with the normalizing action of cathergen on autoregulation in the "mast cell–microvessel" system. Administration of cathergen lowered the intensity of degranulation of the mast-cell apparatus, with an increase in the proportion of 0-forms of mast cells (Table 1; Fig. 2). It can be tentatively suggested that cathergen has an angioprotective action when the circulation of the blood is disturbed.

ACID HYDROLASE ACTIVITY AND CYCLIC NUCLEOTIDE CONTENT IN THE RAT HEART DURING MYOCARDIAL ISCHEMIA AND POSTISCHEMIC REPERFUSION

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Myocardial ischemia causes significant changes in cyclic nucleotide levels [2, 6, 13], destabilizes lysosomal membranes, reduces the latency of lysosomal enzymes, and enhances hydrolase activity in the cell cytoplasm [3, 6, 8, 12]. Cyclic nucleotides play an important role in the regulation of the state of the lysosomal membranes; cAMP, moreover, has a stabilizing action on lysosomal membranes whereas cGMP has the opposite effect [1, 4, 5, 9]. Since methylxanthines, by blocking phosphodiesterase [5], may lead to an increase in the cAMP concentration in the cardiomyocytes, it was thought worthwhile to use substances of this group to study the possible mechanisms of damage to lysosomal membranes in myocardial ischemia and to develop methods of correcting these lesions. In the light of the view that damage to cardiomyocytes is aggravated on restoration of the blood flow along the coronary vessels after myocardial ischemia lasting about 40 min or more [2, 3, 10, 11], there is also an urgent need for a study of the effect of methylxanthenes on cyclic nucleotide concentrations and activity of lysosomal enzymes during postischemic reperfusion.

The aim of this investigation was to study activity of lysosomal hydrolases and cyclic nucleotide levels in tissues of the heart associated with ischemic damage to the myocardium and during the period of postischemic reperfusion, and also to examine the effects of caffeine, a phosphodiesterase blocker, on the state of the lysosomes and cyclic nucleotide levels.

EXPERIMENTAL METHOD

Experiments were carried out on 60 isolated hearts of noninbred male albino rats, perfused by Langendorff's method. The hearts were perfused with Krebs' solution, aerated with a gas mixture consisting of 95% O_2 and 5% CO_2 (temperature 37°C, pH 7.4, perfusion flow rate 10 ml/min, isovolumic cardiac contractions). In the experiments of series I, after perfusion for 20 min under the conditions described above, the perfusion rate was reduced to 1 ml/min, and 40 and 60 min after the beginning of ischemia, tissues of the left ventricle were removed for investigation. In some experiments, after 40 min of ischemia, reperfusion was carried out in the course of 20 min, the perfusion rate being increased to its initial value. In the experiments of series II, 30 min after the beginning of ischemia the hearts were switched to perfusion with Krebs' solution containing 50 μM of caffeine (from "Serva," USA). The conditions of perfusion of the control hearts remained unchanged throughout the experiment. Concentrations of cAMP and cGMP in tissues of the left ventricle were determined by radioimmunoassay using kits from the firm "Amersham International" (England). Activity of cathepsin D and acid phosphatase was determined spectrophotometrically [7]. Two forms of hydrolase activity were investigated: the so-called free activity (FA), i.e., activity of the enzyme in supernatant obtained by centrifugation of the homogenate at 30,000g for 30 min, and bound (lysosomal) activity (LA), or activity of the enzyme in the lysosome-rich fraction obtained by differential centrifugation followed by treatment of the lysosomal fraction to release the bound enzyme with Triton X-100 in a final concentration of 0.1%.

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TABLE 1. cAMP and cGMP Levels in Tissue of Left Ventricle, pmoles/g Tissue

Procedure	cAMP	cGMP
Perfusion, 40 min	350,8±26,8	25,4±1,8
Ischemia, 40 min	486,1±37,1*	33,5±5,0*
Perfusion, 60 min	304,2±30,6	23,5±3,5
Ischemia, 60 min	443,7±38,8*	26,5±2,1
Ischemia, 60 min + caffeine	567,7±47,6***	28,2±1,7
Ischemia, 40 min + 20 min reperfusion	416,3±32,2*	29,2±1,1*
Ischemia, 40 min + 20 min reperfusion + caffeine	530,0±47,5***	26,7±2,0

Legend. Here and in Table 2: *) results differing significantly from control, ***) differing significantly from results obtained during perfusion without caffeine. Number of observations at each point 6-12.

TABLE 2. FA and LA of Acid Phosphatase and Cathepsin D in Tissue of Left Ventricle, nmoles/min/g Tissue

Procedure	Acid phosphatase		Cathepsin D	
	FA	LA	FA	LA
Perfusion, 40 min	237,0±24,2	877,2±76,1	222,2±12,7	852,5±60,8
Ischemia, 40 min	458,0±17,6*	445,4±29,0*	229,2±9,3	714,8±28,5*
Perfusion, 60 min	269,0±6,5	869,8±33,2	208,8±6,8	840,0±54,7
Ischemia, 60 min	428,4±44,8*	288,7±19,5*	253,0±18,4*	741,0±22,9*
Ischemia, 60 min + caffeine	285,0±25,9**	642,8±29,2***	230,6±7,8*	765,4±23,0
Ischemia, 40 min + 20 min reperfusion	376,6±9,4*	263,3±24,4*	240,6±11,9*	566,9±38,2*
Ischemia, 40 min + 20 min reperfusion + caffeine	252,6±16,9**	626,0±18,8***	228,5±4,5*	692,9±37,3***

EXPERIMENTAL RESULTS

The cAMP concentration (Table 1) increased considerably after 40 min of ischemia. After 60 min of ischemia and reperfusion the cAMP level fell a little, but still exceeded the control value significantly. The cGMP concentration also rose after 40 min of ischemia and reperfusion, but after 60 min of ischemia it did not differ significantly from the control. Perfusion with caffeine led to a marked increase in the cAMP (but not the cGMP) concentration during ischemia and reperfusion.

FA of acid phosphatase (Table 2) almost doubled after 40 min of ischemia, remained at the same level after 60 min of ischemia, and after reperfusion it exceeded the control values by about 40%. LA of the enzyme after 40 min of ischemia was only half, and after 60 min of ischemia and reperfusion about one-third of the control values. Against the background of caffeine, FA of acid phosphatase during ischemia and reperfusion did not differ from that in the control; LA during ischemia and reperfusion was lower than in the control, but significantly higher than in experiments using Krebs' solution without caffeine.

FA of cathepsin D increased after 60 min of ischemia and reperfusion; caffeine limited the rise of FA a little, but the differences were not statistically significant. A decrease of LA took place after only 40 min of ischemia, but with an increase in the duration of ischemia, it virtually did not progress. Reperfusion caused a further fall of LA. Caffeine had hardly any effect on LA during ischemia but reduced the degree of its fall during reperfusion.

The changes discovered point to increasing destabilization of the lysosomal membranes and a progressive increase in the concentration of lysosomal enzymes in the cytosol during ischemia, and also aggravation of damage to lysosomes during reperfusion. The lowering of FA of acid phosphatase during reperfusion may have been due to outflow of the enzymes from the cardiomyocytes as a result of a considerable increase in permeability of the sarcolemma.

Against the background of perfusion with caffeine there was an increase in the resistance of the lysosomal membranes to ischemic and reperfusion damage. Allowing for the rise of the cAMP level in the myocardium and an increase in the cAMP/cGMP ratio it can be concluded

that phosphodiesterase blockade leads to stabilization of the lysosomes of the cardiomyocytes of the damaged myocardium.

This conclusion conflicts with ideas on the role of catecholamines, cAMP, and Ca^{++} in the pathogenesis of ischemic heart damage [3], but it is in agreement with results [9] demonstrating the marked antiarrhythmic effect of caffeine during ischemia and reperfusion.

As has been pointed out in the literature [8], in the concentration which we used, caffeine does not affect the contractile function of the myocardium or, probably, intracellular Ca^{++} metabolism.

The view has been expressed [6] that during ischemia the stabilizing role of cAMP is not realized to the full. It can therefore be tentatively suggested that on account of profound disturbances of metabolism and of the intracellular mechanisms of regulation, the stabilizing effect of cAMP is manifested at higher concentrations of this nucleotide than are usually found during ischemia.

Administration of substances raising the cAMP level in the heart cells and, in particular, phosphodiesterase blockers, may prove to be an effective way of stabilizing the lysosomal membranes of the myocardium during its ischemia and postischemic reperfusion.

LITERATURE CITED

1. B. F. Korovkin, V. I. Gosudarskii, K. K. Zaitseva, et al., Vopr. Med. Khim., No. 3, 403 (1980).
2. P. F. Litvitskii, L. I. Vinnitskii, L. I. Zhidkov, et al., Byull. Éksp. Biol. Med., No. 1, 37 (1984).
3. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Myocardial Damage [in Russian], Moscow (1984).
4. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
5. N. A. Fedorov, The Biological and Clinical Importance of Cyclic Nucleotides [in Russian], Moscow (1979).
6. V. I. Shumakov, N. A. Onishchenko, and V. I. Kirpatovskii, Pharmacological Protection of the Transplant [in Russian], Moscow (1983).
7. A. J. Barret, Lysosomes: A Laboratory Handbook, Amsterdam (1972), p. 46.
8. R. S. Decker, A. R. Poole, J. T. Dingle, and K. Wildenthal, J. Mol. Cell. Cardiol., 11, 189 (1979).
9. L. J. Ignarro and S. Y. Cech, Proc. Soc. Exp. Biol. (New York), 151, 448 (1976).
10. R. B. Jennings and C.-E. Ganote, Metabolism of the Myocardium [Russian translation], Moscow (1975), pp. 325-351.
11. R. B. Jennings, J. Mol. Cell. Cardiol., 12, Suppl. 1, 63 (1980).
12. T. Katagiri, J. Sasai, N. Nakamura, et al., J. Mol. Cell. Cardiol., 12, Suppl. 1, 69 (1980).
13. E. G. Krause and A. Wollenberger, Adv. Cycl. Nucleotide Res., 12, 49 (1980).