DISTURBANCE OF MECHANISMS MAINTAINING THE RESTING POTENTIAL OF CARDIOMYOCYTE MEMBRANES DURING STRESS AND ITS PREVENTION

I. L. Yasinskii, V. V. Malyshev, and F. Z. Meerson

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Emotional and painful stress itself can induce some decrease of the resting potential (RP) of the cardiomyocyte membrane, but it has a more marked depressive effect on recovery of RP after its reduction caused by cooling of the heart. In fact, during stress the rate of recovery of RP is reduced by 2.5-3 times, and the hyperpolarization phase, which usually completes the recovery process, is not realized [8]. A study of the mechanism of this phenomenon showed that activity of the main enzyme system responsible for restoration of RP, namely Na,K-ATPase, is only slightly reduced by stress (by 15-20%), but the rate of its thermoinactivation rises sharply. The antioxidant ionol radically prevents these disturbances [9]. It has therefore been suggested that antioxidants and other factors limiting the stress reaction can, in principle, prevent a disturbance in the system maintaining RP of the cardiomyocyte membrane after stress. The aim of this investigation was to assess the possibility of preventing disturbances of recovery of the cardiomyocyte membrane potential in animals exposed to stress with the aid of sodium γ-hydroxybutyrate (GHBA), a metabolite of the stress-limiting system, and also of the antioxidants α-tocopherol and ionol.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 200-240 g. Stress was induced by fixing the animal in the supine position for 6 h at ordinary room temperature. The animals were decapitated 2 h after immobilization under ether anesthesia, the hearts were removed and transferred into a thermostatically controlled chamber at 36°C, in which they were perfused by Langendorff's method under a hydrostatic pressure of 100 mm Hg with oxygenated (95% O_2 + 5% CO_2) Krebs–Henseleit solution of the following composition (in mM): NaCl – 118, KCl_4 – 85, $CaCl_2$ – 1.67, KH_2PO_4 – 1.2, $MgSO_4$ – 2.45, $NaHCO_3$ – 25.2, glucose – 11.0. Processes responsible for maintaining RP of the cardiomyocyte membranes were assessed by a test consisting of incubation of the isolated heart in the cold, leading to "loading" of the cardiomyocytes with Na^+, lowering of RP, and on subsequent heating, to hyperpolarization of the membranes [10-12]. For this purpose, after preliminary recording of RP of the cardiomyocytes on the surface of the working left ventricle with a glass microelectrode, the isolated hearts were incubated for 1.5 h in a solution at 4°C, and then reperfused at 36°C. Investigation of the dynamics of the changes in membrane potential after heating perfusion gave information on the transitional characteristics of recovery of membrane potential. The rate of its recovery was assessed in the period of half-maximal development of the membrane hyperpolarization effect. To assess the possibility of preventing poststress disturbances of the processes responsible for maintenance and recovery of RP, we used GHBA, which was injected intraperitoneally 3 times in a dose of 100 mg/kg: 0.5 h before immobilization of the animals, and 2 and 4 h later in the course of creation of the stress model. The antioxidants also were injected intraperitoneally: ionol in a dose of 60 mg/kg 48, 24, and 1 h before stress, and α-tocopherol in a single dose of 100 mg/kg 24 h before stress.

EXPERIMENTAL RESULTS

The results showed that the transitional characteristics of the change in RP in the control were as follows: the rate of recovery of the cardiomyocyte membrane potential after

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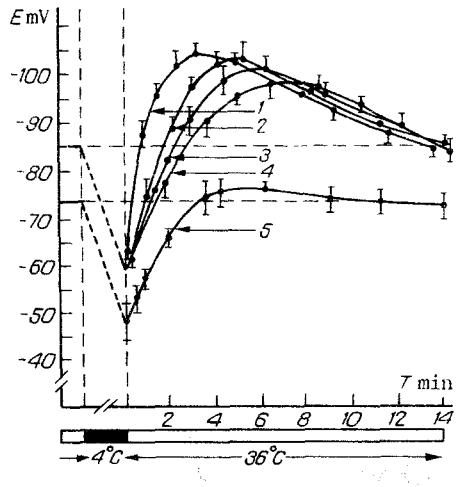

Fig. 1. Transition characteristics of recovery of cardiomyocyte membrane potential of the working heart after incubation for 1.5 h at 4°C (M ± m). 1) Control, 2) action of GHBA + stress, 3) action of ionol + stress, 4) action of α-tocopherol + stress, 5) after immobilization stress.

incubation in the cold was 0.35 ± 0.5 mV/sec and the maximal value of the hyperpolarization effect was 103 ± 3 mV, within 3 min of heating reperfusion of the heart (Fig. 1, curve 1). RP fell 2 h after immobilization (−75 ± 2 mV) during cold incubation to −48 ± 3 mV, and its recovery during heating reperfusion took place more slowly (0.15 ± 0.01 mV/sec, $p < 0.05$). The hyperpolarization phase of the membranes in the recovery period was absent, and recovery of the membrane potential to its original values was not observed until the 5th-6th minute (Fig. 1, curve 5). In animals of the control group the original RP was higher (−86 ± 2 mV, $p < 0.05$) and it fell during cold incubation to −60 ± 2 mV ($p < 0.05$). The fact will be noted that cold incubation in intact and stressed animals depolarized the cardiomyocyte membranes by virtually the same amount, namely 26-27 mV.

Analysis of the protective effect of the antioxidants showed that their preliminary administration to the animals largely prevented the poststress reduction of RP before and after incubation in the cold and significantly normalized the restoration of cardiomyocyte membrane potential during reheating prefusion. Thus the rate of restoration of the membrane potential after stress, produced against a background of ionol, increased (0.25 ± 0.03 mV/sec, $p < 0.05$), whereas hyperpolarization of the membrane reached −100 ± 4 mV (Fig. 1, curve 3). The protective effect of α-tocopherol was weaker than that of ionol.

Stress-induced inhibition of activity and changes in the molecular properties of Na,K-ATPase [9] are thus realized as corresponding changes in processes maintaining RP of cardiomyocytes, and antioxidants prevent these changes. In agreement with this conclusion are our data indicating that ionol can prevent inhibition of activity and the change in molecular properties of Na,K-ATPase during stress [7, 9].

GHBA more effectively prevents inhibition of restoration of RP during stress than antioxidants (Fig. 1, curve 2). In animals exposed to stress against the background of GHBA the rate of recovery of the cardiomyocyte membrane potential was 0.3 ± 0.03 mV/sec, and the maximal value of membrane hyperpolarization was 101 ± 4 mV, which is the closest to the corresponding parameters of their recovery in intact animals.

The results of this investigation revealed significant disturbances in animals exposed to stress in the system maintaining RP of the cardiomyocytes, in which it had substantially smaller values. Under poststress conditions, a transmembrane balanced ionic current, essential for stabilization of RP, can be established only in the presence of a lower electrochemical membrane potential, and the fall in RP by itself is determined by a transient unbalanced ion exchange. The real causes of this decrease may be connected both with lowering of the specific resistance of the injured sarcolemmal membranes, which have nonspecific transmembrane leakages of ions, and with disturbance of the activity of Na,K-ATPase itself, realizing the pumping currents. In fact, considerable disturbances of cardiomyocyte activity

of Na,K-ATPase itself, realizing the pumping currents. In fact, considerable disturbances of cardiomyocyte activity are known during stress, in which they are expressed as damage to the cardiomyocyte membranes [1, 4], the development of conditions of energy deficit [2], and lowering of activity and disturbance of the conformational stability of Na,K-ATPase [7, 9]; taken as a whole these lead to depression of contractile activity and to focal necrosis of the myocardium [3-6]. Probably the hypothetical causes of lowering of the cardiomyocyte membrane RP are not alternatives. Consequently, in rats cold incubation of myocardial tissues leads to an initial fall of RP followed by hyperpolarization of the membranes during heating. These effects can be explained by cold inhibition and subsequent reactivation of the Na-pump, which exhibits electrogenic properties [10-12]. Meanwhile, under conditions of quick heating reperfusion, it was possible to assess the transition characteristics of restoration of the membrane potential, expressed through the rate of the recovery process. The absence during stress of the hyperpolarization effect and the more than twofold decrease in the rate of repolarization of the membrane, when depolarized by cold incubation, is evidence of considerable disturbances of the processes responsible for restoration of the membrane potential. Finally, analysis of the results also shows that cold incubation of cardiomyocytes depolarizes the membrane virtually by the same amount. This, in our view, is evidence that the permeability constants of the membrane for ions, whose concentration gradients determine the decrease in RP, are similar. Consequently, early stress-induced disturbances of mechanisms maintaining and restoring the cardiomyocyte membrane potential are determined by a greater degree by a disturbance of activity of the sarcolemmal ion pump than by a disturbance of integrity of the cell membranes.

Assessment of the possibility of preventing disturbances of the mechanisms maintaining and restoring MP of the cardiomyocyte membranes showed that α-tocopherol and, to an even greater degree, ionol possess a marked protective action by preventing disturbances of electrogenesis during stress. However, the protective effect of antioxidants proved to be incomplete, suggesting that besides the damaging action of products of lipid peroxidation on the membrane and membrane-bound enzymes, other pathogenetic factors giving rise to disturbances may also be involved.

In fact, a metabolite of the central stress-limiting system such as GHBA virtually abolishes disturbances caused by stress. It has been suggested that the greater efficacy of GHBA is due to its stronger antistress action than antioxidants, limiting the stress reaction, and consequently, the excessive release of catecholamines and leukocorticoids [7], which determine both activation of lipid peroxidation and inhibition of other metabolic processes maintaining the cardiomyocyte membrane potential.

The study of the role of all the stages in the stress-induced mechanism giving rise to functional and structural changes in cardiomyocyte membranes will evidently help to make further progress in the analysis of methods of preventing stress-induced heart damage.

LITERATURE CITED

1. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow 91972).
2. V. V. Malyshev, V. I. Lifant'ev, and F. Z. Meerson, Kardiologiya, No. 5, 113 (1982).
3. V. V. Malyshev, E. N. Ekimov, L. M. Belkina, et al., Byull. Éksp. Biol. Med., No. 8, 148 (1984).
4. F. Z. Meerson, V. V. Malyshev, V. E. Kagan, et al., Arkh. Patol., No. 2, 9 (1980).
5. F. Z. Meerson, L. N. Medvedev, L. Yu. Golubeva, et al., Byull. Éksp. Biol. Med., No. 8, 61 (1982).
6. F. Z. Meerson, N. V. Samosudova, E. V. Glagoleva, et al., Arkh. Anat., No. 2, 42 (1983).
7. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Damage [in Russian], Moscow (1984).
8. F. Z. Meerson, I. L. Yasinskii, Yu. P. Koslov, et al., Byull. Éksp. Biol. Med., No. 2, 674 (1986).
9. T. G. Sazontova, Byull. Éksp. Biol. Med., No. 12, 685 (1986).
10. H. O. Glitsch, Am. J. Physiol., 236, 189 (1979).
11. H. O. Glitsch, Basic Res. Cardiol., 79, 611 (1984).
12. T. Tamai and S. Kagaijama, Circulat. Res., 22, 423 (1968).