Introduction The basic calcium store of striated muscles of mammals - are the sarcoplasmic reticulum. The sarcoplasmic reticulum is the branched tubules and vacuoles that are linked with each other - between them into the continual net inside of the muscle cell interior. It exists from cell periphery up to central compartments. The peripheral parts of reticulum are the terminal cysternaes. They are 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 - acto-miozine 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 cysternaes 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 Ca2+-Mg2+-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 Ca2+-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) 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 Ca2+-releasing channel. RyR’s activity is regulated by Ca2+ 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 methylksantin - caffeine and its agonists [1]. The luminal, transmembrane and cytoplasmic satellite proteins: calsequestrin, triadin, janctin and others, modulate the function of RyR also under the certain values of the extra vesicular - cytoplasm ([Ca2+] cyt) and the luminal Ca2+-concentrations ([Ca2+]lum). The necessary values of [Ca2+]cyt, [Ca2+]lum are based on the functional activity of the RyR and Ca2+-pump - Ca2+-ATPase. The gradient of Ca2+ - [Ca2+]lum/[Ca2+]cyt , is created and maintained by coordination of Ca2+-ATPase and RyR activities. Data [2] suggested, that [Ca2+]lum/[Ca2+]cyt may be the key factor, involved in the regulation of the functional states of Ca2+-ATPase and RyR of Ca2+-store - SR. The maximal value of the calcium loading of the Ca2+-store (3mM) induced the Ca2+ -releasing process through the Ca2+ -channel of RyR, inducing the muscle contraction. In this case the Ca2+-ATPase is inhibited completely, and no pumps the

amount of the released Ca2+ to the reticular lumen. Minimal Ca2+-concentration at the reticular lumen (0-0.3mM) completely inhibits the Ca2+ release through the RyR, and activates the Ca2+-ATPase, resulting in the Ca2+-reuptake from the cytoplasm, and followed by muscle relaxation. The middle Ca2+-concentration (1 mM) corresponds with the equilibrium position of the system Ca2+-releasing/Ca2+-pumping, when RyR and Ca2+-ATPase may be activated both one after other. [3]. The efficiency of Ca2+-accumulation (Ca2+/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 Ca2+ ions pumping inside to the FSR lumen. The value of Ca2+/ATP reflects the amount of pumped Ca2+ 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, KH2PO4 (Merck); histidine, imidasol, caffeine (Merck); NaCl, MgCl2 (Merck); DTT (Serva); glycerol (Serva); CaCl2 (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 2-4o 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 CaCl2, 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 cysternaes (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 CaCl2, 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, Ca2+ -ATPase and calsequestrin predominantly. LT contained the Ca2+ -ATPase predominantly and lesser amount of calsequestrin [7]. The protein concentration was

determined by the fast method [8]. Efficiency of Ca2+-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 MgCl2, 2,5 mM imidasol (pH 6,8 37o C) with intensive mixing. The pumping reaction was stimulated by additions of 80 nmoles CaCl2. The Ca2+ ions, accumulated into FSR, bounded with oxalate, and the calcium oxalate stored into the SR lumen. Thus capacitance FSR for Ca2+-ions increased markedly. It becomes to perform the registration of absorption Ca2+-ions by FSR eventually. In native muscle cells under the SR activation Ca2+-ions communicates with luminal proteins - calsequestrin and others, and with phosphate. So that capacitance SR for Ca2+-ions was maintained. The value of Ca2+-ions saturating of Ca2+-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 CaC12 were held as Ca2+-ions was accumulated into the FSR lumen. Reticulum (5 mg of protein) was loaded up to 500 nmol Ca2+/1 mg protein. Then the mixture has cooled up to 4o 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 4o 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 4o C). The measurement of Ca2+ 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 37-42o C). The Mg2+ 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 Ca2+-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 (25-30oC). 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 Ca2+ 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 (10-12o C) for more uniform distribution of fatty acid in FSR membrane Results and discussion As the Ca2+-gradient play the great role for the SR functions regulation, we were needed to investigate some factors influences to the Ca2+-store actions. One of these factors - is the membrane permeability for ions. Because, the Ca2+-ions penetrate through the membrane by several ways, and Ca2+-pumping by SERCA2 activity, and Ca2+-releasing by RyR activity are under widespread intensive investigations, we reversed our attention on the nonspecific way passive membrane permeability for Ca2+-ions. The part of our interests was deal with the influence of passive permeability on RyR that was expressed in sensibility of Ca2+-transport to caffeine. Caffeine is the activator of intermediate state Ca2+-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 Mg2+, without which Ca2+-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 cysternaes, in the presence of caffeine, reduce the efficiency of Ca2+-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 Ca2+ 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 Ca2+ 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 Ca2+, it is possible to calculate the effectiveness of transport process (the mean the value of Ca2+/ATP). Than, the larger amounts of phosphate are accumulated at incubation medium when transport additional amount of Ca2+ occurred, that effectiveness quantity of transport Ca2+ - the value Ca2+/ATP, become are smaller. The value Ca2+/ATP, become are smaller or bigger in dependence of passive permeability changes. And the disagglutinating action caffeine (unconjugation action to Ca2+-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 Ca2+-releasing from FSR under the low Ca2+ concentrations in registration medium (lowered 10-8 M) and without ATP. At this case the Ca2+ accumulating action of Ca2+-pump can not be activated. The FSR was loaded by Ca-oxalate. Than the kinetic of Ca2+ release was registrated by pH-metric method with EGTA at medium. Under the binding of 1 Ca2+-ion by EGTA, two H+ are released from EGTA molecule (pH 7,0-7,1). The pH lowered. The graphics of depending of passive Ca2+-releasing rate/Mg2+ concentration from FSR are presented: FSR - TC (Fig. 1). Fig. 1 - The action of Mg2+ to the caffeine influence to the Ca2+-passive leaks from vesicular fragments of fractions of terminal cisterns. 1 - control; 2 - + 5mM caffeine The passive Ca2+-releasing from FSR TC (Fig.1) is in bimodal depending of Mg2+ concentrations at medium. The variation of Mg2+ concentrations from 0 to 5 mM leads to lowering, and than to increasing of passive Ca2+-releasing rate. The caffeine additions increased the passive Ca2+-releasing rate, but the variation of Mg2+ concentrations operate weakly. The passive permeability of FSR LT changed very negligible under the Mg2+ concentrations from 0 to 5 mM. The caffeine (5 mM) additions not changed the passive Ca2+-releasing too. Thus, caffeine influenced to the passive permeability at FSR TC. The rate of passive Ca2+-releasing was increased to 20%. But, this value doesn’t very essential for value of Ca2+/ATP-lowering under the caffeine actions. The value of Ca2+/ATP lowered by 3 times under the 5mM caffeine additions to the FSR TC. Also the value of Ca2+-transition by ATPase Ca2+-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 Ca2+/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 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 Mg2+ to the caffeine influence to the Ca2+-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 Ca2+-releasing from ion concentration Mg2+ 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 Ca2+/ATP from free fatty acids presence at membranes of terminal cisterns when different ion concentrations Mg2+. 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 Ca2+-transport, and on the Ca2+-transport dependence from ion concentration Mg2+. But this treatment increased the value of Ca2+/ATP. So that becomes clear that extraction of free fatty acids from membranes of terminal cisterns in practice does not contribute to Ca2+-ATPase function. Thus, the increase of passive membrane permeability for ions Ca2+ do not give distinguished contribution to tentatively observed the effectiveness of lowering of Ca2+-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 Ca2+ that leads to increasing of the work efficiency of Ca2+ - pump ATPase SERCA2. Table 1 - The dependence of value of Ca2+/ATP on the free fatty acid presence at terminal cisterns membrane under the two Mg2+ ions concentrations Prepared FSR 1 mM Mg2+ 4 mM Mg2+ - 5 mM caffeine - 5 mM caffeine FSR TC cleaned* 0,8+_0,05 0,3+_0,05 1,8+_0,05 0,8+_0,05 FSR TC 0,7+_0,05 0,25+_0,05 1,2+_0,05 0,35+_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 Mg2+ at registration medium. Maximum of caffeine effect on the passive the permeability occurs when 2мM Mg2+. (Fig. 4). The dependence of rate of passive release Ca2+ from vesicles FSR TC from ion concentration Mg2+ 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 Mg2+ to the caffeine influence to the Ca2+-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 10oC) the vesicles of FSR with linoleic acid (20 γ by 1 mg protein) was occurred, the passive releasing of Ca2+ from FSR increased. However in the presence of caffeine the rate of ion passive releasing Ca2+ becomes in depending on ion concentration Mg2+ in high degree. The curve had the extremum (maximum) in middle region of used concentrations Mg2+. When ion concentration of Mg2+ increased from 0 up to 0, 5 MM, the caffeine in practice does not exert any effect at a rate of ion Ca2+ releasing by ion passive permeability under the presence of fatty acid at membranes. However at concentration of 2мМ Mg2+ the rate of passive permeability by ion passive releasing Ca2+ increased in practice in twice. When the following increasing ion concentration Mg2+ occurred up to 5 MM, the reduction of caffeine effect on the passive permeability to ions Ca2+ by ion passive releasing Ca2+was seen When we compared the data, presented at Fig. 3 and Fig. 4, we may support that the passive ion Ca2+ releasing from vesicles of terminal cisterns decreases when extraction of free fatty acids exists. The caffeine effect on the passive permeability to ions Ca2+ by ion passive releasing Ca2+ 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 Ca2+, decreased; 2) the Ca2+-pump ATPase (SERCA-2) conjugation increased; 3) the ion yield of transport Ca2+ through the Ca2+-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 firs 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 Ca2+ in the environment leads to increasing of the work efficiency of Ryanodine receptor. Ca2+ ion releasing through the Ca2+-channel of Ryanodine receptor increased. We have to note that the Mg2+-ions concentration varied from 0,1 mM up to 10 mM was the main regulator factor for the passive permeability FSR for Ca2+. 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 Ca2+-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 Ca2+-transitions in/out the SR-store and its crosstalk are the famous problem of interrelationships between RyR and Ca2+-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 Ca2+ATPase-pump activation, mediated by increased luminal calcium. The Ca2+-content at SR store and Ca2+-releasing were regulated by luminal Ca2+-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, Ca2+-ATPase or Ca2+-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 Ca2+-pump activity by decreasing in luminal calcium, which may induce calcium dissociation from the luminal Ca2+-binding site on the Ca2+-pump. This mechanism, as the authors [20] believe, should play an essential role in precise control of intracellular Ca2+-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.