DYNAMICS OF NUCLEOTIDE COMPOSITION OF THE MYOCARDIAL RNA IN COMPENSATORY HYPERFUNCTION AND HYPERTROPHY OF THE HEART

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During hyperfunction of differentiated cells, activation of synthesis of nucleic acids and proteins is observed in them [2, 3, 7, 9-11, 15], and serves as the basis for cell growth and proliferation. It is not yet clear whether changes occur in the nucleotide composition of the RNA in these circumstances.

The present investigation was accordingly carried out to study the dynamics of the nucleotide composition of total Na of the heart muscle during prolonged compensatory hyperfunction.

EXPERIMENTAL METHOD

Compensatory hyperfunction of the heart was reproduced in 14 dogs weighing 15-20 kg by the method described previously [4], in which the cross section of the aortic orifice was reduced by half. With this degree of aortic stenosis the strength of the cardiac contractions rose and the systolic pressure in the left ventricle increased by 50-60%. The dogs were sacrificed 4, 7, and 45 days after the beginning of this hyperfunction. Control investigations were made of 9 intact animals and two dogs sacrificed 4 days after application of a ligature to the aorta without causing stenosis. The relative weight of the ventricles of the animals (the ratio between the weight of the ventricles and the body weight of the dog) 4 and 7 days after the beginning of hyperfunction was increased by only 6-12%, but after 45 days the increase reached 30% and was significant, i.e., myocardical hypertrophy had developed.

The heart was taken for investigation from living dogs anesthetized with ether and maintained on artificial respiration, placed in ice-cold physiological saline, washed several times to remove blood, and dried with filter paper, the myocardium of the left ventricle was removed, cut into small pieces, and the fat discarded. The dry defatted weighed sample was freed from acid-soluble compounds and hydrolyzed in 0.5 N KOH solution for 18 h at 37°. The hydrolyzate was neutralized with 50% $HClO_4$ solution to give a final acid concentration in the hydrolyzate of 3%. After centrifugation, the supernatant together with the washings contained the whole of the RNA in the form of mononucleotides. Subsequent treatment took place as described by Schmidt and Thannhauser [16]. The solution obtained was evaporated in cold air to 3-4 ml and used for the quantitative study of the RNA mononucleotides. Chromatographic fractionation of the mononucleotides was carried out by descending chromatography on paper [13], followed by spectrophotometry by Spirin's method [6]. The results of the quantitative analysis of the RNA nucleotides were expressed in molar percentages (the total number of moles of all the nucleotides was taken as 100).

EXPERIMENTAL RESULTS AND DISCUSSIONS

Data for the nucleotide composition of the total myocardial RNA of the left ventricle of the experimental and control animals are given in the table. They show that the ratio between the purine and pyrimidine nucleotides in the total myocardial RNA of all the investigated animals was close to 1. The ratio between the pairs of complementary nucleotides – coefficient of specificity $\left(\frac{G+C}{A+U}\right)^*$ – was 1.86 in the control animals and showed that the total myocardial RNA was RNA of the GC type. The operation of application of

*G – guanine, C – cytidine, A – adenine, U – uridine.

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Nucleotide Composition of Total Myocardial RNA in Compensatory Hyperfunction of the Heart (M ± m)

Experimental conditions and No. of animals	Contents of nucleotides in moles %				Purines / Pyrimidines	$\frac{G+U}{A+C}$	$\frac{G+C}{A+U}$	P for ratio $\frac{G+C}{A+U}$
	G	A	C	U				
Intact animals (9)	32,98±0,50	18,78±0,44	31,38±0,37	16,84±0,26	1,07±0,012	0,99±0,064	1,86±0,091	$P_{I-II} > 0,2$
Mock operation (2)	32,2±0,59	21,4±0,25	29,3±1,30	17,10±1,71	1,15±0,016	0,89±0,019	1,50±0,14	
Four days after stenosis (6)	22,8±1,35	27,4±2,3	31,0±0,59	15,4±1,48	1,08±0,063	0,87±0,071	1,27±0,035	$P_{II-III} < 0,001$
Seven days after stenosis (4)	30,0±0,12	23,0±0,95	28,3±0,22	18,7±0,53	1,12±0,022	0,95±0,036	1,40±0,045	$P_{II-IV} > 0,05$
Forty-five days after stenosis (4)	30,1±1,2	20,1±0,76	33,5±1,35	16,0±1,21	1,01±0,023	0,91±0,014	1,76±0,062	$P_{II-V} > 0,2$

the ligature without causing stenosis of the aorta and hyperfunction of the heart had no effect on the nucleotide composition of the myocardial RNA. Hyperfunction of the heart caused by aortic stenosis, on the other hand, caused substantial changes in the nucleotide composition of the total myocardial RNA.

Four days after the beginning of hyperfunction,the content of guanine nucleotide in the RNA had fallen by 10 mole % while that of adenonine nucleotide had increased by 6 mole %; the coefficient of specificity had fallen correspondingly by 31.8%-1.27. Seven days after the beginning of hyperfunction changes were observed in the nucleotide composition in the same direction. However, quantitatively they were less marked. The coefficient of specificity had fallen by only 24.8%-1.4. Forty five days after the beginning of hyperfunction, the marked hypertrophy was present, the nucleotide composition of the RNA was fully restored to normal—the coefficient of specificity was 1.76.

The main results of the experiment was that in the early stage of hyperfunction and of rapidly developing hypertrophy, marked changes in the nucleotide composition of the myocardial RNA were observed—a change in its nucleotide composition from the GC type toward the AU type. Later, as the process of hypertrophy progressed, the normal nucleotide composition was restored. In a recent investigation by Hyden and Egyhazy [12] it was shown that physiological hyperfunction of the neurons in layers V and VI of the cortex, responsible for the process of learning a particular skill, results not only in activation of RNA synthesis in these cells, but also in changes in the nucleotide composition of the RNA, expressed in particular by a decrease in the ratio $\frac{G+C}{A+U}$, i.e., by a decrease in the coefficient of specificity; during continued hyperfunction these changes disappead comparatively quickly. Hyden and Egyhazy conclude that information reaching the cortical neurons from the external environment is coded in the RNA molecules and this process is expressed by changes in the nucleotide composition of the RNA.

However, the results now described suggest that the change in the nucleotide composition of the RNA toward the AU type is not specific for hyperfunction of the neuron and is not associated with the coding of information arising in the RNA molecules from external environment, but is a characteristic feature of activation of the genetic apparatus of the differentiated cell during an increase in its physiological function.

When the possible mechanism of this change is being assessed, the well-known fact must be remembeered that the ribosomal RNA of animal cells, constituting 80-90% of their total RNA, has a coefficient of specificity of 1.6-1.7, i.e., it belongs to the GC type; the messenger RNA of the same cells belongs to the AU type and has a coefficient of specificity of 0.7-0.75 [1]. This suggests that the marked activation synthesis of nucleic acids and protein, regularly observed in the initial stage of hyperfunction of the heart [5], is accompanied by a change in the ratio between ribosomal and messenger RNA, with an increase in the content of messenger RNA. Subsequently the developing hypertrophy leads to distribution of the increased function of the myocardium among its increased masss: the myocardial function per unit of mass, the intensity of functioning of the myocardial structures, whereupon returns to the normal level and the activation of

nucleic acid and protein synthesis in the myocardium accordingly comes to an end [5]. In this period the content of messenger RNA may fall to the normal level.

This explanation is hypothetical, but at the same time it is in agreement with the results of recent investigation showing that activation of protein synthesis in animal cells regularly involve inclusion of a large proportion of the ribosomes into the polysomes, which are protein-synthesizing complexes of messenger RNA and ribosomes [8, 18]. Probably the incorporation of "free" ribosomes into these complexes is facilitated by an increase in the content of messenger RNA in the cells. On the conclusion of activation of protein synthesis in the cell [14] or on inhibition of RNA synthesis by actinomycin D [17], the polysomes regularly break up into free ribosomes, and this may be accompanied by a decrease in the content of messenger RNA.

Hence, the hypothesis that a relative predominance of messenger RNA may arise and intensive formation of polysomes may take place during the hyperfunction of differentiated cells and the activation of their genetic apparatus clearly requires experimental verification.

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