action of cytoplasmic glycopeptide on transport of HCO_3^- ions through the IMM is intensified. A further increase in pH of the incubation medium increased the basal permeability of the IMM for HCO_3^- ions, but reduced the cytoplasmic glycopeptide-dependent component of transport (Fig. 2). A similar increase in permeability of IMM for NO_3^- and Cl^- ions with an increase in pH of the incubation medium has been described in the literature [10, 11].

The experimental results are evidence that, like transport of NO_3^- and Cl^- ions, electrogenic transport of HCO_3^- ions through the IMM is effected by a pH-dependent anionic pore; functioning of this pore, moreover, is controlled by cytoplasmic glycopeptide. To make this action of cytoplasmic glycopeptide on HCO_3^- ion transport manifest, the MC must be preincubated in its presence, under conditions of energization of the organelles. Without preincubation, the effect of stimulation of HCO_3^- transport does not appear. Addition of respiration inhibitors or of 2,4-DNP to the preincubation medium of MC abolished the action of the glycopeptide on HCO_3^- ion transport (Fig. 1A). Meanwhile inhibitors of respiration and 2,4-DNP do not affect glycopeptide-induced bicarbonate ion transport.

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MEMBRANE-BOUND Ca^{2+} IN MITOCHONDRIA OF EHRLICH'S ASCITES TUMOR CELLS

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KEY WORDS: Ca^{2+} ions; mitochondria; Ehrlich's ascites carcinoma.

Ehrlich's ascites tumor cells, like cells of many other malignant tumors, are characterized by significant changes in energy metabolism [13] and the Ca^{2+} transport system [10]. Investigations have shown that changes in the Ca^{2+} transport system are the primary causes of changes observed in energy metabolism of tumor cells [6, 7]. We know, for instance, that mitochondria (MC) of tumor cells can assimilate and retain unusually high quantities of Ca^{2+} and that even low concentrations of this ion inhibit their oxidative phosphorylation [2, 7, 15].

The concentration of bivalent ions in MC is an important parameter determining their functional activity [11]. However, insufficient attention has so far been paid to the study of the mechanisms regulating the free Ca^{2+} level in MC.

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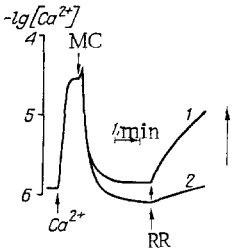

Fig. 1

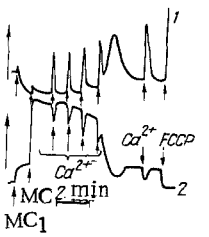

Fig. 2

Fig. 1. Changes in Ca^{2+} ion activity in suspension of MC from liver (1) and from Ehrlich's ascites tumor cells (2) during energy-dependent accumulation and efflux of Ca^{2+}. Substances added: $CaCl_2$ 50 μM, ruthenium red (RR) 10 μM. Arrow on right indicates increase in Ca^{2+} concentration in medium. Here and in Fig. 2, incubation medium: sucrose 0.15 M, KCl 0.05 M, P_i 1 mM, Tris-HCl buffer 10 mM, succinate 5 mM, glutamate 5 mM, MC 2 mg protein/ml.

Fig. 2. Changes in activity of Ca^{2+} ions (1) and fluorescence of pyridine nucleotides NADH (2) during Ca^{2+} transport in suspension of a mixture of MC from tumor cells (MC_t) and from liver cells (MC_l). Substances added: $CaCl_2$ 200 nM in each case, FCCP 1 μM.

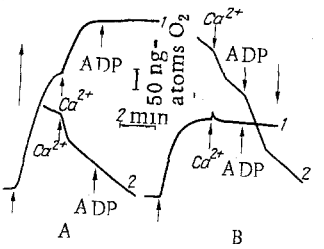

Fig. 3. Changes in intensity of fluorescence of CT (1) and O_2 consumption (2) of MC from tumor cells (A) and MC from liver cells (B) in response to addition of Ca^{2+} (50 nanomoles/mg protein) and 200 μM ADP. Incubation medium the same as in Fig. 1, + 20 μM CT.

This paper describes a study of certain effects of Ca^{2+} ions on permeability and oxidative phosphorylation in MC of Ehrlich's tumor cells with an increased concentration of membrane-bound (bound in hydrophobic regions of membranes and proteins) Ca^{2+}.

EXPERIMENTAL METHOD

The diploid strain of Ehrlich's ascites carcinoma, transplanted intraperitoneally into noninbred albino mice, was used. The cells were harvested 10 days after transplantation, separated from ascites fluid by centrifugation (125g, 5 min), and MC were isolated from them [15].

To estimate accumulation of Ca^{2+} by MC we used chlortetracycline (CT), a fluorescent chelate label of membrane-bound Ca^{2+} [1, 9], the intensity of fluorescence of which was measured in the 550 nm region, with excitation at 405 nm. Changes in fluorescence of pyridine nucleotides were recorded at 450 nm. The Ca^{2+} ion concentration in the incubation medium was measured with an ion-selective electrode, and the O_2 concentration by a closed electrode of Clark's type. Measurements were made on the apparatus described previously [3], whereby changes in the above parameters could be recorded simultaneously.

EXPERIMENTAL RESULTS

The Ca^{2+} concentration in the cytoplasm is a critical value for many cell functions. MC, with their high affinity for Ca^{2+}, can play an important role in regulation of the free Ca^{2+} concentration in the cytoplasm.

The Ca^{2+} concentrations which enabled MC from liver and tumor cells to be maintained in the external medium were compared by means of the Ca^{2+}-sensitive electrode. On the addition of MC from tumor cells to a suspension of liver MC, a fall in the steady-state Ca^{2+} concentration was observed on average by 0.4 μM (from 1.1 to 0.7 μM). To show that the Ca^{2+} level in the medium was independent of the quantity of MC over a wide range of concentrations, further additions of the same MC were made. The steady-state Ca^{2+} level was found to remain unchanged under these circumstances. MC of tumor cells thus can maintain a lower Ca^{2+} concentration in the external medium than liver MC. This may be due both to activation of the electrogenic Ca^{2+} accumulation system and also to inhibition of the electrically neutral Ca^{2+} efflux system in MC from tumor cells.

Activity of the electrically neutral Ca^{2+} transport system was estimated from the rate of Ca^{2+} efflux from MC in the presence of ruthenium red (RR), an inhibitor of energy-dependent Ca^{2+} accumulation [8]. It will be clear from Fig. 1 that the rate of Ca^{2+} efflux from tumor MC was many times slower than from liver MC.

We know that liver MC, even in the presence of ATP, are uncoupled on massive loading with Ca^{2+} ions, swell, and quickly lose Ca^{2+} ions [2]. This was not observed in MC from Ehrlich's ascites tumor cells. Increased resistance to Ca^{2+} and increased Ca^{2+}-capacity of MC of Ehrlich's ascites tumor cells compared with liver MC are clearly illustrated by Fig. 2. A mixture of liver MC and of tumor MC, equalized with respect to protein, was introduced into the measuring cell and the Ca^{2+} concentration gradually raised. After the fourth addition, release of Ca^{2+} from liver MC followed by their influx into tumor MC were observed; after this had taken place, the latter were still capable of transporting newly added Ca^{2+}. Transfer of Ca^{2+} from liver MC into tumor MC was demonstrated by fluorescence of pyridine nucleotides, which was much lower in tumor MC. Fluorescence of pyridine nucleotides of tumor MC was on average 3.5-4 times lower than that of liver MC. The data on Ca^{2+} accumulation in a mixture of MC from control and tumor cells, shown in Fig. 2, are of fundamental importance in connection with new information on the mechanism of Ca^{2+} efflux from MC. It has recently been found that spontaneous Ca^{2+} efflux in a suspension of MC is determined by their heterogeneity, so that the most labile MC are destroyed at the beginning of efflux [14]. The Ca^{2+} thus liberated is later reaccumulated in more stable MC, and this also leads to destruction of their membranes. As will be clear from Fig. 2, tumor MC are not subject to this avalanche process.

The Ca^{2+}-capacity of tumor MC, their resistance to loading with Ca^{2+} ions, and also activity of the system of electrically neutral Ca^{2+} efflux may be determined by the particular kind of Ca^{2+} distribution found in MC of tumor cells.

To assess the state and redistribution of Ca^{2+} in MC, we used CT, which forms a highly fluorescent complex with bivalent metals in the hydrophobic region of membranes [9]. The writers demonstrated previously [1] that Ca^{2+} is redistributed between the matrix of MC and their membrane, depending on the inorganic phosphate concentration (P_i) and pH. During Ca^{2+} transport in liver MC in the presence of high concentrations of P_i (3-5 mM) no increase in fluorescence of CT took place. A complex of Ca^{2+} with P_i evidently is formed and the quantity of membrane-bound Ca^{2+} is not increased. In tumor MC, under similar conditions, the concentrations of free and membrane-bound Ca^{2+} were considerably increased, and the Ca^{2+} was probably fixed on the membrane enzymes.

Investigation of dependence of the intensity of CT fluorescence and the rate of Ca^{2+} efflux, in the presence of RR, on the Ca^{2+} concentration accumulated by MC of Ehrlich's ascites tumor cells showed that, with an increase in Ca^{2+} concentration of 50 nanomoles/mg protein the rate of its efflux from the tumor MC fell, and this correlated with the increase in the intensity of CT fluorescence, i.e., with the membrane-bound Ca^{2+} concentration. It can be tentatively suggested that binding Ca^{2+} with the membrane of the tumor cell MC induces definite structural changes in phospholipids of the bilayer, leading to reduced activity of the H^+/Ca^{2+} exchange system. Incidentally, there is evidence in the literature that binding of bivalent ions with bilayer lipid membranes increases their rigidity and reduces their permeability [12].

Another important property of tumor MC is the high sensitivity of ADP-induced respiration to small quantities of Ca^{2+} [15]. We found that the same 50 nanomoles Ca^{2+}/mg protein almost completely prevented the phosphorylating respiration of Ehrlich's ascites tumor cell MC (Fig. 3A), but this was not observed in liver MC (Fig. 3B). The degree of inhibition correlated with the intensity of fluorescence of CT, which evidently indicates that this process depends on the concentration of membrane-bound Ca^{2+}.

It can be postulated that membrane-bound Ca^{2+} plays a key role both in the regulation of energy processes and in the stabilization of permeability of the tumor cell MC membrane. What distinguishes tumor MC from liver MC is that even low concentrations of Ca^{2+} lead to an increase in membrane-bound Ca^{2+}. As a result of this, the viscosity of the membrane may be increased and activity of membrane-bound enzymes and ion-transporting systems increased.

The high concentration of membrane-bound Ca^{2+} may be determined by the abnormal phospholipid composition of the tumor MC membranes, which are distinguished by higher affinity for Ca^{2+} [4]. Low activity of ruthenium-sensitive Ca^{2+} transport, in turn, readily explains the long familiar phenomenon of lowering of the steady-state Ca^{2+} level in tumor cells.

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MECHANICAL NOISE OF THE MYOCARDIUM AS AN INDICATOR OF ACTIVITY OF THE SARCOPLASMIC RETICULUM

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Mechanical noise (MN, spontaneous oscillations of tone) in the mammalian myocardium was first described and studied about 10 years ago [8]. During this period research into relations between MN and oscillations of intracellular calcium ion concentration [4, 13], the level of contracture of the preparation [3], fluctuations of membrane potential [10], and also the ability of a myocardial preparation to undergo spontaneous excitation [2] have been published. It has been shown [1, 4, 13] that substances which inhibit activity of the sarcoplasmic reticulum (SPR) lead to disappearance of MN. This suggested that the appearance of MN is linked with activity of SPR. We know that the rate of relaxation of the myocardial contractile response can be used as an indicator of the ability of SPR to bind calcium. It was therefore interesting to compare changes in MN and the rate of relaxation under various conditions modulating activity of SPR, and the investigation described below was undertaken for this purpose.

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