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INCREASED RESISTANCE OF THE MYOCARDIUM TO STRESS AND TO EXCESS CALCIUM IN SPONTANEOUSLY HYPERTENSIVE RATS

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Animals of different genetic lines differ in their sensitivity to stress [3] and in their resistance to injuries caused by prolonged stress [2]. The further study of the mechanism of genetically determined resistance to stress is undoubtedly of great importance for an understanding of the pathogenesis of stress injuries and their prevention.

This paper describes a study of the mechanisms of increased resistance of the myocardium of spontaneously hypertensive rats (SHR) to stress injury and evaluation of the role of calcium transport in this mechanism.

EXPERIMENTAL METHOD

Male normotensive Wistar-Kyoto (WKY) and August lines of male rats and SHR rats aged 7-8 months were used. Half of the animals of each line served as the control, and the rest were subjected to immobilization stress (IS) by fixation in the supine position for 6 h. The animals were decapitated after 2 h of IS, the heart was removed, the left ventricle and right atrium were weighed, and these were used for subsequent physiological study. Contractility of the isolated atrium was recorded in oxygenated Krebs–Henseleit solution on an F-50 "Physiograph DMP-4B" myograph (Narco-Biosystems, USA), by the method fully described previously [1]. The atrium contracted spontaneously for 40-50 min, after which it was gradually stretched by stepwise increasing loads to a length of l_{max}, at which it developed maximal tension. Extensibility of the atrium was judged from the increase in its length in response to a standard load of 100 mg, and this increase of length was described as Δl. Contractility of the atrium was judged from the maximal value of developed tension (T_d) during isometric contraction and from the graph of length versus developed tension, i.e., the Starling curve. To assess calcium transport the response of the atrium to an excess of this ion in the working so-

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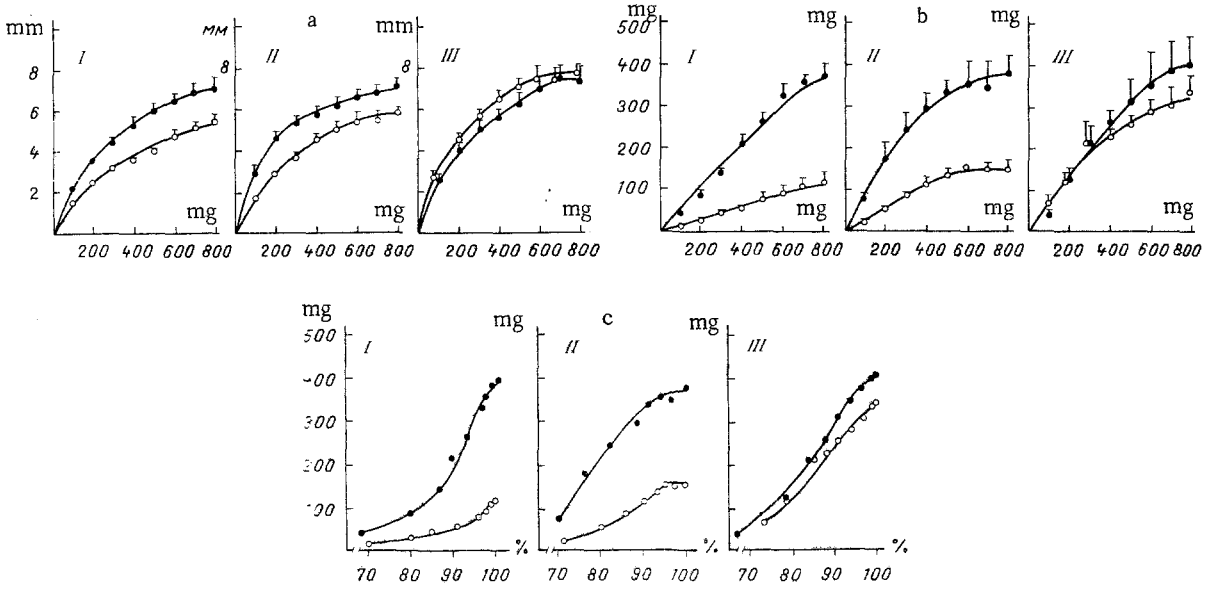

Fig. 1. Effect of stress on extensibility of myocardium (a), on tension developed by atrium (b), and on Starling curve (c) in WKY (I), August (II), and SHR (III) rats. a: Abscissa, external load applied T_e (in mg), ordinate, increase in length of atrium Δl (in mm); b: abscissa, external load applied T_e (in mg); ordinate, developed tension T_d (in mg); c: abscissa, increase in length of atrium (in % of l_{max}); ordinate, tension developed by atrium T_d (in mg).

lution was determined. The response to a threefold increase in Ca^{++} concentration (to 7.5 mM) in the working solution was estimated from the value of the positive inotropic effect, and also from the increase in resting tension T_0, i.e., from the development of Ca-contracture. The index of contracture (IC), equal to the ratio of the increase in T_0 taking place during contracture and the developed systolic tension T_d before the beginning of exposure to an excess of calcium:

$$IC = \frac{T_0}{T_p} \cdot 100\,\%.$$

EXPERIMENTAL RESULTS

The data shown in Fig. 1 enable the extensibility of the myocardium, the developed tension, and the Starling curve to be compared for animals of the various lines and the effect of IC on these parameters to be determined. It will be clear from Fig. 1 that the initial parameters of myocardial contractility were the same in animals of all lines. However, the effect of stress on the parameters of contractility differed significantly in animals of different genetic lines. A substantial decrease in extensibility was observed after stress in WKY rats, and was most marked when the external load was small and the developed tension decreased and the plateau of the Starling curve was depressed by more than half, in agreement with earlier observations [6]. Similar depression of extensibility and contractility developed under the influence of stress in August rats. In spontaneously hypertensive SHR rats, on the other hand, stress caused no significant changes in these parameters.

The results of the next stage of the experiment to study the effect of an excess of calcium in the myocardium agreed with these, at first sight, paradoxical findings and, at the same time, they revealed an essential feature distinguishing the myocardium of SHR rats, detectable even before exposure to stress. This feature is an increase in duration of the inotropic effect of an excess of calcium and an increase in resistance of the control SHR rats to calcium contracture compared with normotensive animals. In fact, at the first minute of calcium contracture the tension developed by the isolated atrium of normotensive WKY and August rats increased by 200 mg, whereas this increase for the atria of hypertensive SHR rats was 604 mg (Table 1). At the 5th minute of action of an excess of calcium this difference was still present. Meanwhile the magnitude of the calcium contracture, characterized by resting tension, was 300 mg or more for the atria of normotensive rats but under 300 mg for the atria of SHR rats ($P < 0.05$). These data agree with the fact establish-

TABLE 1. Response of Atrial Myocardium of Rats of Different Genetic Lines to an Increase in Calcium Concentration in the Working Solution

Parameter studied	Line of rats	Initial level (Ca^{++} = 2.5 mM)	Duration of calcium loading (Ca^{++} = 7.5 mM), min	
			1	5
Developed tension (T_d), mg	WKY	345,0±15,0	545,0±55,6	504,0±57,1
	SHR	296,7±37,6	900,0±117,2	720,5±98,0
	August	293,0±29,1	511,7±43,2	405,7±40,2
Heart rate (F), beats/min	WKY	210,0±11,3	213,3±11,2	256,7±9,6
	SHR	176,0±7,5	177,5±19,3	224,0±20,4
	August	256,7±10,9	266,7±13,3	293,3±21,1
Magnitude of contracture (T_o), mg	WKY	—	0	344,0±62,7
	SHR	—	0	193,3±43,7
	August	—	21,7±6,0	300,0±28,3

TABLE 2. Effect of Prolonged Stress on Index of Contracture (in %) of Atrial Myocardium of Rats of Different Genetic Lines during Increase in Calcium Concentration in Working Solution

Line of rats	Series of experiments	Calcium loading (Ca^{++} = 7.5 mM) for 5 min
WKY	I. control	86,72±21,93
	II. stress	217,26±38,85
	P_{I-II}	<0,01
SHR	III. control	57,43±10,76
	IV. stress	43,95±12,87
August	V. control	111,19±12,37
	VI. stress	153,19±12,85
	P_{V-VI}	<0,05

ed by the same experiment that the latent period of calcium contracture is 65 and 93 sec for intact rats of the normotensive lines but 140 sec for SHR rats, i.e., significantly longer ($P < 0.05$).

The effect of stress on development of calcium contracture of the myocardium of the various lines of rats is illustrated by the data in Table 2. After exposure to stress the index of calcium contracture of the myocardium rose significantly in WKY and August rats, whereas in SHR rats the potentiating effect of stress on the development of contracture was absent.

Thus in SHR rats prolonged stress did not depress the extensibility, developed tension, or increase in calcium contracture of the myocardium. The myocardium of intact SHR rats responded to excess of Ca^{++} by a more marked positive inotropic effect; it showed greater resistance to calcium contracture than the myocardium of normotensive lines of rats.

The fact that the myocardium of hypertensive rats was more resistant to calcium loading can be explained in terms of the distinguishing features of the myocardium of SHR rats which have recently been established. Data in the literature [9] indicate that the sarcoplasmic reticulum (SR) isolated from the left ventricular myocardium has a tendency toward increased binding and uptake of Ca^{++}. The authors cited also demonstrated experimentally that phosphorylation of membrane proteins in SR of SHR rats, which is usually coupled with Ca^{++} transport, also is increased, and this may point to increased activity of the Ca-pump of the cardiomyocytes in hypertensive rats. Hypertrophy of the myocardium, known to exist in SHR rats [4], also was confirmed by the present experiments. According to observations by Lushnikova et al. [4], this type of hypertrophy is accompanied in SHR rats by an almost twofold increase in the relative area of SR membranes. Under these circumstances there is a specific increase in the surface area of SR relative to volume of myofibrils. This compensatory hypertrophy may be the basis for increased calcium transport into the myocardial SR and, consequently, of the greater resistance to calcium loading.

When the increased resistance of the myocardium to stress in SHR rats is assessed it must be recalled that stress injuries of the heart and, in particular, disturbances of calcium

transport develop through β-adrenoreceptors [6, 7]. The myocardium of SHR is known to have depressed adrenoreactivity on account of a reduction in the number of β-adrenoreceptors [11] or of their affinity [12] for catecholamines and a lower cAMP level in the heart muscle [8].

In the mechanism of the increased resistance of the heart to stress injury we can thus now identify two components: first, a reduction in adrenoreactivity of the myocardium and, consequently, a quantitative reduction of the stress reaction in the heart, and second, increased efficiency of operation of the calcium pump of SR in the cardiomyocytes, disturbance of which plays an important role in the development of stress-induced damage to the heart.

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