EFFECT OF SHORT- AND LONG-TERM IMMOBILIZATION STRESS ON Ca-PUMPING FUNCTION OF THE SARCOPLASMIC RETICULUM AND RESISTANCE OF THE HEART TO Ca^{++} EXCESS

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There are sharp functional differences in the dynamics of development of two types of stress: stress of fight and the stress of capitulation. The stress of fight, which is most widespread in the animal world, is characterized in its initial stages by mobilization of the physiological capacity of the body as a whole and of the myocardium in particular, and this is expressed by improvement of the parameters of its contractile function [3]. With an increase in the duration of the stress of fight, damage to the heart is observed [2, 4]. On the other hand, as has recently been shown [6], capitulation stress differs sharply from the stress of fight in the dynamics of its development. The contractile function of the isolated hearts of animals subjected to short-term stress and their resistance to contracture and to the arrhythmogenic action of high Ca^{++} concentrations are depressed, whereas during long-term capitulation stress these parameters do not differ from those in the control. It is logical to suggest that this dynamics is determined by the state of the membrane systems of Ca^{++} transport and, above all, the Ca-pump of the sarcoplasmic reticulum (SPR) of the cardiomyocytes.

The aim of this investigation was accordingly to compare contractile function of the myocardium and the Ca-transporting system of SPR during long- and short-term capitulation stress.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-230 g. Immobilization capitulation stress was produced by fixing the animal by all four limbs in the supine position. The physiological and biochemical investigations were conducted 2 h after the end of exposure to stress. The ECG and mechanical activity were recorded on the isolated heart, perfused by Langendorf's method, as described previously [5, 8]. The recording system included an isotonic TD-112S movement transducer and specialized modules of the PM-6000 polygraph (Nihon Kohden). The amplitude of apicobasal shortening of the heart, the rate of contraction and relaxation, and changes arising in response to a 4-min increase in the $CaCl_2$ concentration in the solution from 1.36 to 10 mM (the magnitude and area of contracture, the number of extrasystoles, the number of cases of fibrillation of the heart) were measured. The magnitude of contracture was expressed as a percentage, the amplitude of stable contractions being taken as 100%. The area of contracture was determined planimetrically and also expressed as a percentage, taking the area of contraction as 100%.

Ca^{++} transport in the vesicles of SPR was determined in homogenates of rat myocardium, using a Ca-selective electrode, on an Orion EA 940 ionometer, as described by the writers previously [7].

Calculations based on the experimental curves of Ca^{++} transport and statistical analysis were carried out on an "Olivetti" minicomputer.

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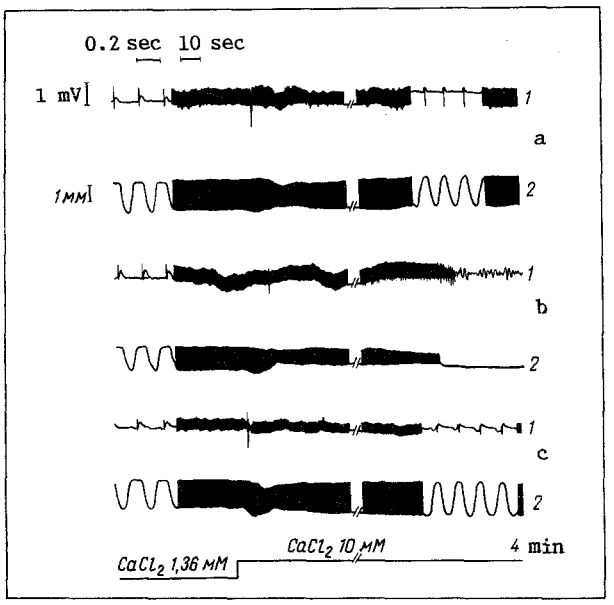

Fig. 1. Effect of immobilization stress of different duration on mechanical and electrical activity of the isolated rat heart under the influence of excess Ca^{++} concentrations. 1) Electrocardiogram, 2) amplitude of isotonic contraction (upper border of curve corresponds to level of diastolic relaxation). a) Control, b) 2 h of stress, c) 6 h of stress. Time of replacing normal solution by hypercalcium solution indicated by a step.

EXPERIMENTAL RESULTS

The data in Fig. 1 and Table 1 show that in the presence of physiological calcium concentrations stress for 2 h caused a significant decrease in the amplitude of contraction and the rate of relaxation. With an increase in the Ca^{++} concentration by 7.3 times, the peak value of contracture and also its area in these animals were increased almost threefold compared with the control. In more than half of these animals, irreversible ventricular fibrillation developed after 3-4 min of perfusion with the hypercalcium solution. This showed conclusively that after immobilization stress of the capitulation type for 2 h the resistance of the heart to the arrhythmogenic and contracture-inducing action of an excess of Ca^{++} considerably reduced. During stress lasting 6 h, the parameters of contracture and extrasystoles were virtually identical with the control, and ventricular fibrillation was not recorded.

The principal stage of the study was to compare physiological parameters of the contractile function of the heart and of the function of the Ca-transporting system of SPR during capitulation stress of varied duration. It will be clear from Fig. 2 that, with an increase in the Ca^{++} concentration in the medium, the rate of its transport into SPR of the myocardium of the control animals increased, and flattened out on a plateau in the region of 10-20 μM Ca^{++} increased, and flattened out on a plateau in the region of 10-20 μM Ca^{++}. After 1 h of stress the appearance of the curve was significantly altered. The maximal velocity of Ca^{++} transport was observed when Ca^{++} was present in a concentration of 7.5 μM, but it was only 9.4 nmoles/min/g protein compared with 16.2 in the control. With a further increase in the concentration, a phenomenon not observed in the control took place: inhibition of Ca^{++} transport. Since in the presence of 20 μM Ca^{++} the rate of its transport was reduced by more than one-third compared with the peak value in this series, and it was 3.5 times less than in the control. In long-term stress (curve 2 in Fig. 2) improvement of the parameters of functioning of the Ca-pump of SPR was observed. The maximal rate of Ca^{++} transport achieved during 6 h of stress was almost twice as high, and it was reached only with Ca^{++} in a concentration of 12 μM, compared with 7.5 for 1 h of stress. In addition, besides depression of the rate of Ca^{++} transport at high concentrations, only a tendency for this phenomenon to develop was observed in this situation. As a result, the rate of Ca^{++} transport after immobilization stress for 6 h, and in the presence of the highest Ca^{++} concentration used (20 μM), was reduced by only 25% compared with a reduction by 78% of the control level in the case of stress lasting 1 h.

TABLE 1. Effect of Immobilization Stress of Varied Duration on Parameters of Contractility, Arrhythmia, and Contracture of Isolated Heart with an Increase in Ca^{++} Concentration in the Perfusion Solution ($M \pm m$)

Parameter	Control (n = 15)	Immobilization stress, 2 h (n = 11)	Immobilization stress, 6 h (n = 8)
HR, beats/min	274±8	269±8	272±6
Ampl. of shortening, mm	2,60±0,08	2,07±0,16*	2,66±0,14
Vol. of contraction, mm/sec	51,0±2,8	42,8±3,4	54,6±3,6
Vol. of relaxation, mm/sec	57,6±3,0	46,8±4,2*	64,6±3,8
Magnitude of contracture, % of initial ampl. of contraction	21,0±4,2	59,2±17,1*	18,3±6,0
Area of contacture, % of area of contraction	10,0±1,8	35,8±10,1*	9,4±3,9
Extrasystoles	5±2	7±3	3±2
Ventricular fibrillation, number of cases	0	6*	0

Legend. *$p < 0.05$: significance of differences compared with control; n) number of experiments.

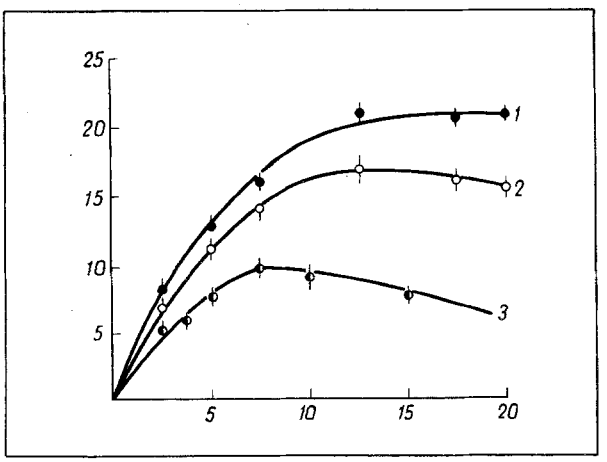

Fig. 2. Effect of duration of immobilization stress on Ca-transporting system of myocardial SPR. Abscissa, Ca^{++} concentration in medium (in μM); ordinate, rate of transport (in nmoles Ca^{++}/mg protein/min). 1) Control, 2) Stress for 6 h, 3) stress for 1 h.

Since the efficiency of Ca^{++} transport in SPR depends both on the intensity of working of the Ca-pump and on the degree of integrity of the SPR membranes, which may be influenced, in particular, by lipid peroxidation (LPO) products, our previous findings [1] relating to the intensity of LPO induced in vitro in heart homogenates from rats exposed to immobilization stress of varied duration, are interesting. It was shown that during long-term (6-h) immobilization stress accumulation of malonic dialdehyde did not differ from that in the control, whereas during stress for only 1 h, a much higher oxidative capacity was found than in the control.

The biochemical data thus show conclusively that the ability of SPR to take up Ca^{++} is disturbed during short-term immobilization stress. This is evidently not some biochemical, in vitro phenomenon, for it was shown above that it is short-term immobilization stress which greatly potentiates the ability of the isolated heart to respond by contracture to an increase in the

Ca^{++} concentration in the perfusion fluid. Consequently, the results of the physiological and biochemical investigation are in complete agreement in that it is short-term immobilization stress which disturbs the ability of SPR to take up Ca^{++} and potentiates the development of the calcium contracture to a greater degree than long-term stress.

Another important circumstance, discovered on comparison of the biochemical and physiological parameters of the work of the heart, connected with calcium homeostasis of the myocardium, must also be mentioned, namely the fact that the significant damage observed to the Ca-transporting system arises distinctly earlier (during 1 h of stress) than disturbance of functioning of the myocardium, revealed by the physiological investigations (2 h of stress). This fact can be explained on the grounds that damage connected with activation of LPO, phospholipases, and so on, has already appeared in the early stages of the damaging action of stress on cell membranes and, in particular, on the membrane of SPR. However, in the intact cell, these lesions are blocked and have no effect whatever on cardiomyocyte function. During destruction of the cells by homogenization, these lesions, which remain potential, are realized and lead to the disturbances of Ca^{++} transport in SPR of the myocardium described above.

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