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THE INFLUENCE OF THE TREATMENT BY THE CLEANED SOLUBLE PROTEINS TO THE FRAGMENTED SARCOPLASMIC RETICULUM

Key words: Human serum albumin; sarcoplasmic reticulum, passive permeability.

The main goal of this work was the supporting of the essential role of free fatty acids to the passive permeability of the basic muscles calcium store - sarcoplasmic reticulum, for the calcium ions. The probability of albumin molecules to absorb and release the free fatty acids and another absorbed substances under the certain variable environments was used for this problems decide. In order to solve this task the aqueous solution of human serum albumin (HSA) was freed from adsorbed hydrophobic ligands fully. The albumin molecules were treated by medium with low pH in presence of pharmaceutical activated charcoal. After deposition of coal by means of centrifugation, the pH of medium was changed back on physiology. In this case the albumin molecule bought its native globular form, but already has been free from all adsorbed substances, which were moved away together with charcoal. Further the preparations of the fragmented sarcoplasmic reticulum were treated with such clean albumin. The passive sarcoplasmic reticulum permeability for calcium ions decreased considerably. So, that the essential role of free fatty acids for the efficiency of basic calcium store of muscles - sarcoplasmic reticulum for the calcium ions was confirmed. And, than the albumin's absorption ability was confirmed by the cleaning of sarcoplasmic reticulum preparations from free fatty acids.

Ключевые слова: Сывороточный альбумин человека; саркоплазматический ретикулум; пассивная проницаемость.

Основной целью работы было подтверждение существенной роли свободных жирных кислот в проявлении пассивной проницаемости мембран саркоплазматического ретикулума, являющегося основным кальциевым депо в скелетных мышцах млекопитающих. Для решения этой задачи была использована способность молекул альбумина адсорбировать и освобождать свободные жирные кислоты и другие вещества при изменениях окружающей среды. Альбумин сыворотки крови человека (САЧ) в виде водных растворов полностью освобождали от адсорбированных гидрофобных веществ с помощью активированного фармацевтического древесного угля при низких значениях pH. После удаления угля центрифугированием возвращали pH раствора альбумина к нейтральному – физиологическому. Молекулы альбумина принимали нативную глобулярную форму, полностью освобожденную от адсорбированных веществ. Инкубация с «чистым» САЧ фрагментированного саркоплазматического ретикулума значительно снижала пассивную проницаемость мембран для ионов кальция.

Introduction

The basic calcium store of striated muscles of mammals spaced at 40 Å from plasmalemma, and are mechanically connected by means of long proteins with potential-depended channels at the cell surface. The longitudinal tubules exist at the muscle cell interior at middle parts. So, that all contractile apparatus - actomyosine complex of striated muscle, were braided of sarcoplasmic reticulum net.

For the initiation of contractile apparatus activity the bursts of the calcium ions concentration are need obligatory. The relaxations of muscle are needed by calcium ions concentration lowering. The net of sarcoplasmic reticulum (SR) is exercising both functions. It activates the muscle contraction, increasing the concentration of calcium ions, when release of ions from terminal cisternae occurred. The relaxation of the muscle begins, when pumped the ions in to inside lumen of reticulum store. The calcium release occurred by the ryanodine receptor (RyR) activation. And pumping of calcium ions occurred by the Ca^{2+} - Mg^{2+} -dependent ATPase (SERCA2) activation. SERCA2 is located previously at the longitudinal tubules, and smaller portions of SERCA are located at the terminal tubules for the maintaining of Ca^{2+} -gradient through the SR membrane. RyR is located at the terminal cistern only. It is exhibited apart to the junction space between SR and plasmalemma.

Our work deals with two model objects that imitated the sarcoplasmic reticulum structure and

activity. The two fractions of fragmented reticulum consist of the suspension of isolated membrane bubbles. Fragmented SR (FSR) are the sarcoplasmic reticulum. The sarcoplasmic reticulum is the br. Fragmented SR (FSR) was prepared: from the terminal cistern – the heavy caffeine-sensitive fraction. From the longitudinal tubules we prepared the light fraction that was separated into the 2 sub-fractions – caffeine-sensitive and caffeine-insensitive. The ryanodine receptor (RyR) is the main Ca^{2+} -releasing channel. RyR's activity is regulated by Ca^{2+} and a numerous of the endogenous and the pharmacological ligands (RyR was blocked or activated by the plant alkaloid – ryanodine, and only was activated by methylxanthin – caffeine and its agonists [1]).

The luminal, transmembrane and cytoplasmic satellite proteins: calsequestrin, triadin, juncin and others, modulate the function of RyR also under the certain values of the extra vesicular – cytoplasm ($[\text{Ca}^{2+}]_{\text{cyt}}$) and the luminal Ca^{2+} -concentrations ($[\text{Ca}^{2+}]_{\text{lum}}$). The necessary values of $[\text{Ca}^{2+}]_{\text{cyt}}$, $[\text{Ca}^{2+}]_{\text{lum}}$ are based on the functional activity of the RyR and Ca^{2+} -pump - Ca^{2+} -ATPase. The gradient of Ca^{2+} - $[\text{Ca}^{2+}]_{\text{lum}}/[\text{Ca}^{2+}]_{\text{cyt}}$, is created and maintained by coordination of Ca^{2+} -ATPase and RyR activities. Data [2] suggested, that $[\text{Ca}^{2+}]_{\text{lum}}/[\text{Ca}^{2+}]_{\text{cyt}}$ may be the key factor, involved in the regulation of the functional states of Ca^{2+} -ATPase and RyR of Ca^{2+} -store - SR. The maximal value of the calcium loading of the Ca^{2+} -store (3mM) induced the Ca^{2+} -releasing process through the Ca^{2+} -channel of RyR, inducing the muscle contraction. In this case the Ca^{2+} -ATPase is inhibited completely, and no pumps the

amount of the released Ca^{2+} to the reticular lumen. Minimal Ca^{2+} -concentration at the reticular lumen (0-0.3mM) completely inhibits the Ca^{2+} release through the RyR, and activates the Ca^{2+} -ATPase, resulting in the Ca^{2+} -reuptake from the cytoplasm, and followed by muscle relaxation. The middle Ca^{2+} -concentration (1 mM) corresponds with the equilibrium position of the system Ca^{2+} -releasing/ Ca^{2+} -pumping, when RyR and Ca^{2+} -ATPase may be activated both one after other. [3].

The efficiency of Ca^{2+} -accumulation ($\text{Ca}^{2+}/\text{ATP}$) by heavy FSR, and light FSR was recorded by the registration of the of incubation medium acidifications. These measurements we made, when we used Mg-ATP for Ca^{2+} ions pumping inside to the FSR lumen. The value of $\text{Ca}^{2+}/\text{ATP}$ reflects the amount of pumped Ca^{2+} ions to one molecule of hydrolyzed ATP.

The bilayer integrity of membrane provides the efficient operation of calcium stores. However when pathologies, in the process of ageing of membrane, when action of damaging factors, the regular structure of bilayer breaks down and the passive leakage of ions occurred, without any related work of specific channels. This is why it was necessary to show that when extraction from reticular membranes of free fatty acids occurred, which were accumulated in membrane at above case, the passive leakage decreased, that promoted the successful work store.

Materials and methods

The materials: KCl, KH_2PO_4 (Merck); histidine, imidasol, caffeine (Merck); NaCl, MgCl_2 (Merck); DTT (Serva); glycerol (Serva); CaCl_2 (Merck); sucrose (Merck); EGTA (Serva); PMSF (Helicon); HSA (Sigma).

The standard methods of isolation and the purification of FSR were modified. The first step was realized in the presence of DTT, PMSF and 10 mM caffeine, and with addition of aggregation stage in glycerine medium at final step [4-6]. FSR were prepared from white muscles back legs of rabbit. Muscle was cooled in physiological solution, and was crushed on meat grinder. Crushed muscles 200 g had placed in 600 ml of medium, containing 0,3 M sucrose, 10 mM caffeine and 10 mM histidine (pH 7,7 4⁰ C). Then it was homogenized at a temperature of 2-4⁰ C by means of homogenizer "Politron". The homogenate was centrifuged under 10 000g during 20 min. The supernatant was pelleted again by centrifugation 36 000 g during 60 min. The pelleted total fraction of membranes was extracted 60 min in the cold medium, containing: 0,6 M KCl, 0,1mM EDTA, 0,2 mM CaCl_2 , HSA (0,6mg/ml), 5 mM histidine (pH 7,4 4⁰ C). The suspension again centrifuged 11 000 g for the pelleted of fragments of terminal cisternae (TC). Then supernatant pelleted by centrifugation 40 000g during 60 min for the deposition of fragments of longitudinal tubules (LT). The obtained pellets in practice not contained of heavy mitochondrial fragments. For FSR holding pellets were suspended at storage medium, contained 25% glycerin (in volume of), 0,1mM EDTA, 0,2 mM CaCl_2 , 5 mM histidine (pH 7,4 4⁰ C). For following cleaning of factions, fractions were layered on storage medium and centrifuged 36 000 g during 60

min. Pellet - was the fragments, responsive to caffeine (TC), low layer of suspension - light fraction (LT). Protein content was standard for the FSR: TC contained the RyR, Ca^{2+} -ATPase and calsequestrin predominantly. LT contained the Ca^{2+} -ATPase predominantly and lesser amount of calsequestrin [7].

The protein concentration was determined by the fast method [8].

Efficiency of Ca^{2+} -accumulation by heavy FSR, and light FSR was recorded by potentiometric method (pH-metric) [9]. FSR vesicles (3-4 mkg/ml) were incubated in 4 ml medium, contained 2 mM ATP, 5mM sodium oxalate, 0,1M NaCl, 4 mM MgCl_2 , 2,5 mM imidasol (pH 6,8 37⁰ C) with intensive mixing. The pumping reaction was stimulated by additions of 80 nmoles CaCl_2 . The Ca^{2+} ions, accumulated into FSR, bounded with oxalate, and the calcium oxalate stored into the SR lumen. Thus capacitance FSR for Ca^{2+} -ions increased markedly. It becomes to perform the registration of absorption Ca^{2+} -ions by FSR eventually. In native muscle cells under the SR activation Ca^{2+} -ions communicates with luminal proteins - calsequestrin and others, and with phosphate. So that capacitance SR for Ca^{2+} -ions was maintained. The value of Ca^{2+} -ions saturating of Ca^{2+} -binding proteins is the essential regulator factor for RyR activity.

The loading FSR by the calcium oxalate into the SR lumen carried out in 40 ml of medium that used for the activity registration in standard conditions. The additions of CaCl_2 were held as Ca^{2+} -ions was accumulated into the FSR lumen. Reticulum (5 mg of protein) was loaded up to 500 nmol $\text{Ca}^{2+}/1$ mg protein. Then the mixture has cooled up to 4⁰ C and centrifuged during 1 hour. The FSR pellet was washed out on medium, contained: 2 mM ATP, 0,1 M NaCl, 5 mM Na oxalate, 0,5 mM imidasol (pH 7,0 4⁰ C). Then the loaded FSR was suspended in 1 ml of storage medium, contained 0,1 M NaCl, 0,5 mM imidasol (pH 7,0 4⁰ C).

The measurement of Ca^{2+} passive release was held by the pH-registration method [9], at incubation medium, contained: 0,1 M NaCl, 0,5 mM EGTA, 0,5 mM imidasol (pH 7,0 37-42⁰ C). The Mg^{2+} concentration varied from 0,1 mM up to 10 mM.

The cleaning of human serum albumin (HSA) from calcium and free fatty acids carried out by next method. Albumin 1g was solubilized in distilled water 5 ml, pH up to 3, 0-3, 5 (on ice).The pharmaceutical activated charcoal was added to albumin-water solution. This mixture was incubated 1 hour under the constant mixing. Then the charcoal was pelleted by centrifugation or filtering. And pH-value of supernatant (that contained of cleaned albumin) was led up to physiology (7,0). The dialysis against solution EDTA 10⁻⁴ M was held the daily on ice. The change of the solution occurred a few times. The cleaned albumin solution kept in frozen condition.

The cleaning FSR from free fatty acids (FFA) was held by means of cleaned albumin by the incubation way of membranes FSR at medium for the Ca^{2+} -transport registration, containing 2-5 mg FSR protein and 10-20 mg /ml of human serum albumin, refined from fatty acids, and 1,9mM ATP during 2 min (25-30⁰C). Then mixture was cooled centrifuged 40 000

g 60 min. The pellet was suspended in storage medium. The FSR cleanness was controlled by registries the Ca^{2+} transport at medium with low concentration of oxalate (1,5 mM) with and without albumin.

The extraction of lipid from FSR was made by Folch method [80,10] 20-25 mg of FSR protein in the 2 ml was homogenized by homogenizator "Politron" under the ice cooling. The medium (40 ml) for extraction consist of 2 part of chloroform, 1 part of methanol and antioxidant ionol (4 metil-2,6-ditertbutilphenol) (1mg/l). Homogenate was filtrated. Then 10 ml 0,1 M KCl were added. And this mixture was homogenized during 10 min. Homogenate was centrifuged 25000g during 40 min. Then the undersize of phospholipids in chloroform was being selected and was evaporating. The phospholipids were solubilized with methanol-geptan mixture (4: 1)

The building in free fatty acids in FSR membrane carried out by next procedures. The ethanol solution of free fatty acid was added to FSR membranes, suspended in storage medium (15-25 mg free fatty acids/1 mg FSR protein). This mixture was incubated during 3-4 hour (10-12° C) for more uniform distribution of fatty acid in FSR membrane

Results and discussion

As the Ca^{2+} -gradient play the great role for the SR functions regulation, we were needed to investigate some factors influences to the Ca^{2+} -store actions. One of these factors – is the membrane permeability for ions. Because, the Ca^{2+} -ions penetrate through the membrane by several ways, and Ca^{2+} -pumping by SERCA2 activity, and Ca^{2+} -releasing by RyR activity are under widespread intensive investigations, we reversed our attention on the nonspecific way - passive membrane permeability for Ca^{2+} -ions.

The part of our interests was deal with the influence of passive permeability on RyR that was expressed in sensibility of Ca^{2+} -transport to caffeine. Caffeine is the activator of intermediate state Ca^{2+} -channel gate of RyR, and respectively, it is the fine indicator of RyR functioning. However and the caffeine can affect to the manifestation of passive permeability. Also the significant factor can be the variation of ion concentration Mg^{2+} , without which Ca^{2+} -ATPase SR are not activated.

The increase of passive permeability we tried to cause by building in FSR membranes the unsaturated fatty acids. The lowering of passive permeability we tried to cause by extraction of free fatty acids from membranes FSR by means of albumin, cleaned from related materials.

The fractions of FSR, received by the fragmentation of terminal cisternae, in the presence of caffeine, reduce the efficiency of Ca^{2+} -accumulating that was shown by pH metric method [8]. And fractions, received from longitudinal tubules, didn't under the caffeine influences, when caffeine suspension (5-10 mM) was added to the incubating medium. The addition Ca^{2+} on incubation medium, contained Mg-ATP, Na-oxalate and FSR, leads to sharp activating of acidification rate at incubating medium. After a time the rate of acidification drops to datum level, that indicates

the accumulation of all amount of additional calcium by the reticulum bubbles. The rate of acidification of medium in absentia of Ca^{2+} is determined by the ATP hydrolysis by nonspecific enzyme - ATPase by the essentially. On ion increment H^+ and respectively, inorganic phosphate in transport process of Ca^{2+} , it is possible to calculate the effectiveness of transport process (the mean the value of $\text{Ca}^{2+}/\text{ATP}$). Than, the larger amounts of phosphate are accumulated at incubation medium when transport additional amount of Ca^{2+} occurred, that effectiveness quantity of transport Ca^{2+} - the value $\text{Ca}^{2+}/\text{ATP}$, become are smaller.

The value $\text{Ca}^{2+}/\text{ATP}$, become are smaller or bigger in dependence of passive permeability changes. And the disagglutinating action caffeine (unconjugation action to Ca^{2+} -transport/ATP hydrolysis effectively) may be mediated not only RyR activation, But the raise of passive permeability may take some part at this unconjugation action.

By this, we tested the caffeine actions to the Ca^{2+} -releasing from FSR under the low Ca^{2+} concentrations in registration medium (lowered 10^{-8} M) and without ATP. At this case the Ca^{2+} accumulating action of Ca^{2+} -pump can not be activated. The FSR was loaded by Ca-oxalate. Than the kinetic of Ca^{2+} release was registrated by pH-metric method with EGTA at medium. Under the binding of 1 Ca^{2+} -ion by EGTA, two H^+ are released from EGTA molecule (pH 7,0-7,1). The pH lowered. The graphics of depending of passive Ca^{2+} -releasing rate/ Mg^{2+} concentration from FSR are presented: FSR - TC (Fig. 1).

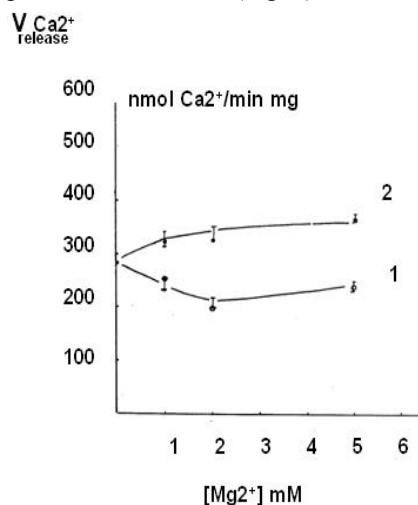


Fig. 1 - The action of Mg^{2+} to the caffeine influence to the Ca^{2+} -passive leaks from vesicular fragments of fractions of terminal cisterns. 1 - control; 2 - + 5mM caffeine

The passive Ca^{2+} -releasing from FSR TC (Fig.1) is in bimodal depending of Mg^{2+} concentrations at medium. The variation of Mg^{2+} concentrations from 0 to 5 mM leads to lowering, and than to increasing of passive Ca^{2+} -releasing rate. The caffeine additions increased the passive Ca^{2+} -releasing rate, but the variation of Mg^{2+} concentrations operate weakly.

The passive permeability of FSR LT changed very negligible under the Mg^{2+} concentrations from 0 to

5 mM. The caffeine (5 mM) additions not changed the passive Ca^{2+} -releasing too.

Thus, caffeine influenced to the passive permeability at FSR TC. The rate of passive Ca^{2+} -releasing was increased to 20%. But, this value doesn't very essential for value of Ca^{2+} /ATP-lowering under the caffeine actions. The value of Ca^{2+} /ATP lowered by 3 times under the 5mM caffeine additions to the FSR TC. Also the value of Ca^{2+} -transition by ATPase Ca^{2+} -transporting activity is 10 times as bigger than the passive permeability. Under different sets of conditions, caffeine doesn't influence to the passive permeability at FSR TC, and it lowered the value of Ca^{2+} /ATP as usually.

It is known that membrane of SR contains 0,65mg lipids/1mg protein. The phosphatidylcholine and phosphatidylethanolamine were the predominant phospholipids of SR. And SR contains FFA - less 2%. [11] [12]. The lipid composition FSR TC and LT is similar [13]. We investigated the FSR TC and LT lipid compositions for our case. Data presented at Fig.2.

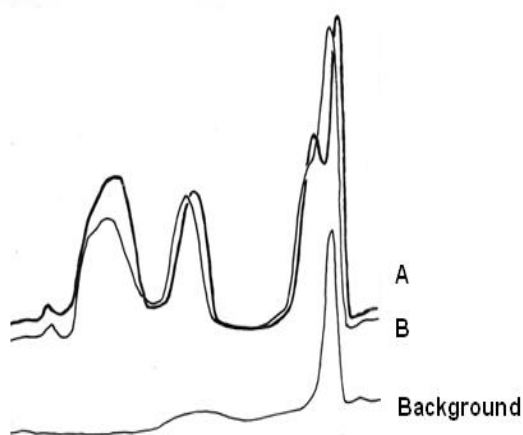


Fig. 2 - Scan of chromatographic plate with lipids, isolated from terminal cisterns (A) and longitudinal tubules (B), prepared from rabbit skeletal muscle. Chromatograms were carried out by methods [14, 15]: 1 - Acidic phospholipids 6,3 % (A); 5,0 % (B); 2 - Phosphatidylcholine 43 % (A); 35,6 % (B); 3 - Phosphatidylethanolamine 22,1 % (A); 25,2 % (B); 4 - Free fatty acids (FFA) 19,4 % (A); 20,1 % (B); 5 - Neutral lipids 9,1 % (A); 14,1 % (B).....

It is known that the FSR contain the 3-8 mg free fatty acids/1 mg protein. The unsaturated FFA exists at those membranes [16] As the membranes were washed from contaminated or integrated into bilayer FFA, the properties of passive permeability were changed. By these manipulations we used the FSR treatment with cleaned albumin.

The serum albumins are known as the carriers for any hydrophobic substances in blood. Respectively albumins also shall adsorb and meet to needed targets the biologically active substance of exogenous origin too. The level of saturation of albumin by hydrophobic molecules may be regulated. In our work the human serum albumin (HSA) was freed fully from adsorbed hydrophobic ligands if HSA was treated with the water suspension of pharmaceutical activated carbon when

low the pH-values (4-5) applied, that allowed <to deploy> the molecule of HSA. Carbon was precipitated by centrifugation. The reverse of pH up to physiology (6,5-7,0) resulted in solution receipt of native HSA, able to connect with a number of hydrophobe molecules. The albumin used for stabilizing membrane preparations by the extracting from bilayer of the free fatty acids (FFA). FFAs are the chaotropic agents, disturbing the crystalline structure of bilayer. HSA extracted of FFA from membranes of intracellular organelles - faction of sarcoplasmic reticulum (SR TC), obtained from white rabbit muscles (Fig. 3).

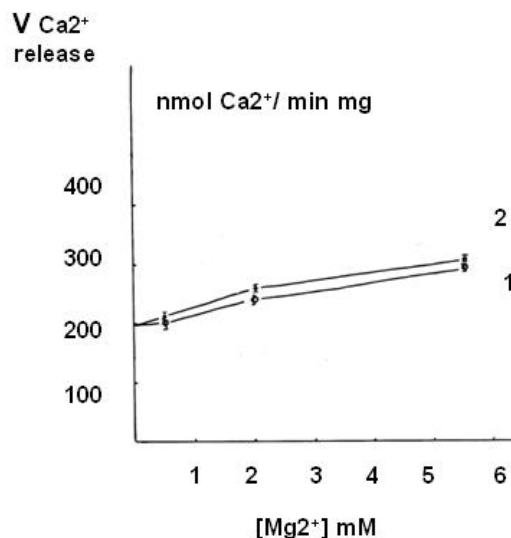


Fig. 3 - The action of Mg^{2+} to the caffeine influence to the Ca^{2+} -passive leaks from vesicular fragments of fractions of terminal cisterns. The free lipid acids were extracted from membranes of FSR with aid of clean albumin HSA. 1 - control; 2 - + 5mM caffeine

The dependence of rate Ca^{2+} -releasing from ion concentration Mg^{2+} with and without caffeine from vesicles of fragmented terminal cisterns are submitted at Fig.3. The FSR TC membranes were washed from free fatty acids.

In table 1 are submitted the data, reflecting the dependence of value of Ca^{2+} /ATP from free fatty acids presence at membranes of terminal cisterns when different ion concentrations Mg^{2+} . Data, presented at tables alludes to the fact that the presence or the absence of free fatty acids at membranes of terminal cisterns in practice didn't influenced as on caffeine effect to the Ca^{2+} -transport, and on the Ca^{2+} -transport dependence from ion concentration Mg^{2+} . But this treatment increased the value of Ca^{2+} /ATP.

So that becomes clear that extraction of free fatty acids from membranes of terminal cisterns in practice does not contribute to Ca^{2+} -ATPase function. Thus, the increase of passive membrane permeability for ions Ca^{2+} do not give distinguished contribution to tentatively observed the effectiveness of lowering of Ca^{2+} -transport under action of caffeine.

We indicated (using the pH-metric method) that in result of treatment by cleaned HSA is the decreasing of the passive permeability SR for Ca^{2+} that

leads to increasing of the work efficiency of Ca^{2+} - pump ATPase SERCA2.

Table 1 - The dependence of value of Ca^{2+} /ATP on the free fatty acid presence at terminal cisterns membrane under the two Mg^{2+} ions concentrations

Prepared FSR	1 mM Mg^{2+}		4 mM Mg^{2+}	
	-	5 mM caffeine	-	5 mM caffeine
FSR TC cleaned *	0,8+ _{-0,0} 5	0,3+ _{-0,0} 5	1,8+ _{-0,0} 5	0,8+ _{-0,0} 5
FSR TC	0,7+ _{-0,0} 5	0,25+ _{-0,0} 05	1,2+ _{-0,0} 5	0,35+ _{-0,0} 05

* - The FSR TC, cleaned from free fatty acids by extraction method under the human serum albumin presence.

The next experiments were - the membranes incubation of FSR with free unsaturated fatty acids. This treatment considerable extent increases the caffeine effect on quantity of passive membrane permeability. It should be noted that the strengthening of influence of caffeine on the passive permeability by fatty acids was polymodal depends on ion concentration Mg^{2+} at registration medium. Maximum of caffeine effect on the passive the permeability occurs when 2mM Mg^{2+} . (Fig. 4). The dependence of rate of passive release Ca^{2+} from vesicles FSR TC from ion concentration Mg^{2+} with and without caffeine was shown at Fig. 4. The FSR TC membranes were enriched by the free fatty acid.

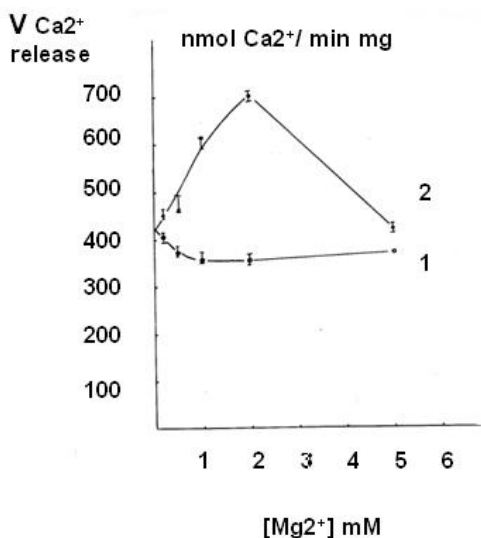


Fig. 4 - The action of Mg^{2+} to the caffeine influence to the Ca^{2+} -passive leaks from vesicular fragments of fractions of terminal cisterns. The free lipid acids were enriched the membranes of FSR. 1 - control; 2 - + 5mM caffeine

As it can be seen from Fig. 4, when incubation (3hours 10°C) the vesicles of FSR with linoleic acid (20 γ by 1 mg protein) was occurred, the passive releasing of Ca^{2+} from FSR increased. However in the presence of caffeine the rate of ion passive releasing Ca^{2+} becomes

in depending on ion concentration Mg^{2+} in high degree. The curve had the extremum (maximum) in middle region of used concentrations Mg^{2+} . When ion concentration of Mg^{2+} increased from 0 up to 0,5 mM, the caffeine in practice does not exert any effect at a rate of ion Ca^{2+} releasing by ion passive permeability under the presence of fatty acid at membranes. However at concentration of 2mM Mg^{2+} the rate of passive permeability by ion passive releasing Ca^{2+} increased in practice in twice. When the following increasing ion concentration Mg^{2+} occurred up to 5 mM, the reduction of caffeine effect on the passive permeability to ions Ca^{2+} by ion passive releasing Ca^{2+} was seen

When we compared the data, presented at Fig. 3 and Fig. 4, we may support that the passive ion Ca^{2+} releasing from vesicles of terminal cisterns decreases when extraction of free fatty acids exists. The caffeine effect on the passive permeability to ions Ca^{2+} by ion passive releasing Ca^{2+} from terminal cisterns membranes, treated by the clean albumin for extraction of fatty acids, was reduced in practice up to 0.

Conclusion

The human serum albumin (HSA) was used for stabilizing membrane preparations by the cleaning of membrane from free fatty acids (FFA), being the chaotropic agents that disturbed the crystalline structure of bilayer.

Aqua solution of HSA extracted FFA from membranes of intracellular organelles - two factions of fragmented sarcoplasmic reticulum (FSR). FSR were prepared of heavy and light fractions that were isolated from white rabbit muscles. It is known that membranes of SR contain 0,65 mg lipids by 1 mg protein. Of them, the FFA containing is less 2%. We obtain that pH-metering method was indicated that in result of HSA treating the several following processes existed: 1) the passive permeability FSR for Ca^{2+} , decreased; 2) the Ca^{2+} -pump ATPase (SERCA-2) conjugation increased; 3) the ion yield of transport Ca^{2+} through the Ca^{2+} -channel of Ryanodine receptor intensified. Similar extraction FFA by albumins from membranes can come about and in the animal's body.

The extraction of FFA from biological membranes in the animal body cells may lead to the more successful works of integrate enzymes (ATP-ase, for example), or ion channels. At first case, the ion pumping occurred more effectively. At second case, the regulation processes for channel's activity may be mediated by ions streams through membrane. Thus the quantity reducing of ions Ca^{2+} in the environment leads to increasing of the work efficiency of Ryanodine receptor. Ca^{2+} ion releasing through the Ca^{2+} -channel of Ryanodine receptor increased.

We have to note that the Mg^{2+} -ions concentration varied from 0,1 mM up to 10 mM was the main regulator factor for the passive permeability FSR for Ca^{2+} . But this problem may be discussed at next works.

We used caffeine in our work, as the test of RyR function activation. RyR activation was reflected in lowering of accumulation efficiency of calcium ions to the SR Ca^{2+} -store. The application of biologically

active substances, which regulating the work of intracellular organelles, is good step for exploration functioning and components interconnection of these organelles. The active and passive Ca^{2+} -transitions in/out the SR-store and its crosstalk are the famous problem of interrelationships between RyR and Ca^{2+} -ATPase pump activations. These transitions are regulated by the many factors. Thus RyR activation was initiated by endogenous factor cADPR [17]. At the other following studies was found that RyR activation by cADPR was a result of Ca^{2+} -ATPase-pump activation, mediated by increased luminal calcium. The Ca^{2+} -content at SR store and Ca^{2+} -releasing were regulated by luminal Ca^{2+} -sensitive leak [18]. These processes are actual, as for mammals, and for insects. Because there are the similarities of insect and mammalian ryanodine binding sites [19]. Thus the investigations of activators and inhibitors machineries mechanisms of RyR, Ca^{2+} -ATPase or Ca^{2+} -nonspecific leaks play the great role for the muscle and cardiovascular problems solutions. And it can help to found the target sites for insecticides.

Recently it was being found novel exogenous activator of RyR – flubendiamide. This substance stabilizes the insect RyRs in an open state in a species-specific manner and desensitizes the calcium dependence of channel activity [20]. Flubendiamide stimulated the Ca^{2+} -pump activity by decreasing in luminal calcium, which may induce calcium dissociation from the luminal Ca^{2+} -binding site on the Ca^{2+} -pump. This mechanism, as the authors [20] believe, should play an essential role in precise control of intracellular Ca^{2+} -homeostasis.

The investigations of each next activator or blocker actions permit us to understand the SR component interaction better, for potential application these substances as pharmacological substances in medicine, so and as insecticides in agriculture.

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