CYTOLOGICAL CHANGES IN THE MYOCARDIUM OF RATS ADAPTED TO HIGH-ALTITUDE HYPOXIA

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During adaptation of rats to high-altitude hypoxia evidence of stimulation of both formative and lytic processes was observed, and either one process or the other was predominant in different cells. The ratios between volumes of mitochondria and myofibrils were substantially unchanged. The relative volume of the microcirculation was increased on account of dilatation of the small vessels with no increase in their number.

KEY WORDS: *Hypertrophy of the myocardium; intracellular regeneration; lysis of myofibrils; heterogeneity of myocardiocytes; nuclear pores.*

There is little information in the literature on ultrastructural changes in the myocardium during adaptation of animals to high-altitude hypoxia. Some workers have found an increase in the number and relative volume of the mitochondria [3], whereas others have observed the maintenance of a constant ratio between the volumes of mitochondria and myofibrils in animals exposed to chronic hypoxia under high-altitude conditions [6, 9, 10, 12].

The object of this investigation was to study structural changes in the myocardium during the period of formation of adaptation of animals to high-altitude hypoxia (up to 4 weeks).

EXPERIMENTAL METHOD

Experiments were carried out on 44 male Wistar albino rats weighing 200 ± 20 g, of which 17 were controls whereas the others were trained in a pressure chamber. Training of 22 rats lasted 6 h daily, 5 days a week, during which the atmospheric pressure was reduced daily by an amount equivalent to an elevation of 1000 m, until an "altitude" of 7000 m was reached, after which subsequent training was carried out at that level. Five rats were killed 2 weeks and 17 rats 4 weeks after the beginning of training. Five rats were trained by a more rigorous program: They were "raised" to an altitude of 8000 m and kept in the pressure chamber for 8 h daily; these animals were killed on the 14th day of the experiment. The heart was cooled and fixed by perfusion of a 4% solution of paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) through the aorta under a pressure of 50 cm water. The right and left ventricles (together with the ventricular septum) were weighed separately and the ratio between their weight and the body weight (Ind) was calculated. Pieces were cut from the myocardium of both ventricles for light and electron microscopy. Material for electron microscopy was fixed with 1% osmium tetroxide solution. Ultrathin sections were stained with uranyl and lead, semithin sections with toluidine blue. By using a stencil of points and lines [1, 14], the volume density of the mitochondria (Vv_{mc}) and myofibrils (Vv_{mf}) was determined for points on ultrathin sections, and their surface density (Sv_{mc} and Sv_{mf}) at intersections. The corresponding parameters of the small vessels (Vv_V, Sv_V) and muscle fibers (Vv_f, Sv_f) were determined by the same method in semithin sections and the number of vessels per square millimeter of surface of the myocardial section (Na_V) also was calculated. Ten

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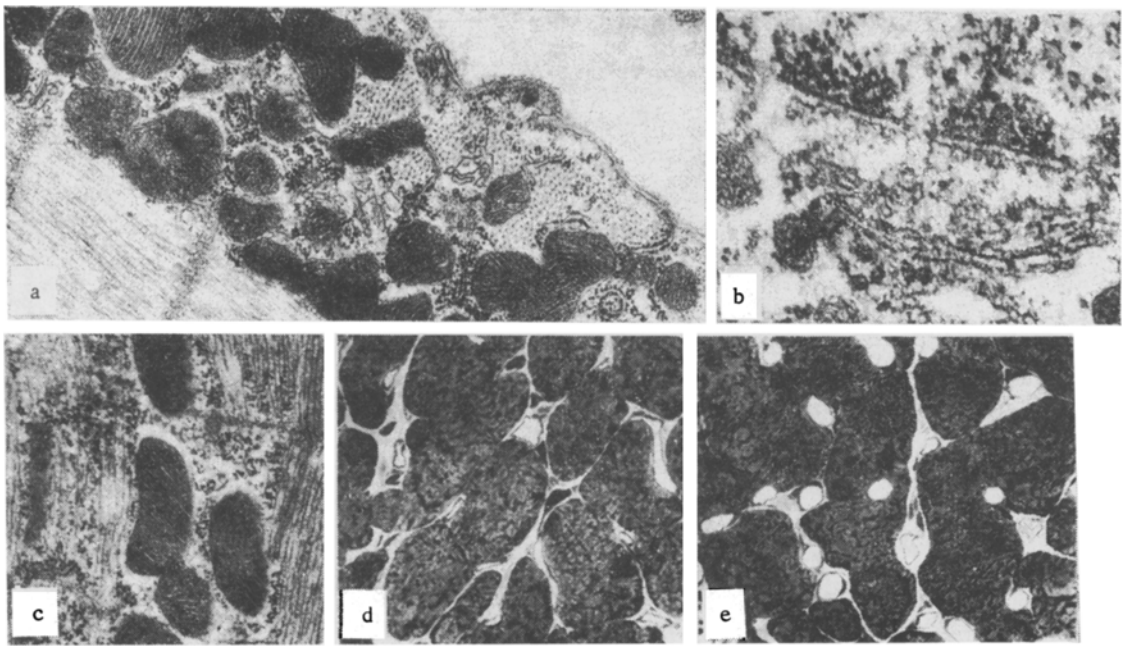

Fig. 1. Ultrathin (a-c) and semithin (d, e) sections through right ventricle of myocardium of rats adapted to high-altitude hypoxia: a) myocardiocyte after 2 weeks of rigorous training. Accumulation of mitochondria, granular endoplasmic reticulum, and ribosomes in subsarcolemmal region, formation of transversely arranged myofibrils. Stained with uranyl and lead, 23,000×; b) animal of same series. Part of nucleus of myocardiocyte with numerous pores in membrane. Alongside — Golgi apparatus. Stained with uranyl and lead, 56,000×; c) animal of same series. Part of myocardiocyte with partly lysed myofibrils. Polysomes visible on some loosely arranged myofilaments. Stained with uranyl and lead, 20,000×; d) myocardium of control animal, stained with toluidine blue, 400×; e) myocardium of rats after training for 4 weeks. Vessels dilated, stained with toluidine blue, 400×.

pieces were taken from each ventricle for stereological investigation and at least 4000 points were counted in each fragment.

EXPERIMENTAL RESULTS

Histological investigation showed no focal lesions of the myocardium in the animals exposed to training of moderate severity, but in rats trained under arduous conditions foci of necrosis of single myocardiocytes were found in the wall of the left ventricle and in the ventricular septum.

Electron-microscopic investigation showed an increase in size of the nucleoli and in the number of pores in the nuclear membrane of the myocardiocytes (Fig. 1) and hypertrophy of the granular reticulum and Golgi apparatus; the cytoplasm included many ribosomes and polysomes bound with myofibrils. These phenomena could indicate stimulation of formative processes [2, 4, 8]. Features reflecting stimulation of lytic processes were found in the same cells: In places the myofibrils were loosely arranged and partially lysed. Manifestations of lysis were usually more marked in the central zone of the cardiocytes, whereas new myofibrils were formed particularly intensively at the periphery of the cell; a transverse arrangement of the newly formed myofibrils was observed beneath the sarcolemma. Heterogeneity was a conspicuous feature of the changes in the muscle cells. In some of them lytic processes were stronger (a less compact arrangement of myofibrils, clearing of the cytoplasmic matrix); in others a denser packing of the structures and a condensed matrix were present. The changes described after 2 weeks of training, especially under rigorous conditions, were more marked than in rats trained for 1 month.

The ratio between the volume of the mitochondria and the volume of the myofibrils was not increased in any of the series of experiments, but in the right ventricle of rats trained for 1 month under moderate conditions and in both ventricles of animals trained un-

TABLE 1. Results of Stereologic Investigations of Myocardium of Right (RV) and Left (LV) Ventricles of Rats Adapted to High-Altitude Hypoxia (M ± m)

Index	Control		Training under moderate conditions				Training under rigorous conditions	
			2 weeks		4 weeks			
	RV	LV	RV	LV	RV	LV	RV	LV
Ind, %	100±4,9	100±2,1	126±7,3†	114±3,9	127±6,2†	115±2,7*	188±7,9†	157±3,8†
Vv_{mc}, %	31,3±0,54	31,0±1,87	31,7±0,76	29,5±1,67	29,4±0,58*	29,4±1,45	26,4±1,17†	23,7±0,66*
Vv_{mf}, %	56,4±0,54	60,8±2,05	56,4±1,14	60,9±1,1	58,0±0,52	59,7±1,38	58,2±1,06	60,5±1,11
V_{mc}/V_{mf}	0,56±0,014	0,52±0,054	0,57±0,023	0,49±0,038	0,51±0,013*	0,50±0,034	0,46±0,027*	0,39±0,016*
Sv_{mc}, mm^{-1}	3 110±179	3010±108	3 290±97	2 935±130	2809±114	2899±141	3 092±80	2 767±114
Sv_{mf}, mm^{-1}	3 157±80	3191±140	2 923±167	3 181±146	3119±173	3440±100	2 952±86	3 845±133
S_{mc}/V_{mc}, mm^{-1}	10 131±616	9692±381	10 393±334	10 022±447	9487±125	9933±241	11 825±446	11 700±368*
S_{mf}/V_{mf}, mm^{-1}	5,481±237	5239±69	5 178±251	5 217±202	5357±289	5766±103	4 946±147	5 628±165
Vv_v, %	4,3±0,22	6,7±0,65	4,0±0,75	8,2±0,87	9,0±0,57†	8,2±0,64	9,3±0,27*	7,6±1,47
Vv_f, %	83,0±2,02	84,4±1,41	84,0±3,86	86,0±1,67	78,3±1,79	83,5±0,69	77,2±3,12	75,8±1,54*
Vv_v/Vv_f	0,05±0,005	0,08±0,008	0,05±0,008	0,10±0,01	0,11±0,01†	0,10±0,008	0,12±0,008†	0,10±0,017
Sv_v, mm^{-1}	51,5±2,7	64,0±5,8	47,3±1,36	67,0±2,8	72,6±4,96*	68,0±3,8	64,8±2,69*	55,0±5,7
Sv_f, mm^{-1}	209±7,35	189±8,5	228±8,5	199±8,8	175±11,2	202±6,0	172±6,96*	183±3,1
S_v/V_v, mm^{-1}	1 212±75	926±54	1 221±131	845±7,1	819±58*	847±54	702±46†	833±115
S_f/V_f, mm^{-1}	252±10,7	224±7,5	272±10,7	230±7,5	224±13,5	241±6,9	224±3,8	242±4,7
Na_v, mm^{-2}	3 424±405	3601±154	3 569±199	3 935±281	3665±189	3665±143	3 318±252	2 353±137†

*P < 0.05.
†P < 0.005.

der rigorous conditions the ratio was actually reduced (Table 1), possibly on account of the more marked hypertrophy in these cases, when development of the myofibrils predominated [5, 7, 11, 13]. The absence of significant changes in the ratios between the surface area of the mitochondria and myofibrils and their volume is evidence that their mean dimensions remained unchanged.

Only in the left ventricle of rats trained under rigorous conditions was the S_{mc}/V_{mc} ratio increased, reflecting a decrease in size of the mitochondria.

The relative volume of the microcirculation (Vv_v) of the right ventricle in the control series and in rats trained for 2 weeks under moderate conditions was significantly less than in the left ventricle. In animals trained for a longer time or under more rigorous conditions this index rose sharply, to exceed that for the left ventricle.

The increase in volume of the circulation took place on account of dilatation of the vessels, the number of which was not increased (Fig. 1). In the left ventricle of intensively trained rats the number of vessels per unit area was actually reduced, evidently on account of the more marked hypertrophy and the edema of the myocardium. The latter was also reflected by a decrease in Vv_f.

The chief morphological manifestation of adaptation to high-altitude hypoxia so far as the heart is concerned was an increase in the relative volume of the microcirculation of the right ventricle associated with its hypertrophy but with no increase in the relative volume of the mitochondria in either ventricle.

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EFFECT OF SYMPATHOMIMETIC AGENTS AND DOPA ON THE ULTRASTRUCTURE OF THE NERVOUS APPARATUS OF THE HEART

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After a single injection of noradrenalin or dopa into albino rats noradrenalin was incorporated into adrenergic axons of the heart and deposited as granules in the small synaptic vesicles measuring about 30 nm in diameter. In this way adrenergic axons could be distinguished from cholinergic. Cholinergic axons were more numerous than adrenergic in the atria. Adrenergic terminals come into very intimate contact with cholinergic terminals and also with capillary endothelial cells and muscle cells of the myocardium. It is postulated that adrenergic fibers may act on heart muscle in three ways: by means of presynaptic inhibition through cholinergic axons, by a humoral mechanism, and directly on the muscle cells of the myocardium.

KEY WORDS: *Innervation of the heart; adrenergic axons; granular synaptic vesicles; noradrenalin synthesis.*

Exogenous noradrenalin is known to be incorporated into adrenergic fibers [7]. Tritiated noradrenalin has been shown to accumulate in unmyelinated axons of the heart containing small synaptic vesicles with a dense core [15]. Vesicles of this sort are seen very rarely in axons of different organs after osmium fixation [6, 9]. They increase in number after injection of labeled noradrenalin into animals [4]. Injection of 5-hydroxydopamine also causes the appearance of granular synaptic vesicles about 40 nm in diameter in the varicose expansions of adrenergic fibers of the heart [5]. What is not yet clear is where the adrenergic synaptic vesicles are formed. It has not been unanimously agreed in which synaptic vesicles noradrenalin is stored or whether it can be deposited as granules in all vesicles of the axon.

The object of this investigation was to study changes in the nervous apparatus of the heart under the influence of noradrenalin, its chemical precursor dopa, and isopropylnoradrenalin (isoproterenol).

EXPERIMENTAL METHOD

Noradrenalin was injected into the caudal vein of 13 male Wistar albino rats in doses of 0.001, 0.03, 0.3, and 0.5 mg/kg. Dopa [β-(3,4-dihydroxyphenyl)-DL-α-alanine; Reanal, Hungary] was injected intraperitoneally into five animals in doses of 20, 50, and 100 mg/kg. Isoproterenol (Izadrin, Spofa, Czechoslovakia) was injected subcutaneously into three animals in a dose of 100 mg/kg. The animals were decapitated 20 or 30 min after the injections. Two animals were given intraperitoneal injections of reserpine in a dose of 2.5 mg/kg 18 and again 2.5 h before the material was taken, and an injection of noradrenalin (0.3 mg/kg) 30 min later. Twelve animals served as the control. Pieces of the atria and of the atrial and ventricular septa were fixed in Caulfield's osmium fixative and embedded in Epon. The atria of four rats were treated by a cytochemical method for the detection of noradrenalin [13].

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