathology of the Cardiovascular System, Kaunas Medical Institute. Department of Biological and Organic Chemistry, Kaunas Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Z. I. Januškevičius.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 1, pp. 40-42, January, 1984. Original article submitted March 3, 1983.

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