Effect of Hydra Morphogen Peptide on the Structure of the Tissue Components of the Myocardium Layers in the Early Development of Heart Hypertrophy in Rats

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The peptide factor derived from sea anemones [8] and according to its functional property named the hydra morphogen peptide (HMP), also called the head activator, was later detected in mammals and humans [12]. It has been established that HMP of invertebrates is a growth hormone regulating cell proliferation [11]. The role HMP plays in mammals is still unknown. HMP is known to be capable of stimulating amylase secretion in isolated pancreatic lobes [9] and of activating peptide biosynthesis in regenerating rat liver [1,5]. There are data attesting to accelerated maturation of rats under the influence of HMP [2]. In our view, the reported results may testify that HMP is a peptide regulator which, having no tissue specificity, is active in the stimulation of a developing or a disrupted function. As has already been noted in our previous investigations, the myocardial layers differ morphologically [3] and present an ambiguous reaction to the increased load caused by a narrowing of the aorta [4].

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The aim of this investigation was to study the morphological response of different myocardial layers to a single injection of synthetic HMP.

MATERIALS AND METHODS

The experiments were carried out on 9 male Wistar rats weighing 180-200 g. HMP was synthesized at the Laboratory of Peptide Synthesis, Research Institute of Experimental Cardiology of the Russian Academy of Medical Sciences. The operation of 50% abdominal aorta narrowing was performed under nembutal anesthesia (0.05 mg/kg). Immediately after the end of the treatment HMP was injected i.p., 20 μg/kg in 1 ml of saline. Control animals received an injection of saline with an equimolar mixture of amino acids. Ten days after treatment the heart was perfused with 2% glutaraldehyde in 0,1 M phosphate buffer and then removed. The myocardial disks, excised from the middle third of the left ventricle, were postfixed with 1% osmium tetroxide solution, dehydrated, and embedded in Epon. The semithin sections were stained with toluidine blue and measured under the microscope at a final magnification of 1350, using a multipurpose ocular morphometric grid

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TABLE 1. Morphological Parameters of Tissue Components of Different Left Ventricle Myocardial Layers of Rat Heart During Formation of Hypertrophy and During Hypertrophy under Peptide Injection ($M \pm m$)

Parameter	Norm	Amino acid control	Peptide injection
		Subendocardial layer	
V(m)	0.820±0.007	0.710±0.007	0.800±0.010
S(m)	48.56±0.85	38.6±2.9	31.95±1.66
V(c)	0.063±0.004	0.077±0.005	0.067±0.003
S(c)	9.84±0.28	18.05±1.38	11.75±0.63
V(ct)	0.173±0.008	0.283±0.008	0.203±0.001
D(m)	12.70±0.02	12.04±0.43	13.50±0.53
S(m)/V(m)	54.90±0.67	47.07±0.35	54.4±0.43
S(m)/S(c)	4.93±0.05	2.14±0.02*	2.14±0.05
V(m)/V(c)	12.95±0.50	10.65±0.36	9.22±0.44
V(c)/V(ct)	0.408±0.025	0.270±0.021*	0.270±0.032
		Intramural layer	
V(m)	0.720±0.008	0.760±0.008	0.800±0.007
S(m)	48.68±0.87	40.15±3.24*	38.35±2.38
V(c)	0.132±0.007	0.073±0.006*	0.060±0.006
S(c)	19.70±0.31	10.60±0.90*	10.60±0.83
V(ct)	0.273±0.009	0.237±0.008	0.205±0.011
D(m)	11.88±0.02	13.80±0.41*	13.81±0.26
S(m)/V(m)	62.60±0.38	52.83±0.19	47.8±0.75
S(m)/S(c)	2.48±0.06	3.79±0.09*	3.61±0.11
V(m)/V(c)	5.76±0.37	10.41±0.28	13.33±0.14
V(c)/V(ct)	0.509±0.051	0.310±0.027*	0.290±0.017
		Subepicardial layer	
V(m)	0.699±0.009	0.790±0.008*	0.690±0.010
S(m)	58.62±1.18	35.20±2.40	39.80±2.50
V(c)	0.169±0.007	0.143±0.008	0.110±0.005
S(c)	27.30±0.50	18.00±1.42	14.90±0.75
V(ct)	0.302±0.010	0.212±0.005*	0.310±0.010
D(m)	9.28±0.02	10.30±0.40 *	10.96±0.41
S(m)/V(m)	79.80±0.29	44.56±0.55	57.7±0.29
S(m)/S(c)	2.15±0.10	2.00±0.07	2.67±0.14
V(m)/V(c)	4.13±0.25	5.53±0.17	6.27±0.65
V(c)/V(ct)	0.570±0.031	0.680±0.019	0.350±0.029

Note: amino acid control in contrast to HMP injection: asterisk – $p>0.05$;, in all other cases $p<0.05$

[4]. Morphometric analysis revealed no difference between the series of treated animals which did and did not receive the amino acid mixture. The following parameters were determined: the relative volume of myocytes V(m); the relative volume of connective tissue V (ct): the relative volume of capillaries V(c); the surface area of the myocytes S(m); the surface area of the capillaries S(c); the surface area of the cardiomyocytes expressed per unit volume of the cardiomyocytes S(m)/V(m); the surface area of the cardiomyocytes expressed per unit surface area of the capillaries S(m)/S(c); the volume of the cardiomyocytes expressed relative to the volume of the capillaries V(m)/V(c); the volume of the capillaries as a ratio of the volume of connective tissue V(c)/V(ct). The average diameter of the cardiomyocytes D (m) was also calculated. An Amstrad PC 1640 computer was used for the calculations and statistical analysis by Student's t test and for distinguishing the primary and secondary objects.

RESULTS

Under HMP injection a reduction of the size of the zone of changes was obtained in the subendocardial layer [4] and its shifting in the direction of the ventricle cavity. Vacuolization of myocytes is still present, but significantly less expressed. Altered nuclei and supercontracted cardiomyocytes occur rarely. The other myocardial layers seem to be practically intact. Edema of the subendocardial layer is more weakly expressed than in the amino acid control. The other

layers have no signs of edema. The results of the measurements and calculations are listed in Table 1.

Under HMP injection normalization of V(m) is observed in the subendocardial layer (differences from the normal parameter are not significant). S(m) decreases reliably in comparison with the control, reportedly due to an increase of cell size [10]. This is also supported by the tendency for D(m) to increase. The diminished connective-tissue share compared to the control evidently stems from the reduced edema. The relative volume of capillaries is normalized. The lowered S(m)/V(c) value in the control, which characterizes the contractility of the myocardium [7], approaches normal. Index S(m)/S(c), testimony to metabolic activity [7], remains on the control level. This fact is also corroborated the lowered V(m)/V(c) index as compared to the control.

In the intramural layer under HMP injection there is a greater increase of the cardiomyocyte contribution than in the subendocardial layer and, probably connected with this, a tendency toaward a decrease of their area. The mean D(m) is unaffected, possibly reflecting arborization of the cardiomyocytes while hypertrophy is developing [6]. The connective-tissue component is also diminished due to reduced edema. S(c) is similar to that in the control.

A comparison of the relative volumes and surfaces of capillaries of the subendocardial and intramural layers suggests an increase of their circulation volume.

In the subepicardial layer under HMP injection the correlation between V(m) and V(ct) is restored to normal. In this case D(m) are markedly increased, indicating an earlier involvement of the cardiomyocytes in the process of hypertrophy. The decrease of S(c) together with the decrease of V(c) can be a manifestation of a reduced blood supply of the subepicardial layer.

Thus, a single injection of HMP facilitates the development of myocardial hypertrophy. This effect is expressed most strongly in the subendocardial layer. The effect of HMP is not only manifested in the muscular and connective-tissue components of the myocardium; it also affects the system of the myocardial wall vessels, leading to a redistribution of the intramural circulation.

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