STRESS-INDUCED MODIFICATION OF THE SARCOPLASMIC RETICULUM Ca TRANSPORT SYSTEM OF THE HEART AND ITS RESISTANCE TO ENDOGENOUS DAMAGING FACTORS

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Prolonged exposure to stress is followed by lowering of the fibrillation threshold of the heart [10] and the syndrome of poststressor myocardial rigidity [2], while dependence of the function of the isolated heart on the Ca^{++} concentration in the surrounding solution rises significantly. Biochemical investigations have shown a decrease in the Ca-ATPase activity of the sarcoplasmic reticulum (SPR) and disturbance of Ca^{++} binding in the SPR membrane [7], and electron-microscopic studies have demonstrated an excess of Ca^{++} in the sarcoplasm of the cardiomyocytes [8]. These facts have led to the view that an extraordinary adrenergic effect, associated with long-term exposure to stress, induces damage to SPR, disturbs the Ca homeostasis of the cardiomyocytes and, as a result, leads to depression of the contractile function and of the electrical stability of the heart.

The aim of the investigation described below was accordingly to study the effect of stress on function of the Ca-pump in medium containing different concentrations of Ca^{++}, and also the resistance of this process to factors causing endogenous damage to the heart associated with autolysis.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200 g. Immobilization stress was induced by fixing the animals by all four limbs on their back for 6 h. The rats were killed by decapitation 2 h after stress, and the hearts were removed, washed with physiological saline, and frozen in liquid nitrogen until required for use. The tissue was homogenized in an "Ultra-Turrok" homogenizer for 30 sec with the 25 N-10 blade in position 8. The homogenization medium contained 100 mM KCl, 20 mM imidazole, pH 7.8, and 20% glycerol; ratio of tissue/medium was 1:4.

Ca^{++} transport was determined on an "Orion EA 940" ionometer with Ca-selective electrodes. The method consisted essentially of measuring the rate of absorption of added Ca^{++} by vesicles of the SPR in the presence of potassium oxalate which, while not entering the vesicles of the sarcolemma, prevented Ca^{++} transport through it, and Ca^{++} accumulation by the mitochondria was prevented by the addition of NaN_3. The velocity of transport was measured for 5 min by adding 50-200 μl of homogenate in 5 ml of medium containing 100 mM KCl, 15 mM potassium oxalate, 20 mM HEPES (pH 7.0 at 37°C), 4 mM $MgCl_2$, and 5 mM NaN_3. ATP and $CaCl_2$ were added to the incubation medium immediately before determination up to final concentrations of 4 mM and 1-20 μM, respectively. The Ca^{++} concentration in the ATP should not exceed 0.002%. Because of the nonlinear characteristic curves of the electrode, calibration was carried out against Ca^{++} in medium with ATP by recording changes in millivolts in response to the addition of Ca^{++} in doses of 25 nmoles. The reaction velocity was determined from an experimental curve by drawing a tangent at the point 1.5 min from the start of the reaction, and taking account of the calibration curves. The calculations and statistical analysis were undertaken on an "Olivetti" computer.

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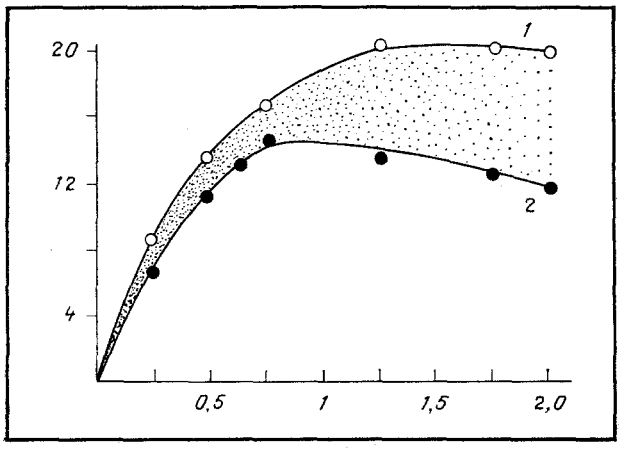

Fig. 1. Dependence of velocity of Ca^{++} transport in myocardial SPR of control (1) and stressed (2) rats on Ca^{++} concentration in incubation medium. Abscissa, Ca^{++} concentration (in $M \cdot 10^{-5}$); ordinate, velocity of transport (in nmoles Ca^{++}/mg protein/min).

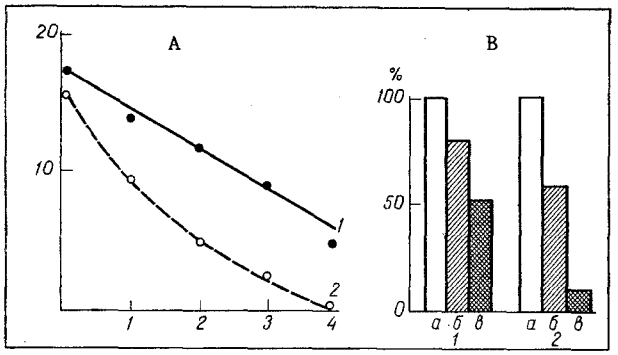

Fig. 2. Effect of duration of keeping heart homogenates from control (1) and stressed (2) rats on Ca-transporting capacity of SPR. A: Abscissa, days of keeping; ordinate, velocity of transport (in nmoles Ca^{++}/mg protein/min). B: Degree of reduction of Ca^{++} transport activity in SPR from initial value (a), taken as 100% both in the control (1) and after stress (2), and after keeping for 1 (b) and 3 (c) days.

EXPERIMENTAL RESULTS

Dependence of the velocity of Ca^{++} transport into SPR on its concentration in the medium is illustrated in Fig. 1. Clearly, with an increase in the Ca^{++} concentration the rate of its transport into SPR of the myocardium of the control animals rose, to flatten out on a plateau in the region of $(1\text{-}2) \cdot 10^{-5}$ M. As a result of exposure to stress the velocity of Ca^{++} transport fell: in 5 μM Ca^{++} from 13.6 to 11.2 nmoles Ca^{++}/min/mg protein, i.e., by 20%, and the maximal velocity (V_{max}) was recalled with a concentration as low as 7.5 μM. With a further increase in the Ca^{++} concentration in the medium the Ca-pump of the myocardium of the stressed animals not only did not increase its activity, but was actually inhibited, thus leading to an increase in the difference between the velocities of Ca^{++} transport in the control and after stress to 40%. This state of affairs was particularly important from the physiological point of view, for we know that cascadelike accumulation of Ca^{++} in the cardiomyocytes takes place as a result of ischemia, reperfusion, myocardial infarction, and stress-induced damage [8, 9, 14]. Intensive Ca^{++} transport inside the SPR and externally through the sarcolemma is necessary to normalize its level. However, during stress, activity of Ca^{++} transport in SPR is inhibited by an excess of Ca^{++}, the concentration of which continues to rise, thus inhibiting its removal from the sarcoplasm even more. A distinctive vicious circle is formed, and may lead to irreversible consequences: focal contractural damage and cardiosclerosis.

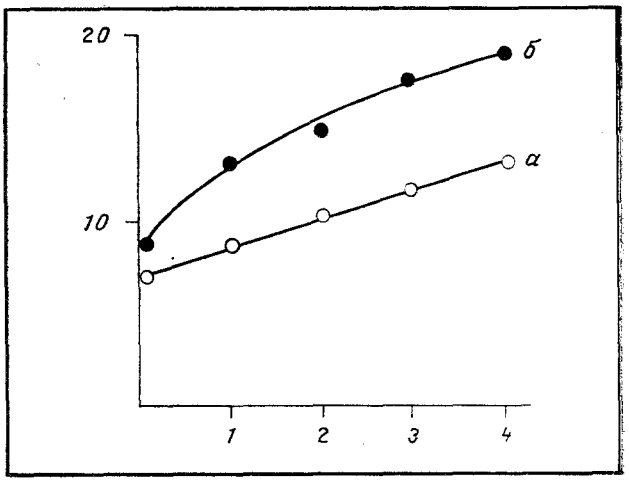

Fig. 3. Accumulation of free Ca^{++} during keeping rat heart homogenates in control (a) and after stress (b). Abscissa, days of keeping; ordinate, Ca^{++} concentration (in nmoles/20 mg tissue).

Values of V_{max} and K_M of the Ca^{++} transport process were calculated by kinetic analysis. As a result of stress, with a very small change in V_{max}, K_M increased by only one-third – from 13.2 to 17.2 μM, evidence of a marked decrease in affinity for Ca^{++}. Modification of the structure of the Ca-pump (K_M) thus makes a greater contribution to the total reduction of the effectiveness of its function in stress than a simple increase in the number of molecules or of turnover of the enzyme. Because of the membrane location of the Ca-pump, such changes can be associated with conformational changes in the protein due to changes in its lipid microenvironment. The latter are very probable in stress, for in that state lipid peroxidation (LPO) and phospholipases have been shown to be activated, and the concentration of free fatty acids and of lysophospholipids in the blood and myocardium are increased, and as has been shown, this leads to increased membrane permeability and uncoupling of transport ATPases [4]. The action of these endogenous factors may be realized to a greater degree in autolysis, in which activation of the same natural factors capable of destroying cellular structures is recorded, namely: activation of LPO [1], of phospholipases [3], and of proteases [11], and labilization of lysosomes [15].

With this in mind we studied inhibition of Ca^{++} transport in SPR in homogenates kept at 4°C. The dynamics of inactivation of this process evidently reflects the action, on the one hand, of proteases, phospholipases, and oxidizing agents, and on the other hand, of antioxidants, and protectors of proteins and lipids against destruction. However, such an integral parameter may give an idea of the unified state of the cell and of relations between the factors mentioned above and other in it.

It will be clear from Fig. 2 that as a result of stress the decrease in the rate of Ca^{++} transport during keeping takes place much faster than in the control. For instance, whereas the initial rate during stress was 90% of the control value, after keeping for 3 days it was only 17%, and by the 4th day no Ca^{++} transport could be recorded. This is evidence of a marked decrease in the resistance of SPR to endogenous degradation factors, in agreement with our data [12] showing a threefold increase in the intensity of LPO during stress when induced in heart homogenates in vitro. Disturbance of the antioxidative status of the cell may perhaps be one cause of the decrease in stability of the process.

During keeping a gradual increase in free Ca^{++} concentration takes place in the homogenates, in connection with autolytic damage to the structures of SPR and mitochondria and the release of Ca^{++} from them. It will be clear from Fig. 3 that the initial Ca^{++} concentration in myocardium was 25% higher after stress than in the control, but during keeping, the Ca^{++} concentration during stress increased almost twice as fast as in the control. Incidentally, the rate of release of Ca^{++} from SPR may be determined both by the degree of integrity of integrity of the membranes and its resistance during keeping, and to the quantity of Ca^{++} absorbed by the membrane depots of this ion in vivo.

The investigation thus showed that as a result of exposure to stress the velocity of Ca^{++} transport in SPR is significantly reduced, and this reduction is more marked if the Ca^{++} concentration is increased. Kinetic analysis demonstrates the more important contribution of internal conformational changes in protein to these processes, changes which, as the data on autolysis confirm, are due to a change in state of the lipid microenvironment of the enzyme. Reduction of the stability of

Ca^{++} transport and disturbance of the integrity of the SPR membrane during keeping are perhaps connected with activation of intracellular damaging factors during stress.

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