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Ca^{2+}-transporting ATP-hydrolyzing systems activity in lymphocytes of peripheral blood from men with erectile dysfunction

Aktywność systemów hydrolizujących ATP zależnych od Ca^{2+} w limfocytach krwi obwodowej mężczyzn z zaburzeniami erekcji

INTRODUCTION

The most widely spread male sexual disorder is erectile dysfunction (ED), which is a stable disability to achieve and keep erection [8, 10]. 40–52% of male from age group 40 plus have different forms of this disease. It is proved that 63–69% have ED of psychogenic origin, 9–20% of natural origin and 6-11% have a combined form [1, 15].

The main risk factors are depression and psychological traumas, diabetes mellitus, alcohol misuse, hypertension, atherosclerosis, hyperlipidhemia [7, 11, 14, 15]. Organism adopts to stress factors (one of which is erectile dysfunction) with the help of nervous, endocrine and immune systems [4, 12, 13].

In recent years we have noticed a increasing interest in lymphocytes of peripheral blood caused by their role in processes influencing the level of homeostasis of the whole organism. Lymphocytes are involved in pathological process not only in the conditions of blood diseases; they suffer serious structural and functional changes in the conditions of different genesis diseases [3, 4].

An important index of the functional condition of cells, especially lymphocytes, is ionic homeostasis, especially homeostasis Ca^{2+}, which is supported by Ca^{2+}-transportation systems. Ca^{2+} is an intracellular messenger and it regulates almost all cell functions, especially contraction-relaxation of muscles [2, 14]. Functions of Ca^{2+}, Mg^{2+}-ATPase in plasmatic membrane and endoplasmatic reticulum which provides the level of intracellular calcium homeostasis are under a modulating influence of both external and internal cellular factors. We assume that lymphocytes can be an appropriate model for studying biochemical indexes in the development of erectile dysfunction.

The aim of the research was to study the activity of Ca^{2+}-dependent ATP-hydrolases on the example of lymphocytes as the model of peripheral blood of males of different age groups with erectile function disorders. Both general Ca^{2+},Mg^{2+}-ATPases activity and the activity of this ferment in plasmatic membrane and endoplasmatic reticulum were studied with the use of appropriate blockers of ATPases.

MATERIAL AND METHODS

The objects of the research were lymphocytes of peripheral blood of clinically healthy men (group 1, control), men with psychogenic mono syndrome erectile dysfunction in the age group 18–29 years (group 2) and men of age group 50–63 years with a mixed form of erectile dysfunction influenced by dyslipidhemia, hypertension, abdominal obesity and partial androgenic deficiency (group 3). Mononuclear lymphocytes of peripheral blood were extracted from heparinized freshly taken blood in the gradient concentration of philol-urographine [3]. The number of cells was counted in Horyayev camera using 0.1% tripane blue as a pigment. Viability of lymphocytes in all tests results was not lower then 95%. To discover latent ATPase activities, a 0.2% solution of saponine was added to, lymphocyte suspension [14].

General Na^+,K^+,Mg^{2+}-ATPases activity was tested in incubation environment (mM): 30 NaCl, 120 KCl, 5 $MgCl_2$, 3 ATP, 3 Hepes-Tris buffer (pH 7,4), 0.2 % saponine solution, 0.5 mg proteine/ml. Testing of Ca^{2+},Mg^{2+}-ATPase activity was conducted in the presence of 10 μM $CaCI_2$, a Ca^{2+}-independent - 1 mM EGTA [9]. For testing Ca^{2+},Mg^{2+}-ATPase activity of plasmatic membrane and endoplasmatic reticulum, a number of non specific and specific blocker of ATPases were used; they were eosine Y (1 mM), tipsigargine (0.1 μM), oubaine (1 mM), sodoum azide (1 mM) [14]. Incubating time – 5 min, temperature 37^0 C. The statistical analysis of the obtained data was made using Student criteria. Statistic and kinetic calculations were made on IBM PC.

RESULTS AND DISCUSSION

Cells Ca^{2+},Mg^{2+}-ATPase activity is one of the indexes which reflect their functional condition [2, 14]. It is known that a cell has two Ca^{2+}-depending ATPases: Ca^{2+},Mg^{2+}-ATPase of plasmatic membrane and Ca^{2+},Mg^{2+}-ATPase of endoplasmatic reticulum [14].

General Ca^{2+},Mg^{2+}-ATPase activity of saponine permiabilized lymphocytes of peripheral blood of clinically healthy men of age group 20–29 years shows 10.7±0.8 μM P_i/h · protein mg (n=48), men of group 2 – 10.5±0.8 (n=27), group 3 - 8.1±0.7 μM P_i/h · protein mg (n=14). It's discovered that diabetes mellitus of 1st and 2nd type the Ca^{2+},Mg^{2+}-ATPase activity is reduced [9]. The level of saturated acid increases and the level of polyunsaturated acid decreases which results in a decreasing level of membrane-joined ATPase activity. Active LP processes in pathological condition results in changes in membrane-joined ferments activity level, especially of Na^+,K^+-ATPase.

Using specific and not specific blockers of different ATPases, we identified the contribution of each of them to the keeping of Ca^{2+}-homeostasis in the cell (Fig. 1).

The component of general ATPase activity which was inhibited by specific blocker Ca^{2+},Mg^{2+}-ATPase of endoplasmatic reticulum 0.1 μM tapsyharine on the background of action Na^+,K^+-ATPase blocker oubaine (1 mM) and H^+-ATPase mitochondrial blocker sodium azide (1 mM) in control point was equal to 2.6±0.3 μM P_i/hour · protein mg. The component which was additionally inhibited by 1 mM eosyne Y was equal to 2.8±0.3 μM P_i/h · protein mg. This is the activity of Ca^{2+},Mg^{2+}-ATPase lymphocytes plasmatic membrane.

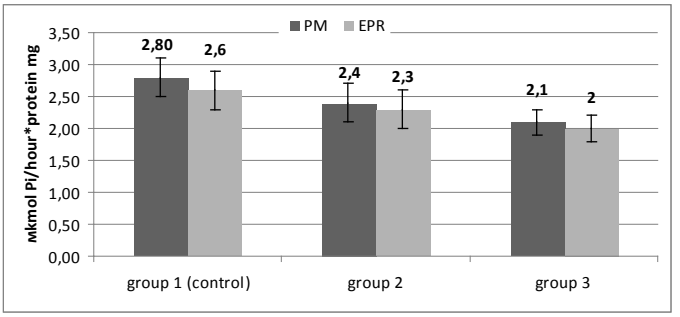

Fig. 1. Ca^{2+},Mg^{2+}-ATPases activities of plasmatic membranes and endoplasmatic lymphocytes reticulum of peripheral blood

The second group of men showed the activity of ferment in PM – 2.55±0.22 μM P_i/h · protein mg, and in EPR – 2.32±0.22 μM P_i/h · protein mg. Group 3 showed the activity of PM Ca^{2+},Mg^{2+}-ATPase equal 2.13±0.2 μM P_i/h · protein mg and the activity of EPR Ca^{2+},Mg^{2+}-ATPase equal – 2.06 ±0.2 μM P_i/h · protein mg. We can see that the activity of Ca^{2+},Mg^{2+}-ATPase both PM and EPR decrease in conditions of erectile dysfunction but a more significant decrease takes place as the age of the patients grow. So in conditions of erectile dysfunction development, the activity of ferment decreases. It results in slowing down of outflow Ca^{2+} from cytosole and this can be evidence of the fact that the concentration Ca^{2+} in the cell increases. Accumulating of Ca^{2+} in the cells and a decrease of ATP level results in a break down of ionic pumps and overload of cytosole with calcium. On the other hand, increasing Ca^{2+} concentration results in activation of Ca^{2+}-dependant phospholipase A_2 which stimulates accumulation of lipophractions and free fatty acids in membranes [2]. These data confirm others which show that high accumulation of calcium ions in lymphocytes in conditions of diabetes mellitus and hypertension is caused by decreasing Ca^{2+},Mg^{2+}-ATPase activity in the plasmatic membrane and by modulation of Na^+/Ca^{2+}-exchange. Increasing of ionized calcium concentration in cytoplasm in different pathologic conditions is widely spread. These data are confirmed by those received on platelets [5, 6]. For example, in condition of hypertension to avoid excessive flow of Ca^{2+} to cells blockers of Ca^{2+}-channels are widely used (diltiasem, nifedipine, nicardipine etc) [2].

Disorder in the functioning of the complicated system of Ca^{2+}-connecting and Ca^{2+}-transporting mechanisms logically causes disorders in calcium homeostasis, regulation function Ca^{2+} and numerous pathological changes and metabolicic shifts which are harmful to the cells.

CONCLUSIONS

Erectile dysfunction of combined genesis in the presence of dislipidemia is accompanied by both decreasing of general Ca^{2+},Mg^{2+}-ATPase activity, and Ca^{2+},Mg^{2+}-ATPase activity of plasmatic membrane and endoplasmatic reticulum of lymphocytes, which indicates the increase of ionized calcium concentration in cells and disorders in regulation mechanisms of the cells.

With aging of the patients with sexual function, disorders decreasing of Ca^{2+},Mg^{2+}-ATPases activity grow.

Lymphocytes of peripheral blood can become a convenient and appropriate model for studying erectile dysfunction mechanisms.

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SUMMARY

Ca^{2+},Mg^{2+}-ATPases properties in lymphocytes of peripheral blood from men with erectile dysfunction were studied. It was shown that with age total Ca^{2+},Mg^{2+}-ATPase activity and its activity in plasmatic membrane and endoplasmatic reticulum decreases, which leads to Ca^{2+} concentration growth in cytosole.

Keywords: Ca^{2+},Mg^{2+}-ATPase, lymphocyte, erectile dysfunction

STRESZCZENIE

Zbadano właściwości Ca^{2+},Mg^{2+}-ATPazy limfocytów krwi obwodowej u mężczyzn z zaburzeniami erekcji. Wykazano, iż wraz z wiekiem całkowita, jak też związana z błonami plazmatycznymi i retikulum endoplazmatycznym aktywność Ca^{2+},Mg^{2+}-ATPazy obniża się, co prowadzi do wzrostu stężenia Ca^{2+} w cytozolu.

Słowa kluczowe: Ca^{2+},Mg^{2+}-ATPaza, limfocyty, zaburzenia erekcji