CALPAINS AND CATHEPSIN D OF THE INJURED MYOCARDIUM: ROLE OF IODOTHYRONINES

E. A. Stroev and E. A. Ryazanova

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In recent years a leading role in the development of injuries to cardiomyocytes has been ascribed to calcium ions, which exert their action through the system for membrane transport and release of calcium, and also enzyme systems reacting to fluctuations in the concentration of this ion. Observations have shown that iodothyronines acting on systems of Ca^{2+} transport and Ca^{2+}-depots [3-5] modify the intracellular calcium ion concentration, and through this, affect calcium-reacting enzyme systems. These include, in particular, the calpains and lysosomal enzymes. Calpains (EC 3.4.22.17) are Ca^{2+}-activated neutral proteinases, whose proteolytic activity is absolutely dependent on Ca^{2+}. Release of lysosomal proteinases also is a Ca^{2+}-dependent process [2]. However, there are virtually no data on the state of these proteinases during changes in the iodothyronine level or on their role in damage to myocardial cells.

The aim of this investigation was to study regulation of the activity of proteinases (calpains and cathepsin D) by iodothyronines in the normal and damaged myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred male rats weighing 160-220 g. Hyperthyroidism was induced in the intact rats by subcutaneous injection of tri-iodothyronine ("Reanal," Hungary) in a dose of 10 μg/kg, or of L-thyroxine ("Reanal," Hungary) in a dose of 50 μg/kg daily for 7 days. Myocardial damage was simulated in euthyroid and hyperthyroid animals by injection of isoprenaline (Novodrin, VEB, East Germany) subcutaneously in a dose of 120 mg/kg 24 h before sacrifice. Control rats received an injected of a corresponding solvent at the same times. The rats were killed by superficial ether anesthesia. The hearts were homogenized in 8 volumes of 50 mM sodium chloride solution containing 4 mM EDTA, 50 mM Tris-acetate, pH 7.6 [10], in a glass homogenizer of Potter–Elvehjem type, with teflon pestle at 1500 rpm for 90 sec. The homogenate was centrifuged successively at 1500*g* for 10 min and at 30,000*g* for 30 min. The last supernatant was used for determination of activity of the calpain and nonsedimented activity of cathepsin D. The residue was resuspended in 0.1% solution of Triton X-100 ("Ferak," West Germany) for determination of the sedimented cathepsin D activity [11]. All procedures were carried out at 0-4°C. Activity of the myocardial calpains was determined after their preliminary separation from endogenous inhibitors by hydrophobic chromatography on octyl-sepharose C1-4B ("Pharmacia," Sweden) [1]. Alkali-denatured casein (after Hlammersten "Reakhim," USSR) was used as the substrate [8]. Cathepsin D activity in the heart was measured as hydrolysis of hemoglobin ("Reanal," Hungary) in the presence of pepstatin ("Sigma," USA) [7]. The protein concentration was determined by Lowry's method [9]. Blood levels of tri-iodothyronine and thyroxine were estimated by radioimmunoassay using test kits from the Institute of Bioorganic Chemistry, Russian Academy of Sciences. The experimental results were subjected to statistical analysis by Student's t test.

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TABLE 1. Effect of Iodothyronines on Activity of Calpains and Cathepsin D in Heart after Injection of Isoprenaline into Animals (M ± m)

Experimental conditions	Concentration, mmoles/liter		Heart		
	T_3	T_4	calpains	cathepsin D	
				NSA	SA
Control (12)	0.81±0.06	76.91±6.72	1.32±0.10	0.100±0.013	0.399±0.024
Myocardial damage (10)	0.36±0.06*	60.45±9.72*	1.77±0.11**	0.120±0.011*	0.312±0.021
Control (16)	0.81±0.06	65.97±9.06	1.44±0.11	0.213±0.012	0.209±0.015
T_3, 10 μg/kg (10)	1.05±0.09**	—	1.20±0.13	0.116±0.010***	0.206±0.019
T_3 + myocardial damage (10)	1.06±0.13**	43.93±1.27**	1.23±0.07*	0.062±0.010*	0.270±0.018
T_4, 50 μg/kg (10)	1.31±0.05*	106.05±10.30*	1.13±0.09	0.062±0.005*	0.136±0.010*
T_4 + myocardial damage (12)	3.21±0.37*	158.99±10.34*	1.74±0.15*	0.134±0.020*	0.214±0.013

Legend. Proteinase activity expressed in μmoles tyrosine·mg protein^{-1}·h^{-1}; NSA) nonsedimented, SA) sedimented cathepsin D activity. Number of determinations shown between parentheses. Significance of changes compared with control: *$p < 0.05$, **$p \leq 0.01$; ***$p \leq 0.001$.

EXPERIMENTAL RESULTS

Blood levels of tri-iodothyronine and thyroxine in the rats fell by 55.6 and 21.4% respectively 1 day after development of necrosislike changes in the myocardium due to isoprenaline [6]. Under these conditions a marked increase in calpain activity was found in the heart (by 34.1%). Meanwhile the sedimented cathepsin D activity in the myocardium decreased by 21.8%, whereas nonsedimented activity rose by 20.0% (Table 1).

In the intact rats a course of injections of tri-iodothyronine inhibited secretion of endogenous thyroxine, as a result of which only tri-iodothyronines were found in the animals blood, in which their level rose by 29.6%. A tendency was found for calpain activity in the heart to decline. A sharp decrease in nonsedimented cathepsin D activity in the myocardium (by 45.6%) was noted, whereas the sedimented cathepsin D activity was indistinguishable from the control.

In response to a combination of myocardial damage and preliminary injection of tri-iodothyronine the blood level of this hormone was increased by 1.3 times. As a result, calpain activity in the heart was equal to that observed under hyperthyroid conditions. At the same time there was a further decline in nonsedimented cathepsin D activity in the myocardium to 29.1%, and a very small increase in sedimented activity of this enzyme. Incidentally, under the experimental conditions described, effects found for T_3-hyperthyroidism on the whole were preserved.

A course of injections of L-thyroxine into intact rats increased the triiodothyronine and thyroxine concentrations in the blood by 1.6 times. Under these conditions a decrease in proteinase activity was found in the heart: calpains by 21.5%, nonsedimented and sedimented cathepsin D activity by 70.9 and 35.2% respectively.

In the T_4-hyperthyroid animals with myocardial damage blood levels of triiodothyronine and thyroxine were increased by 2.9 and 2.4 times respectively. Activity of calpains in the heart rose by 20.8% compared with the control animals. Nonsedimented cathepsin D activity in the myocardium was reduced by 37.1%, which is similar to the effect found in T_4-hyperthyroidism, but to a less marked degree. Sedimented cathepsin D activity was 1.6 times higher than the hyperthyroid level, having regained the control value.

It can be concluded on the basis of these results that iodothyronine levels in the body affect changes in proteinase activity in the presence of myocardial damage. In euthyroid animals the action of isoprenaline on the myocardium was accompanied by activation of calpains and labilization of lysosomes, which are evidently connected with an increase in the intracellular Ca^{2+} concentration. Tri-iodothyronine and thyroxine inhibit calpains and stabilize lysosomal membranes in the intact rat heart. As a result, the lysosomal system of the myocardium in hyperthyroid animals was more resistant to the damaging action of isoprenaline: in T_4-hyperthyroidism the lysosome-stabilizing effect of the iodothyronines was weakened, whereas in T_3-hyperthyroidism it was strengthened. Activation of calpains in the damaged heart of T_4-hyperthyroid rats also was less marked than in euthyroid rats, whereas in T_3-hyperthyroid rats the inhibitory effect of tri-iodothyronine was preserved. Iodothyroninelin physiologi-

cal concentrations evidently prevent excessive accumulation of Ca^{2+} in the myocardiocytes and, consequently, they prevent realization of the damaging action of isoprenaline.

Changes in proteinase activity found in euthyroid rats with myocardial damage thus evidently do not develop or are weakened by the presence of hyperthyroidism, with the greater activity being exhibited by tri-iodothyronine.

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