GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Pathophysiological Aspects of Experimental Myocardial Infarction during Arterial Hypertension

G. S. Yakobson, A. R. Antonov, A. V. Efremov, N. A. Makhanova, M. D. Shmerling, V. F. Maksimov, I. M. Korostyshevskaya, and M. G. Yakobson

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 11, pp. 500-504, November, 2003
Original article submitted December 11, 2002

We compared the results of clinical and experimental studies of endocrine parameters in patients with myocardial infarction and arterial hypertension and NISAG rats with hereditary stress-induced arterial hypertension during experimental myocardial infarction. Changes in the content of corticosterone, aldosterone, insulin, triiodothyronine, and thyroxin were similar in animals and patients with myocardial infarction and arterial hypertension. The disadaptive course of myocardial infarction against the background of arterial hypertension can be explained by reduced compensatory capacity of the myocardium.

Key Words: *myocardial infarction; arterial hypertension; hormones*

Arterial hypertension (AH) and ischemic heart injury are the most common cardiovascular disorders in Russia and other countries [2]. Myocardial infarction (MI) against the background of AH is a complex systemic disease characterized by severe course. Little is known about the pathogenesis of this combined pathology. The neuroendocrine regulation of adaptive processes under conditions of ischemic (catecholamine-induced) injury in the myocardium against the background of hereditary AH is poorly studied [10]. Considerable difficulties in studying this combined pathology are associated with the absence of adequate experimental models of AH and complexity of multifactor correlation analysis in clinical trials [15]. Despite numerous and long-term studies of AH and MI, there are no general pathophysiological models of these disorders.

Here we evaluated peculiarities of the neuroendocrine regulation in NISAG rats with hereditary stress-induced AH and experimental MI (EMI) and comparison of these analogous parameters in patients with MI.

Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; Medical State Academy, Novosibirsk; Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk

MATERIALS AND METHODS

Experiments were performed on 150 male Wistar and NISAG rats (body weight 180-220 g). Hypertensive animals were bred by Prof. A. L. Markel from outbred Wistar rats at the Laboratory of Evolutional Genetics (Institute of Cytology and Genetics). Blood pressure (BP) in the caudal artery was measured by the sphygmographic method. BP in a rubber cuff and pulse fluctuations were recorded on a Biocomb-5 polyphysiograph with Statham P23 and Elema Schonender EMT-510 pressure transducers. Control values of BP were estimated under short-term rausch-narcosis to prevent emotional stress associated with the procedure. ECG was recorded in ether-narcotized rats placed in the prone position in a shielded chamber and connected to a Mingograph-34 cardiograph via needle electrodes. Thin needles were introduced subcutaneously into 4

0007-4888/03/1365-0444$25.00

limbs. ECG was recorded in 3 standard leads and 3 augmented limb leads (tape speed 100 mm/sec, channel sensitivity 20 mm/mV). The width and amplitude of *P*, *R*, *S*, and *T* waves and *QRS* complex and the length of *PQ*, *QRST*, and *RR* intervals were measured. Basal BP in WAG and NISAG rats was 117.00±2.23 and 172±2 mm Hg, respectively. During stress these parameters were 128.20±2.03 and 205±2 mm Hg, respectively [5].

The model of EMI was selected by simplicity, low traumatism, and identity to the corresponding state in humans. The model of catecholamine-induced (metabolic) MI was most adequate. EMI was induced by subcutaneous injection of 0.1% epinephrine in a single dose of 0.2 mg/100 g. The blood was collected in dry centrifuge tubes immediately after decapitation and centrifuged at 900*g* for 10 min. The plasma was stored at -20°C. Blood samples were obtained on days 1, 2, 3, 7, 14, and 21 after EMI.

For morphological study the animals were sacrificed 1, 7, and 30 days after epinephrine administration under ether anesthesia. Myocardial specimens were examined under light and electron microscopes; morphometric analysis was carried out.

We examined 156 patients with MI and positive history of AH. The reference group included 60 healthy donors and 162 patients with MI without AH (25-50 years). The diagnosis of MI was made by criteria of the World Health Organization. Clinical symptoms included severe and typical pain syndrome, pathognomonic ECG signs, and enzyme markers (increase inaspartate transaminase, alanine transaminase, and creatine phosphokinase activities by more than 50%). Clinical studies were performed in collaboration with Prof. A. D. Kuimov at the Department of Cardiology (Municipal Clinical Hospital No. 1, Novosibirsk). The blood was taken from patients with MI (with positive or negative AH history) on days 1 (hospital admission), 3-5 (pronounced necrosis in the myocardium), 5-7 (transition from acute to subacute stage), and 25-30 (complete clinical and functional stabilization by the end of hospital treatment and rehabilitation).

Plasma aldosterone concentration (PAC) in experimental animals and MI patients was measured using commercial kits for radioimmunoassay (RIA, Sorin). Insulin content was determined using RIA kits and I^{125} (Russia). Plasma corticosterone concentration in experimental animals was estimated by the method of competitive protein binding with modifications of A. A. Tinnikov and N. M. Bazhan [7]. Blood hydrocortisone concentration in patients with MI and plasma contents of T_3 and T_4 in experimental animals and MI patients were measured using commercial RIA kits with I^{125}.

RESULTS

Aldosterone concentration in NISAG rats with acute stage of EMI increased to 7.5 nmol/liter (*vs.* 1.5 nmol/liter in intact animals). It should be emphasized that we did not observe a sharp increase in corticosterone concentration, which is typical of stress [6]. These changes reflect extracardiac compensation of cardiac insufficiency that is especially pronounced in hypertensive rats. Cardiac necrosis was accompanied by initial shift of steroidogenesis toward the synthesis of aldosterone, but not of glucocorticoids. It was related to activation of the renin-angiotensin-aldosterone system (RAAS) [12]. These results are consistent with the concept that cardiovascular insufficiency serves as a unifying factor for changes of RAAS in the acute stage of EMI. Activation of RAAS is one of the major stress reactions to acute circulatory arrest in the heart that has a biological adaptive role. However, this is only partly true for hypertensive rats. The reaction of mineralocorticoid function in the adrenal cortex to stress (EMI) disagrees with the Wilder—Leites law of initial value. It postulates that the stimulatory signal produces a weak, zero, or opposite effect when the cell, tissue, organ, or physiological system is functionally active. The inhibitory signal causes maximum response under these conditions. A further decrease in PAC is probably due to reduced sensitivity of glomerular adrenocortical cells to the stimulatory signal and delayed activation of corticosterone synthesis and secretion. In normotensive animals the reaction of the adrenal cortex was traditional and manifested in the increase in plasma corticosterone level on day 1. No changes in PAC in these rats attest to higher resistance to stress and less pronounced cardiac insufficiency. The secondary rise in plasma corticosterone level in normotensive rats on day 21 corresponds to the stress reaction. The absence of these changes in hypertensive rats is consistent with the dynamics of cortisol content in elderly patients with MI [1]. The law of initial state adequately illustrates the directionality of endocrine changes. Without considering specific features of basic metabolism, changes in PAC and corticosterone level appear as the primary adaptive response.

The consistency between the results of experimental and clinical observations is of importance. On day 1 hyperaldosteronism developed in patients with MI and AH anamnesis, but not in patients without AH (Table 1). These changes are considered as an unfavorable prognostic criterion. Hyperaldosteronism was observed in patients having MI of functional classes III-IV with lethal outcome. Previous studies showed that aldosterone sensitizes the myocardium to catecholamines. Moreover, tissue components of RAAS are activated to a greater extent in hypertensive

patients [11]. These data explain high severity of ischemic injury to the myocardium in NISAG rats. K. Lindpaintner and D. Ganten [13] revealed that the myocardium synthesizes angiotensinogen whose gene is expressed under the influence of glucocorticoids in high concentrations. Angiotensin II suppresses energy metabolism in the myocardium. The action of aldosterone is followed by the development of corticosterone-induced damage to the heart, which aggravates the symptoms of ischemia. Published data show that glucocorticoids stimulate expression of genes for angiotensin II receptors in vessels. It increases the sensitivity to vasoconstricting action of angiotensin II [9]. The observed changes probably play a role in the pathogenesis of reperfusion injury to the myocardium [3].

In normo- and hypertensive animals BP and ECG decreased in the acute stage of EMI (Table 2). These changes were most pronounced in NISAG rats. Activation of mineralocorticoid function in NISAG rats probably serves as an adaptive response to progressive BP drop. However, this process has a pseudoadaptive component and can be considered as a manifestation of "adaptation disease" [8]. Signs of transmural MI (increase in *S* wave amplitude and appearance of deep negative *QS* waves) were observed in hypertensive rats at various terms of the experiment. By contrast, the incidence of these disorders in Wistar rats did not exceed 20%. *T* wave inversion was revealed in rats of both strains in the acute period of EMI. It was most pronounced in NISAG rats. Changes in the *T* wave corresponding to diffuse metabolic disorders and typical of epinephrine-induced MI were observed in Wistar rats. Acute focal necrosis developed in some hypertensive rats. Over the first day of EMI the mortality rate in hypertensive rats was 40% (*vs.* 0% in normotensive animals).

TABLE 1. PAC in Patients with MI and AH Anamnesis, nmol/liter (*M*±*m*)

Days	With AH	Without AH
Control	0.250±0.008	0.250±0.008
1	0.41±0.08$^+$	0.29±0.04*
3-5	0.37±0.08$^+$	0.29±0.04*
7-10	0.56±0.10$^+$	0.31±0.05*
25-30	0.46±0.08$^+$	0.47±0.07$^+$

Note. Here and in Table 2: $p<0.05$: *compared to another group; $^+$compared to the control.

TABLE 2. BP in Normo- and Hypertensive Rats during Acute Stage of EMI, mm Hg (*M*±*m*)

Days	Wistar	NISAG
Control	127±2	153±2
1	107±6$^+$	113±4$^+$
2	117±4	131±6$^+$
3	119±6	127±5$^+$

Plasma insulin concentration in hypertensive rats remained high to the 14th day after the incidence of EMI and surpassed that in intact animals (60 and 14 mg%, respectively). This parameter remained practically unchanged in Wistar rats. Changes in the corticosterone/insulin ratio in Wistar rats were within the limits of normal reaction without exhaustion. Taking into account the general anabolic effect of insulin, it can be hypothesized that continuous hyperinsulinemia in hypertensive rats reflects non-economical activity of the organism [1]. The absence of inverse relationships between these hormonal systems suggests that regulatory complexes undergo a change or shift in rats with hereditary AH. Insulin not only produces the metabolic effect, but also maintains pressure equilibrium. In addition to hypercorticism, activation of the insulin system contributes to the increase in BP. This pressor effect is mediated by various mechanisms, including modification of electrolyte metabolism (Na^+-K^+ pump and Na^+-H^+ exchange) and stimulation of the sympathoadrenal system [14].

T_3 concentration in Wistar rats decreased by the end of the recovery period. This is consistent with the course of MI in humans and can be explained by suppressed production of the immediate response hormone triiodothyronine. This reaction probably reflects a new energetically advantageous level of functioning in the thyroid system. Thyroid hormones in low physiological concentrations stimulate the synthesis of various RNAs, which increases production of structural and functional proteins in mitochondria and microsomes. These changes contribute to hypertrophy of intact cardiomyocytes and intensification of tissue respiration and ATP synthesis in mitochondria. It should be emphasized that stimulation of respiratory processes is not accompanied by discoordination between oxidation and phosphorylation.

The decrease in T_4 concentration in hypertensive rats was accompanied by an increase in the T_3/T_4 ratio (compared to normotensive animals). These findings confirm the hypothesis about high energetic value of adaptation in hypertensive rats and are consistent with clinical observations. The observed changes reflect "paracrine synergism" in the realization of hypertensive reactions that return BP to the initial level.

Histomorphometric analysis showed that cardiomyocyte atrophy and contracture death in most cells occur in the early period after epinephrine injection. Degenerative changes were observed in individual cells. Vascular disorders accompanied by edema and

infiltration of the stroma prevailed in the myocardium of NISAG rats. We revealed vasodilation and capillary plethora.

In Wistar rats morphometric indexes of the microcirculatory bed returned to normal by the end of the first week. Ultrastructural signs of intensive protein synthesis were revealed in endotheliocytes [4]. Structural relationships between major cellular organelles were normalized in cardiomyocytes. In NISAG rats vascular disorders persisted in this period. Cardiomyocytes underwent low-intensity reparation. The major sign was activation of stromal cells.

By the end of observations (day 30) histomorphometric indexes of the myocardium in Wistar rats did not differ from normal. Pronounced cardiosclerosis and vascular disorders in the microcirculatory bed (dilation of capillaries and thinning of the vascular wall) were revealed in the myocardium of NISAG rats. Under these conditions the ratio between thickness of the wall and lumen of vessels was 2 times lower than in the initial state. Cardiomyocytes with high volume density of mitochondria and low number of myofibrils prevailed, which reflected impairment of plastic processes. As differentiated from normotensive Wistar rats, epinephrine-induced atrophy and degeneration of cardiomyocytes, vascular disorders, and impairment of reparative processes persisted for a long time in the myocardium of NISAG rats.

We believe that constellation of interendocrine relationships in hypertensive rats is a sign of metabolic resetting determined by basic metabolism and corresponding to the disadaptive course of EMI. Epinephrine-induced damage to the heart in Wistar rats develops against the background of sufficient compensatory reserves in the myocardium. However, compensatory potential is markedly reduced in NISAG rats. An excellent correlation between clinical and experimental data on changes in endocrine and functional parameters of the cardiovascular system during MI indicates that NISAG rats are an adequate model for studying the pathogenesis of this disease.

REFERENCES

1. A. D. Kuimov and G. S. Yakobson, *Myocardial Infarction: Clinical and pathophysiological Aspects* [in Russian], Novosibirsk (1992).
2. M. S. Kushakovskii, *Hypertension* [in Russian], St. Petersburg (1995).
3. P. F. Litvitskii, V. A. Sandrikov, and E. A. Demurov, *Adaptive and Pathogenic Effects of Myocardial Reperfusion and Reoxygenation* [in Russian], Moscow (1994).
4. V. F. Maksimov, I. M. Korostyshevskaya, A. L. Markel, *et al*., *Morfologiya*, **115**, No. 1, 19-23 (1999).
5. A. L. Markel, *Medical Proceedings of the Moscow State University* [in Russian], Moscow (1994), pp. 201-204.
6. F. Z. Meerson, *Adaptive Medicine: Mechanisms and Protective Effects of Adaptation* [in Russian], Moscow (1993).
7. A. A. Tinnikov and N. M. Bazhan, *Lab. Delo*, No. 12, 709-713 (1984).
8. V. G. Shalyapina, *Fiziol. Zh*., No. 4, 9-13 (1996).
9. D. Chansel, C. Llorens-Cortes, S. Vandermeersch, *et al*., *Hypertension*, **27**, No. 4, 867-874 (1996).
10. L. Fei, J. Keeling, J. Gill, *et al*., *Br. Heart J*., **71**, 322-328 (1994).
11. K. F. Hilgers, U. Hilgenfeld, R. Veelken, *et al*., *Hypertension*, **21**, No. 6, Pt. 2, 1030-1044 (1993).
12. B. Jackson, D. Paxton, D. Fabris, *et al*., *Aust. NZJ Med*., **23**, No. 1, 77 (1993).
13. K. Lindpainter and D. Ganten, *Circ. Res*., **68**, No. 4, 905-921 (1991).
14. L. H. Opie, *Cardiovasc. Res*., **26**, No. 1, 20-25 (1992).
15. T. G. Pickering, J. E. Schwarz, and G. D. James, *Clin. Exp. Pharmacol. Physiol*., **22**, No. 3, 226-231 (1995).