Disturbances of Membrane Mechanisms during Heart Stress Damage in Rats of Different Strains

T. V. Bershova, L. V. Simutenko, M. I. Bakanov, G. G. Barsegyan, T. M. Serebryakova, and V. I. Serbin

UDC 616.12-02:613.863]-092.9-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 3, pp. 247-249, March, 1993
Original article submitted October 21, 1992

Key Words: *stress; arrhythmia; membrane destabilization; action potential*

Mental stress is the cause of a number of pathological states, including cardiac arrhythmia. Changes in the electrical stability of the heart are considered to play an important role in the genesis of stress-induced heart damage. Taking into account the peculiar importance of membrane disorders in the changes of the automatism, excitability, and contractility of the heart, it can be assumed that the complex character of stress influence on the heart may be connected with changes of the structural and functional state of the cardiomyocyte biomembranes and with disorders of cell metabolism regulation.

In the present study, in order to elucidate the membrane mechanisms of the arrhythmia arising against the background of stress, the level of lipid peroxidation (LPO), the activity of the protective enzymes of the antioxidant system (APE), total cholesterol (ChS), and the calcium ion (Ca^{2+}) concentration in the myocardial tissue were studied in the rats of different strains.

MATERIALS AND METHODS

The experiments were carried out on mature male rats of the Wistar, August, and Fisher strains weighing 200-270 g. The rats of each strain were divided into two groups: group 1 consisted of control animals and group 2 comprised animals subjected to 24-hour immobilization. All the animals were preliminarily tested "in the open field." A comparative assessement of the organism's tolerance of emotional stress was performed by studying the autonomic reactions and by taking the emotional reactivity of the animals of different strains into account. A varying level of emotionality was observed in the animals of the three strains. The lowest reactivity was discovered in the Wistar rats (0.06), while that found in the August and Fisher rats was 0.11 and 0.15 relative units, respectively. Changes in the weight of the body, adrenals, and thymus, as well as the mortality during immobilization and the electrical stability of the heart were considered as the stress-resistance parameters. The heart's electrical stability was assessed as the bradycardia and arrhythmia thresholds in nembutal narcotized (45 mg/kg) rats during stimulation of the vagus nerve (amplitude duration in the series 0.5 msec, series duration 1 sec, interval between two series 1 sec). The biochemical parameters were also determined in rats undergoing vagus stimulation but not exposed to stress (immobilization). During 60 sec of electrostimulation, the degree of bradycardia and the nature of the disturbances of the heart rate and conductivity were assessed according to the ECG standard lead II. The ECG analysis showed that diverse conductivity blockades and ectopic rhythms arose in the animals of all strains. The sinus bradycardia and blockade observed in the rats only subjected to vagus stimulation had rhythms more frequent than degree I (a

Laboratory of Clinical Biochemistry, Research Institute of Pediatrics, Russian Academy of Medical Sciences; Laboratory of Neurobiology, Scentific Center of Medical Diagnostics, Ministry of Health, Russia. (Presented by M. Ya. Studenikin, Member of the Russian Academy of Medical Sciences)

0007-4888/93/0003-0258$12.50

TABLE 1. Parameters of Cell Metabolism in Myocardial Tissue of Rats with Diverse Types of Arrhythmia

Rat strain	Model of arrhythmia	MDA, nmol/ml	HP, rel. U/ml	SOD activity, U/ml×min	GR activity, U/ml×min	Ca^{2+}, μmol/mg protein	Cholesterol, mmol/ml
August	Stress + stimulation	5.51±0.36* n=8	1.48±0.13* n=8	17.84±0.89* n=18	1.54±0.09* n=21	3.25±0.09*,** n=25	65.81±0.02* n=18
	Electrical stimulation	5.86±0.37* n=8	1.38±0.13* n=8	21.58±1.18* n=15	1.91±0.10* n=20	1.28±0.09* n=14	73.14±0.13*,** n=16
	Control	3.43±0.24 n=9	1.68±0.06 n=9	34.74±1.82 n=15	2.74±0.09 n=21	1.59±0.12 n=25	38.61±0.31 n=12
Wistar	Stress + stimulation	5.19±0.28* n=10	1.08±0.09* n=10	16.23±0.34* n=12	1.45±0.06* n=10	2.89±0.11* n=9	57.21±0.15* n=10
	Electricalal stimulation	6.35+0.30* n=14	0.98±0.03* n=9	22.00±1.34* n=10	2.02±0.03* n=12	1.40±0.03* n=9	68.41±1.10* n=8
	Control	2.63±0.36 n=12	0.68±0.05 n=12	33.61±1.12 n=9	2.50±0.05 n=10	1.58±0.02 n=10	37.21±0.90 n=10
Fisher	Stress + stimulation	5.56±0.27* n=7	1.51±0.16*,** n=7	16.71±0.41* n=6	1.50±0.02* n=7	3.10±0.01* n=7	63.04±1.12* n=7
	Electric stimulation	7.20±0.37*,** n=6	1.54±0.15*,** n=6	21.73±1.00* n=6	1.99±0.05* n=6	1.45±0.02 n=6	61.05±1.74* n=6
	Control	3.08±0.34 n=5	0.66±0.08 n=5	33.92±1.54 n=6	2.70±0.07 n=7	1.49±0.01 n=5	38.92±1.15 n=7

Note. Asterisks denote the significance of differences with respect to: *) the control animals; **) the groups of other strains in the same pathophysiological model.

rhythm of 2:1), whereas an ideoventricular rhythm with intermittent extrasystole against the background of bradycardia was registered in the rats exposed to immobilization stress. The thresholds of bradycardia and arrhythmia were lower in Fisher rats than in the other strains. Diverse degrees of somatoregulatory disorders in rats of the strains investigated were also noted. For example, the lowest percentage of adrenal hypertrophy and thymus involution, the greatest amplitude of fluctuations of the heart rate, and cases of lethal outcome were observed in response to 24-hour immobilization in the Fisher rats, this attesting to a reduced stress-resistance in this strain. The LPO intensification was studied by measuring the malonic dialdehyde (MDA) and hydroperoxide (HP) content in the myocardial tissue. The concentration of MDA was determined by its reaction with thiobarbituric acid. The level of lipid HP was determined according to the characteristic absorption of diene conjugates in a methanol-hexane solution. The activity of superoxide dismutase (SOD) was assessed by the reaction to nitrotetrazolium blue, and glutathione reductase (GR) activity was measured as the quantity of reduced glutathione using $NADPH_2$ as a reduced equivalent. The ChS content was measured by the enzymatic method with the aid of a Boehringer Mannheim standard kit; the Ca^{2+} concentration was determined with ion-selective electrodes on a Ciba-Corning-634 analyzer.

RESULTS

Analysis of the results showed that the heart damage induced by stress was accompanied by marked disturbances of LPO, as well as by changes of the ChS and Ca^{2+} level in the myocardial tissue, the amplitude of these changes being most pronounced in the Fisher rats. This correlated with the morphophysiological peculiarities of this group and confirmed the considerable species-specific sensitivity of these animals (Table 1). At the same time, no appreciable changes of the content of these substances were noted in the myocardial tissue of the control animals of the three strains. It was found that the LPO intensification was determined to a certain extent by a decrease of APE activity, this indicating a decompensated functional state of the antioxidative system reserve capabilities and restricted detoxication of the superoxide anion-radical. Accumulation of the latter can induce continuously relapsing processes in the pathogenetic chain of heart rhythm disturbances. It is typical that intensification of free-radical oxidation in rats with induced disturbances of the heart rhythm is accompanied by a significant increase of the ChS level, this increase being pronounced in arrhythmias caused by "pure" vagus nerve stimulation. In this group of animals a decrease of the Ca^{2+} concentration was observed vis-a-vis both the control group of rats and the other group. When arrhythmia was caused by vagus nerve stimulation against the background of stress, a more than twofold increase of the Ca^{2+} concentration in the myocardial tissue was noted. At the same time, the dependence of the Ca^{2+} level upon the degree of LPO intensification was confirmed by the correlation discovered between the Ca^{2+} content and the MDA and HP concentration (r=+0.69 and r=+0.71, respectively).

An important role has been assigned to the intensification of the adrenergic effect during heart stress damage [13]. It cannot therefore be excluded that the LPO intensification is due to an increased catecholamine content in the myocardial tissue. LPO initiation in the rats with arrhythmia caused by vagus nerve stimulation can also be realized through a rise in the level of the unsaturated fatty acids that are LPO substrates in ischemized myocardial tissues [12]. The LPO intensification and rise of the ChS level is evidence of cell membrane disintegration [1,12], leading, in turn, to disturbances of ion transport. Thus, in the rats with arrhythmia induced by vagus nerve stimulation against the background of stress, a marked increase of the Ca^{2+} concentration in the heart tissue homogenates was observed, this being evidence of the activation of its passive entry into the cell. Furthermore, an accumulation of free-radical oxidation products leads to destruction or inactivation of the membrane-bound enzymes, including Ca^{2+}, Mg^{2+} and Na/K-ATPases [2,4,14]. At the same time it cannot be ruled out that a decrease of Na/K-ATPase activity promotes a rise of the intracellular Na^+ content and a fall of the K^+ content [4]. When the Na^+ gradient is diminished, the activity of Na^+/Ca^{2+} exchange drops to a certain extent, as a consequence of which the Ca^{2+} release from the cardiomyocytes is slowed [7,11]. By the above mechanisms, the disturbances of active and passive Ca^{2+} transport lead to an increase of its intracellular pool. In turn, a high intracellular Ca^{2+} concentration promotes destabilization of the membrane potential and disturbances of excitability and automatism, because in the cells of the sinus node the ion current responsible for the spike of the action potential is primarily a calcium current [5]. A significant decrease of the intracellular Ca^{2+} level can be the result of acetylcholine (AC) influence on the cardiomyocyte during conductivity disturbances caused by "pure" vagus nerve stimulation. The inhibitory effect of AC is manifested in an increase of membrane permeability for K^+ ions [8,10], this causing an increase of the maximum diastolic potential and action potential shortening, these both being secondary electrical phenomena leading to a negative inotropic effect [6,10].

Thus, it may be concluded that stress damage to the heart is, to an appreciable degree, induced by changes in the membrane processes, followed by the formation of pathological cell-level interactions capable of evoking disturbances in cardiomyocyte electrical stability. The changes of the myocardial electrophysiological properties are determined largely by the intracellular Ca^{2+} level and depend upon the species-specific and emotional reactivity of the experimental animals.

REFERENCES

1. T. S. Balashova, N. K. Khitrov, and A. M. Gerasimov, *Fiziol. Zh. SSSR*, № 10, 1312-1316 (1990).
2. Yu. A. Blyuzdin, L. Zh. Osadchaya, and A. A. Boldyrev, *Biokhimiya*, **51**, № 9, 1499-1505 (1986).
3. V. I. Veksler, *Byull. Vsesoyuz. Kardiol. Nauch. Tsentra*, № 1, 72-75 (1987).
4. R. J. Froud, J. M. East, O. T. Jones, and A. G. Lee, *Biochemistry (Wash.)*, **25**, № 3, 7544-7552 (1986).
5. J. M. Quaeqebeur, Y. J. Schonten, and H. E. Ter Keurs, *J. Physiol. (Lond.)*, **377**, 119 (1986).
6. Li Jia and De Hui Hou, *Acta Pharmacol. Sin.*, **9**, № 5, 421-426 (1988).
7. J. Kajita, H. Sako, S. Imauichi, and M. Arita, *J. Molec. Cell. Cardiol.*, **22**, Suppl. 2, 42 (1990).
8. D. Kim, *J. Physiol. (Lond.)*, **437**, 133-135 (1991).
9. G. A. Langer, T. L. Rich, and F. B. Orner, *J. Molec. Cell. Cardiol.*, **21**, Suppl. 2, 177 (1989).
10. Hon-Chi Lee and W. T. Clusin, *Circulat. Res.*, **61**, № 6, 934-939 (1987).
11. J. Morgan, S. Warren, G. Manrice, and T. Biggs, *J. Cell Biochem.*, Suppl. 13-E, 171 (1989).
12. Bo K. Siesjo, *J. Molec. Cell. Cardiol.*, **20**, 7 (1988).
13. F. T. Trandrojen, K. H. Muntz, L. M. Buja, and J. T. Willerson, *Circulation*, **82**, № 3, Suppl., 30-37 (1990).
14. N. Vrbyar, A. Breier, A. Ziegelhoffer, *et al.*, *Gen. Physiol. Biophys.*, **5**, № 5, 545-549 (1986).