Delayed Effect of Hypotensive Drugs on Blood Pressure and Structure of the Myocardium in Hypertensive ISIAH Rats during Chronic Stress

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Administration of antihypertensive drugs α_1-adrenoceptor antagonist and Ca^{2+} channel blocker during early ontogeny had various delayed effects on blood pressure in ISIAH rats with inherited stress-induced arterial hypertension under resting conditions and chronic stress. The drugs did not prevent the development of myocardial hypertrophy. Chronic stress had little effect on myocardial structure in adult animals.

Key Words: *arterial hypertension; antihypertensive drugs; myocardium; stress; rats*

Male rats with inherited stress-induced arterial hypertension (ISIAH) are characterized by increased blood pressure (BP) at rest (up to 180 mm Hg) and, particularly, during acute emotional stress (up to 220 mm Hg). All signs of hypertrophy are observed in the heart of adult male ISIAH rats, including greater relative weight, moderate cardiosclerosis, cardiomyocyte hypertrophy, thick walls, and reduced lumen of coronary arteries (compared to normotensive Wistar rats of similar age) [4]. Morphological signs of left ventricular hypertrophy appear from the 3rd to the 5th week of postnatal development [3], when genetically determined hypersensitivity to stress manifests in persistent increase in BP [2]. Administration of various antihypertensive drugs to ISIAH rat pups during the 2nd month of life did not prevent the development of genetically determined arterial hypertension and myocardial hypertrophy [1]. However, they had a modifying effect on signs of these disorders. Qualitative and quantitative differences were revealed in the delayed effect of drugs on BP and structure of the myocardium.

This work was designed to study the pathogenesis of inherited stress-induced arterial hypertension. We evaluated the effect of treatment with hypotensive drugs during early ontogeny on reactivity of the cardiovascular system in adult ISIAH rats to chronic stress (CS).

MATERIALS AND METHODS

ISIAH rats were maintained in a vivarium under standart conditions and had free access to water and food. Finely ground suspension of hypotensive drugs in 0.25-0.50 ml water was given daily (orally) to rat pups.

The animals were divided into 5 groups of 5 males each. Group 1 rats received angiotensin-converting enzyme inhibitor enalapril maleate (KRKA) in a daily dose of 25 mg/kg on days 30-60 of life. Group 2 rats received type 1 angiotensin receptor

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antagonist potassium losartan (E. I. du Pont de Numours Co.) in a daily dose of 10 mg/kg on days 30-60 of life. Group 3 rats received α_1-adrenoceptor antagonist terazosin hydrochloride (Abbott Lab.) in a daily dose of 2 mg/kg on days 20-30 of life. Group 4 rats received Ca^{2+} channel blocker Corinfar (nifedipine, AWD Pharma) in a daily dose of 15 mg/kg on days 30-60 of life. Group 5 rats received an equivalent volume of water (placebo) on days 30-60 of life. The animals aging 6 months (body weight 357.0±5.3 g) were subjected to CS. CS was induced by 60-min daily immobilization in a cage for 10 days. Basal systolic BP was measured indirectly by the tail-cuff method under resting conditions (short-term ether narcosis to avoid stress) and after CS.

Experiments were performed in accordance with the principles of humanity (European Community Directive, 86/609/EC). The rats were decapitated under ether anesthesia.

For electron microscopy, the left ventricular myocardium was fixed in 2.5% solution of glutaraldehyde and 2% paraformaldehyde, postfixed in 1.5% OsO_4, and embedded in a mixture of Epon and araldite. Semithin cross-sections were stained with toluidine blue. The diameter of the nuclear region in cardiomyocytes was measured with an ocular micrometer (increment 1.54 μ, ×700). Stroma-parenchyma ratios in the myocardium were evaluated using a 289-point standard grid (8 superpositions, total area 0.15 mm^2). Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 100SX electron microscope. The relative volume of cardiomyocyte organelles, numerical density of mitochondria, and thickness of myofibrils were estimated with a square grid (72 points, initial magnification ×5000).

The significance of differences was evaluated by Student's *t* test and nonparametric sign test.

RESULTS

The hypotensive effect of enalapril, losartan, and terazosin in adult animals persisted after drug withdrawal. Corinfar had no delayed effect (Fig. 1). CS-induced elevation of BP in rats receiving losartan and terazosin during early ontogeny was similar to that in control animals (by 15-20 mm Hg). Enalapril and Corinfar completely abolished this response.

Previously described changes at the organ (increase in the relative weight), histological (cardiomyocyte hypertrophy, cardiosclerosis, and increased vascularization of the myocardium), and ultrastructural levels (increase in the volume and thickness of myofibrils) were revealed in the heart of experimental animals. They are typical of the hypertensive state and age of ISIAH rats [4]. Morphological signs of acute structural alteration in animals of various groups were not found after CS.

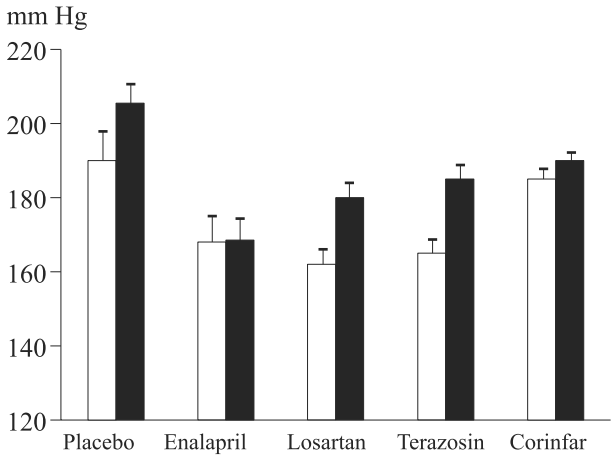

Fig. 1. Systolic BP in 6-month-old ISIAH rats of various groups under resting conditions (light bars) and after CS (dark bars).

Qualitative characteristics and morphometric indexes of the left ventricular myocardium were studied in rats of various groups (Table 1). These parameters were better in animals receiving inhibitors of the renin-angiotensin system (compared to the placebo group). For example, severe hypertrophy of the heart was accompanied by intense vascularization of the myocardium in enalapril-treated animals. Large cardiomyocytes were characterized by a proportional intracellular architectonics. The mitochondrion/myofibril volume ratio approached 1. Intracellular indexes in losartan-treated rats did not differ from those in control specimens. However, structural characteristics of myocardial tissue in these animals were better than in the placebo group. The size of myocytes was smallest in losartan-treated rats. The amount of stroma was minimum in these animals.

Other results were obtained in experiments on rats receiving terazosin and Corinfar. The diameter of cardiomyocytes and the amount of the stroma in the ventricular wall of these animals were greater than in the placebo group. Studying the intracellular composition of hypertrophic myocytes in rats of these groups showed that contractile proteins are accumulated and exist as thick myofibrils in the sarcoplasm. The relative volume of mitochondria in these rats was smaller than in the placebo group. The mitochondrial volume was not proportional to the volume of myofibrils. The severity of cardiosclerosis was highest in terazosin-treated rats. The fibrous stroma around each myocyte contained a considerable number of capillaries. These capillaries were often located in deep invaginations of

TABLE 1. Morphometric Indexes of the Left Ventricular Myocardium in 6-Month-Old ISIAH Rats after CS and Pharmacological Correction of Hypertension ($M \pm m$)

Parameter	Placebo (control)	Enalapril	Losartan	Terazosin	Corinfar
Heart weight /body weight, mg/g	2.98±0.09	3.58±0.07*	3.100±0.026	3.140±0.042	3.380±0.144*
Myocyte diameter, μ	22.90±0.37	22.40±0.61	21.20±0.44*	24.30±0.48*	25.10±0.41*
Stromal V_V, %	23.50±0.52	23.70±0.78	17.80±0.37*	25.20±0.58	23.60±0.72
Stromal V_V/myocyte V_V	0.310±0.009	0.320±0.014	0.220±0.005*	0.340±0.011*	0.310±0.013
Myofibrillar V_V, %	56.10±0.63	51.9±0.4*	53.40±0.64*	59.50±0.53*	61.70±0.63*
Mitochondrial V_V, %	37.90±0.59	42.60±0.42*	40.80±0.63*	34.0±0.5*	33.00±0.61*
Mitochondrial N_A, 10 μ^{-2}	6.370±0.141	6.870±0.124*	6.30±0.12	5.40±0.14*	6.940±0.154*
Myofibril diameter, μ	1.410±0.026	1.0300±0.0196*	1.460±0.024	1.540±0.033*	1.810±0.033*
Mitochondrial V_V/myofibrillar V_V	0.690±0.018	0.930±0.014*	0.790±0.021*	0.580±0.013	0.550±0.016*

Note. V_V, relative volume; N_A, numerical density of profiles. *$p<0.05$ compared to the placebo.

muscle cells. The lumen of capillaries was widened. The wall was layered with thin endothelial cells with a considerable number of pinocytotic vesicles. Muscle cells have an unusual shape; their processes were connected via composite intercalated disks and deep interdigitations. These structures had typical signs in ultrathin sections (Fig. 2). The diameter of cardiomyocytes was maximum in Corinfar-treated rats. These animals were characterized by the greatest thickness of myofibrils (Table 1).

Hypertension and myocardial hypertrophy in ISIAH rats are probably associated with a continuous psychosomatic response to exogenous stimulation, which serves as a stress factor for genetically predisposed animals. The hyperresponse to CS contributes to a certain neuroendocrine status, which is typical of adult hypertensive ISIAH rats (high concentration of corticosterone, low activity of angiotensin-converting enzyme in blood plasma, *etc*.) [2]. The absence of significant changes in the cardiovascular system under stress conditions can be explained exhaustion of reserves during hypertension development in ontogeny.

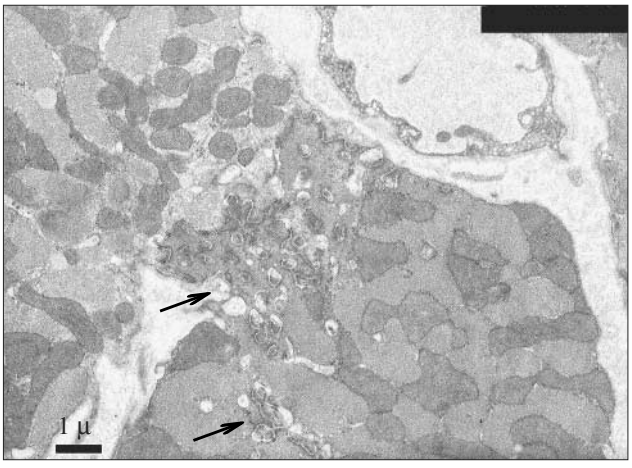

Fig. 2. Left ventricular myocardium in the adult ISIAH rat receiving terazosin on days 20-30 of life. Arrows: composite intercalated disks between processes of hypertrophic cardiomyocytes. Widening of the capillary lumen and thinning of the endothelium.

No qualitative and quantitative differences in the myocardial structure were revealed during modification of hypertension by pharmaceutical products in the absence of stress [1] and after CS. It concerns enalapril and losartan, which have a delayed hypotensive effect. This is also true for Corinfar, which does not produce a delayed hypotensive effect. Previous studies showed that administration of terazosin during the 2nd month of life was not followed by a decrease in basal BP in adult animals [1]. In our experiments, this effect was achieved after treatment with terazosin during the earlier ontogenetic period (last decade of the 1st month of life). However, no changes were found in the myocardial structure. Structural characteristics of the myocardium under resting conditions and after CS can be described as "preterm aging" (cardiosclerosis, severe hypertrophy of cardiomyocytes, and disproportional composition of organelles). Our findings confirm the notion that high-efficacy antihypertensive drugs can produce a delayed effect on the myocardium. These drugs improve stroma—parenchyma interactions, prevent hypertrophy of myocytes (losartan), maintain balanced composition of organelles in hypertrophic cells (enalapril), or increase the severity of cellular hypertrophy during the imbalance in the tissue and intracellular structure (terazosin).

No correlation was found between structural characteristics of the myocardium and BP changes in CS after pretreatment withthe test drugs. BP elevation was observed in rats of the placebo, losartan,

and terazosin groups (as differentiated from animals receiving enalapril and Corinfar; Fig. 1). Morphologically, the animals receiving anti-angiotensin drugs had a more favorable prognosis compared to rats of the placebo, terazosin, and Corinfar groups. Therefore, hypertension is always accompanied by myocardial hypertrophy. However, significant differences are observed in the development and regulation of these processes during ontogeny [5-7].

We conclude that administration of hypotensive drugs to ISIAH rats during early ontogeny had various delayed effects on the cardiovascular system in adult animals. Some drugs decrease basal BP (enalapril, losartan, and terazosin), while others not (Corinfar). Some drugs prevent BP elevation during CS (enalapril and Corinfar), while others produced no such effect (losartan and terazosin). Some drugs abolish the development of prognostically unfavorable signs in the hypertrophic myocardium (enalapril and losartan), while others were ineffective in this respect (terazosin and Corinfar). CS has little effect on myocardial structure in rats of various groups.

REFERENCES

1. I. M. Korostyshevskaya, V. F. Maksimov, A. L. Markel, *et al*., *Ros. Fiziol. Zh.*, **93**, No. 2, 169-179 (2007).
2. L. N. Maslova, V. V. Bulygina, and A. L. Markel', *Ibid*., **88**, No. 6, 774-780 (2002).
3. M. D. Shmerling, E. E. Filushina, I. M. Korostyshevskaya, *et al*., *Byull. Sib. Otd. Ros. Akad. Med. Nauk*, No. 2, 91-94 (1997).
4. M. D. Shmerling, E. E. Filushina, I. M. Korostyshevskaya, *et al*., *Byull. Eksp. Biol. Med.*, **122**, No. 9, 271-273 (1996).
5. J. S. Gottdiener, D. J. Reda, B. M. Massie, *et al*., *Circulation*, **95**, No. 8, 2007-2014 (1997).
6. A. Himmelmann, A. Svensson, A. Bergbrant, and L. Hansson, *J. Hum. Hypertens*., **10**, No. 11, 729-734 (1996).
7. J. Zicha and J. Kunes, *Physiol. Rev*., **79**, No. 4, 1227-1282 (1999).