EFFECT OF STRESS ON CARDIOMYOCYTE TRANSMEMBRANE POTENTIAL OF THE WORKING HEART AND ITS RECOVERY AFTER HYPOTHERMIA

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UDC 612.172.4-06:613.863

KEY WORDS: emotional-painful stress; myocardial damage; electrogenic sodium pump; effect of membrane hyperpolarization.

Emotional-painful stress (EPS) lowers the electrical threshold of fibrillation and may cause arrhythmias and cardiac arrest in man [13]. In complete agreement with this fact it has been shown that EPS damages cardiomyocyte membranes [7] and regularly depresses activity of membrane-bound enzymes, namely Na,K-ATPase [5, 6] and Ca-ATPase [2], which play the principal role in active cation transport and maintenance of the transmembrane potential (TMP).

The aim of this investigation was to study how the damaging action of stress is reflected in the value of TMP and the ability of the cationic pumps to restore this potential after its temporary fall.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 220-230 g. EPS was produced over a period of 6 h by the method in [8]. The rats were decapitated 30 min after exposure to stress, simultaneously with control animals, and the hearts were removed and perfused by Langendorf's method under a constant hydrostatic pressure of 100 mm Hg at 36°C, so that both electrical and contractile activity was preserved. Krebs–Henseleit solution (pH 7.4), oxygenated with a mixture of 98% O_2 and 2% CO_2, was used for perfusion. TMP were derived by glass microelectrodes with a resistance of 25-50 MΩ. The potential difference, i.e., the TMP, was measured by the voltage compensation method and was recorded after puncture of the cardiomyocyte membrane. For this purpose a voltage of opposite sign, from an I-01 imitator, was led to the input of a source follower, through a relay. The accuracy of measurement of TMP was ±1 mV. The principal experimental method was based on the known hypothesis that heart muscle, like any other tissue with high energy metabolism, responds by a varied degree of activation of the Na,K-pump to an increase in the intracellular Na concentration. As a rule, this leads to the development of a membrane hyperpolarization effect [9, 10]. A simple and easy procedure, leading to inhibition of energy metabolism and accumulation of intracellular Na, is incubation of the preparation in a solution of 4°C for a few hours. As has been shown on cat [14] and guinea pig [11] heart papillary muscles, rewarming of the preparation leads to an increase of TMP as a result of an increase in the pump flow and an increase of Na removal from the cells. Accordingly, after measuring the initial TMP, we cooled the heart to 4°C, and then rewarmed it to 36°C. The initial value of TMP, the rate of its recovery during rewarming of the heart, and the level of hyperpolarization after rewarming were used as the criteria of activity of the cationic pumps.

EXPERIMENTAL RESULTS

In the control (n = 9) incubation of the isolated hearts for 1.5 h at 4°C initially depressed TMP at rest from 80 ± 2 to 45 ± 2 mV (Fig. 1a). Changing the temperature conditions from 4 to 36°C led to recovery of TMP and of hyperpolarization, the peak value of which was 110 ± 3 mV after 2.5 min. The rate of recovery of TMP at the time corresponding to half of the maximal development of the membrane hyperpolarization effect was about 0.7 mV/sec. When

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 0007-4888/86/0012-1658$12.50

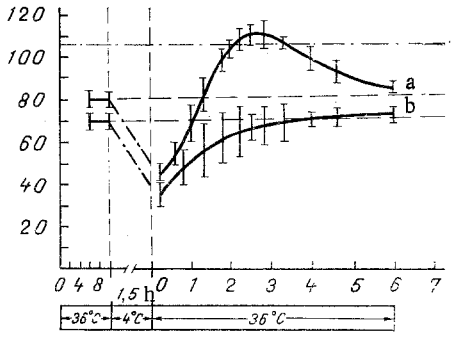

Fig. 1. Recovery of TMP at 36°C after incubation of heart at 4°C for 1.5 h. Abscissa, duration of experiment (in min); ordinate, negative value of TMP (in mV). a) control; b) stress.

hyperpolarization reached its maximum it began to fall and became stabilized after 3.5-4 min, when TMP was close to the resting potential (84 mV). Spontaneous cardiac rhythm was restored when TMP reached a value of 60-65 mV.

Changes in TMP 30 min after EPS (n = 11) were different in character (Fig. 1b). Before incubation in the cold the mean value of TMP fell, and at rest it was 70 ± 2 mV. After incubation in the cold, it was initially 35 ± 2 mV, but later increased and regained its initial value after 5-6 min. During recovery the hyperpolarization effect of TMP was not present and the maximal rate of recovery did not exceed 0.2 mV/sec, which is less than one-third of the control value. Rewarming of some of the preparations led to the onset of fibrillation when TMP was 60-65 mV. In these cases no further increase in TMP took place.

EPS thus significantly disturbs the electrogenic capacity of the cationic pumps and, evidently of the Na,K-pump in particular. This is reflected both in the absence of membrane hyperpolarization effect and the sharp fall in the rate of recovery of TMP and, consequently, the density of the pump flow also. When this fact is evaluated two considerations must be borne in mind. The first is that one probable cause of the observed decrease in the rate of recovery of TMP and absence of hyperpolarization during rewarming of the hearts of animals exposed to stress is a disturbance of transport of high-energy phosphate groups from mitochondria to the cationic pumps of the plasma membrane. Evidence that this phenomenon does actually occur during stress-induced myocardial damage is given by the fact that the normal ATP concentration is combined with a fall in activity of creatine kinase and acid phosphatase and in the glycogen concentration [1], i.e., with disturbances in systems responsible for energy transport to membrane cationic pumps. The contribution of direct damage to the membrane and of an increase in its permeability for cations is evidently small initially, as also is the decrease in TMP at rest, and according to some data, it is realized in the later period after exposure to stress [3, 4].

The second consideration is that the clinical importance of latent disturbance of the work of cationic pumps and the maintenance of membrane potential which we observed during stress may most probably be realized through strong adrenergic influences on the heart, which have been shown to play a role in the genesis of stress-induced arrhythmias and fibrillation of the heart [13]. Tachycardia, developing under these conditions, is initially coupled with an increase in the intracellular concentration of sodium, which enters the cardiomyocytes with each action potential. In the intact myocardium the response of activation of the Na,K-pump is so great that a hyperpolarization phenomenon takes place, with an increase in TMP and depression of the intrinsic ventricular automation [15]. However, in foci of stress-induced damage the absence of sufficiently effective response activation of the Na,K-pump may lead to a fall of TMP, with the development of ectopic beats and fibrillation of the heart. It is important to note that a similar situation may be realized with even greater probability in acute myocardial infarction, where circumscribed focal injury to the heart is combined with EPS and stress-induced damage to the heart as a whole [1]. It can be tentatively suggested that disturbance of activity of the cationic pumps by stress plays an essential role in the genesis of disturbances of electrical stability of the heart and of dangerous disturbances of the cardiac rhythm.

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ANTIAGGREGATING ACTION OF SODIUM HYPOCHLORITE ON PLATELETS

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UDC 615.281:546.133.1].015.4:612.111.7-064

KEY WORDS: platelets, plasma, aggregation, hypochlorite, ADP.

Sodium hypochlorite (NaClO), obtained by electrolysis of sodium chloride solutions, possesses bactericidal properties and is therefore used in medicine as an external disinfectant [1, 2]. To study the possibility of further application of NaClO in medicine and, in particular, the possibility of its percutaneous absorption, its action on blood components must be studied.

EXPERIMENTAL METHOD

Platelet-enriched plasma (PEP) from rabbit blood was used [7]. Platelet aggregation induced by addition of 0.1 ml of ADP solution (pH 7.4, 10 μM) to 1 ml of PEP, was recorded by a turbidimetric method [3] on an aggregometer made in the writers' laboratory [5]. The main quantitative parameter of platelet aggregation was the maximal change of light transmission by PEP (degree of aggregation), recorded 7-8 min after addition of ADP.

Absorption spectra of ADP and mixtures of it with NaClO were recorded on the DU-7 spectrophotometer (Beckman, USA) in a cuvette 1 cm thick.

EXPERIMENTAL RESULTS

NaClO, added to PEP in a final concentration of 2-4 mM approximately 1 min before addition of ADP, had a strong antiaggregating action, leading to a decrease in the initial rate of platelet aggregation (the tangent of the angle of slope of the initial segment of the curve showing an increase in transmittance) and the degree of aggregation (Fig. 1; Table 1). A sim-

Research Institute of Physicochemical Medicine, Moscow. N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 12, pp. 676-678, December, 1986. Original article submitted July 9, 1985.

 0007-4888/86/0012-1660$12.50