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THE EFFECT OF ^{22}Na UPTAKE UPON IONIC COMPARTMENT IN AGING RAT SKELETAL AND CARDIAC MUSCLE

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Abstract

Aim of study is to point out the role of charges distribution at the level of contractile proteins in Contraction and Relaxation state of skeletal and cardiac muscle cells from rats of different ages ,by means of ^{22}Na uptake method in order to bring new arguments in the favor of swelling theory (lateral expansion of filaments network) of muscle contraction in skeletal and cardiac muscle during aging process .Material and method: The study was done on 16 male Wistar rats divided into two groups of 8 rats each: I) 6 months old and II) 37 months old. The animals were anesthetized and then sacrificed and samples from sartorius skeletal muscle and from papillary muscle from left ventricle were excised and placed in glycerol solution in order to remove cell membranes and to expose the contractile apparatus. Our Na22 uptake studies pointed out another structural aspect of functional implication of contractile proteins with modified features from 37 months old skeletal and cardiac muscle and the data concerning the charges distribution at the level of contractile proteins in Contraction and Relaxation which bring new arguments in the favor of swelling theory(lateral expansion of filaments network) of muscle contraction in skeletal and cardiac muscle during aging process.

Key words: *^{22}Na radioisotope, myosin, actin, swelling mechanism, hexagonal lattice, contraction*

INTRODUCTION

Molecular mechanisms of heart muscle contraction have been described by numerous scientists in order to find out drugs able to improve any intermediary phases of the disease affected by the pathological process (Popa et al., 2017).

At each contraction, Ca^{2+} enters in cardiac cells, inducing liberation of a high quantity of Ca^{2+} from the sarcoplasmic reticulum in order to activate contraction and to prevent overloading the myocyte with Ca^{2+}, the quantity of Ca^{2+} which has induced contraction must be reexported in extra cellular space (Rüegg, 1987). Because Ca^{2+} from myocyte cytosol is of micromoles order or even less, while in extra cellular space it is found in millimoles concentrations, its re exporting requires an active transport. There are two systems in charge with this function: an ionic exchanger Na^{+}/Ca^{2+} and a specific ATP-ase; these are working in parallel, but have different kinetics properties, being qualified for full filing different roles (Marin and Goga, 2018a).

The ATP-ase has a great affinity for Ca^{2+}, but a decrease rate of pumping, and can be in such a way considered fine regulator of calcium of cell calcium; it functions in the same manner in all cells where is present (Marin et al., 2017).

The ionic exchanger Na^{+}/Ca^{2+} has a weak affinity for Ca^{2+}, but a great rate of transport, which determine its activation when it is necessary to be ejected high quantity of Ca^{2+}.

There are studies (Curtin et al., 1988; Marin et al., 2018b) which pointed out a dependency of force developed by muscle contraction on intracellular pH - i.e. the muscle force enters into decline with the change into pH - towards acid values. This finding put forward for analysis two possibilities: (1) there are less cycles of cross bridges binding, each producing the same integral force-time, and (2) each cycle produces a less integral force-time.

The second possibility is sustained by the fact that the reduction of pH-ul results in reduction of maximum speed of shortening of muscle fiber, that points out that the kinetics of cycles of binding cross bridges is altered, not only their number.

Almost ¼ from the total consumed ATP- in muscle contraction is used by the calcium pump of the sarcoplasmic reticulum (Curtin and Woledge, 1981).

This ATP-ase activity is diminished by the increased of acidity *in vitro* (MacLennan, 1970), and *in vivo*; it is possible that this effect to be responsible for the obvious slowing down of relaxation produced by the increase in CO_2.

The conclusion of their study was that the mechanical impulse generated by the splitting of a ATP molecule increasing with the value of intracellular pH.

The action potential of heart muscle usually has a duration of more hundreds of milliseconds. In majority of cases the contraction time is approximately equal with duration of action potential. For this reason, is adequate to consider the excedent of action potential as a trigger of contraction, and rapid repolarisation as a shutting down of it. Each contraction is lengthening enough in order to be maximal; in such a way, that the tension corresponds to a titanic contraction in skeletal muscle.

An interesting aspect of cardio myocyte is that the duration of action potential depends on cardiac frequency: as high the frequency is, the duration is less. The duration of potential action is approximately ½ from the interval between two beats.

The action potential of cardiac muscle differs of that of a nerve or of skeletal muscle, in the sense that has a greater duration and a great variability of duration in rapport with frequency.

The rhythm dependency and the exceeding of action potential on external concentration of natrium (Na) demonstrates that the excess of action potential is determined by a great increase in membrane permeability for Na.

But, the nature of permeability modifications which accounts for a much longer depolarization platau phase is not known.

The total resistance of membrane is increases in platau phase, that means that in this phase the permeability to (Na) is increased, and permeability to potassium (K) and/or chloride (Cl) is reduced.

It can be given simple explanation for long duration of action potential and its dependency on rhythm, if we suppose that exists two types of Na conductance.

The first type of conductance responsible for platau phase of action potential is initially high but, is rapidly inactivated during milliseconds after depolarisation and as far as rapid activated after repolarisation.

The second type of conductance responsible for platau phase is in comparison much smaller, but it is slowly inactivated (during seconds) and as slow as activity after repolarisation.

After platau phase of action potential and inactivation of rapid conductance to (Na), the slow inactivated conductance, even reduced of Na persists.

This keep membrane potential towards zero, because conductance of K and Chloride (Cl) have decreased, because it is supposed that these depends on the membrane action potential.

As far as the inactivated conductance of slow Na decreases, the potential decreases slowly, up to a potential at which one or more conductance start to change rapidly in accord with membrane voltage. The (Na) conductance is “stopped”, and the conductance of K and Cl are “triggered on”, in such a way that repolarisation is performing faster and faster. After repolarisation, excitability returns once with activation of fast conductance of (Na), but the action potential will be short because the lent conductance of Na is slowly activated. The platau will appear at a smaller voltage in such a way that the potential at which is produced rapid repolarisation will be reached faster.

Muscle cell is a polyelectrolyte system made up of proteins with many ionizable groups in the same molecule. Basic groups are in charge with positive charges and those acidic with negative charges.

Usually the acidic groups are much more numerous and the net charge at the surface of filaments is negative. The lateral side chains of amino acids from contractile proteins make up a network by weak H bonds with H_20 molecules at the proteins surface leading to an increase in electric load at the level of filaments.

Heterogeneity of protein surface suggests that numerous H bonds fix water at the protein surface some being stronger than others. Such a network of lateral side chains electrically charged exist between 2 myosin tails or

between two polypeptides chains from a single tail.

Concerning the swelling mechanism of muscle contraction the pioneer research of Elliot et al. (1968) has led to the conclusion that the estimation of dimensions of hexagonal lattice of actin and myosin depends on the relation between electrostatic repulsive and attraction forces between the filaments.

Those the last ones seem to be mainly caused by the transverse structural restrictions such as attached cross bridges presented in Rigor, line Z and line M.

An expansion of filament lattice such as is produced at high salt concentrations may be achieved if the repulsive forces are increased or if the forces which determines structural restrictions diminish or due to a combination of those two.

Interactions at the level of contractile proteins are either pure electrostatic, or interactions due to ionic polarizing or they may be caused by the dipoles contribution.

Paper aim was to study the role of charges distribution at the level of contractile proteins in Contraction and relaxation state in contractile apparatus from skeletal and cardiac muscle cells from rats of different ages, using the ^{22}Na uptake method in order to bring new arguments in the favor of swelling theory (lateral expansion of filaments network) of muscle contraction in skeletal and cardiac muscle during aging process

MATERIALS AND METHODS

The study was done on 16 male Wistar rats divided into two groups of 8 rats each: I) 6 months old and II) 37 months old.

The animals were sacrificed and sartorius skeletal muscle and papillary muscle from left ventricle were removed and placed in glycerol solution in order to remove the membranes according to the published method (Revnic et al., 2013).

Glycerinated muscle samples 50-100 mg each were washed in bidistiled water for 15 minutes in 3 successive baths for 5 minutes each. Then the biological samples were placed in test tubes with 1 ml of:

1) Contraction medium with the following composition: KCl 150 mM, CaCl 2.2 mM, $MgCl_2$ 2 mM, TRIS buffer 10 mM, pH 7.2, ATP 2 mM.

2) Relaxation solution with the same composition as contraction solution, excepting CaCl2 which has been replaced with EDTA 4 mM.

3) Rigor solution with the same composition as contraction solution excepting the ATP.

The next step included incubation with ^{22}Na (cat.NENRes. Products, Du Pont) according to Zak et al. (1979) method of skeletal and cardiac rat muscle of different ages.

Characteristics of ^{22}Na radioisotope:

- It is found in aqueous solution of NaCl; the half life time = 2.602 years;
- type of gamma radiation; the energy of radiation 511,1275; specific activity between 100-2000 Ci/gram; the radionuclide power>99%. Concentration approximative 1-10 mCi/mil; it was used the isotope concentration of 0.5 mCi/ml.

This concentration was diluted I: 500 mCi in 5 ml bidistiled water to a concentration of 100 mCI/ml;

Dilution II: 0.1 ml (10 mCi) + 20 ml bidistiled water = 0.5 mCi;

The working dilution was 0.05 mCi/0.1 ml.

The incubation of biological samples was performed for 2 hours at 37°C, then the samples were washed two times for a minute each in the corresponding solutions of Contraction, relaxation and rigor in order to remove ^{22}Na non-specifically absorbed.

Then the tissue fragments were placed in extraction medium (1 ml HCI), following then the recording of radioactivity in incubation and extraction media with a Gamma Counter Beckman, with a multiplier with NaI crystals.

The results of 5 experiments (of 24 samples each, total-120 samples) from which (60 were incubations and 60 extractions) were calculated using the same formula as for Beta isotopes used in our previous studies (Revnic et al., 2018).

E/I x G = cpm/gram tissue, where: E = extraction, I = incubation, G = weight in grams.

RESULTS AND DISCUSSIONS

The distribution of charges at the level of contractile filaments in contraction, relaxation and rigor following the uptake of ^{22}Na (Figure

1) in striated muscle from 6 and 37 months old rats in the presence of ionic strength of 150 mM KCl pointed out an increase uptake of Na22 quantity in young rat skeletal muscle in comparison with 37 months old rat skeletal muscle which is accounted for the presence of high quantity of negative charges at the level of contractile proteins in young rat at the skeletal muscle in comparison with old rat skeletal muscle .

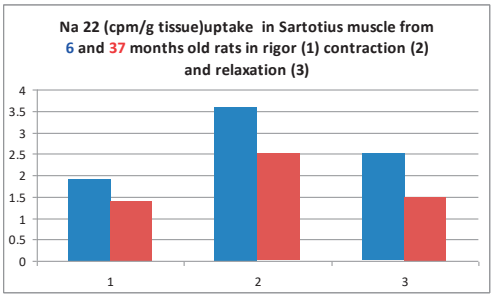

Figure 1. The uptake of ^{22}Na in striated muscle from 6 and 37 months old rats in the presence of ionic strength of 150 mM KCl

Molecular movement of actin on myosin is of Brownian type motion. In Ri the cross bridges are relatively mobile (mobility is strictly Brownian). The water from the surface of proteins is different from that of total water in different ways. Proteins include oxygen which arranges water molecules at the level of protein surface. Chemical change of protons between water and ionisable proteins groups from the around protein molecules have a preferential orientation to the protein surface and a quick movement identified through local water liberated from the lateral side chains.

According to Offer (1984) studies in the presence of high ionic strength there is a higher quantity of water retention because of the binding of Cl ions to myofibril proteins increasing in such a way the negative charges on muscle filaments.

Other studies of Millan et al. (1980) pointed our that the repulsive forces have increased when there is an increase in charge density, taking into account that a certain density of charges there is at the level of untreated muscle. On the other hand, the salt concentration will increase the shielding of these charges according to Leward (1983). It seems that the only way in which the repulsive forces will increase will be is the ray of these charges toward the filament axis will be increased, because the repulsive forces are extremely sensitive to this parameter according to Elliot et al. (1982).

It is not clear at the moment if the increase or not in electrostatic force are partially responsive of the swelling contraction phenomenon (the lateral expansion of hexagonal lattice). The fact that swelling at the high concentrations of salt is highly cooperative suggests that the removal of transverse structure as could play a major role in determination of swelling.

Offer et al. (1983) studies have shown that the removal of cooperative restrictions due to cross bridges of myosin filaments heads it can be produced either by dissociation of myosin heads upon the actin filaments as it is achieved in the presence of pyrophosphate and of increase concentration of Cl ions, or by de polymerization of thick myosin filaments as is produced in the presence of Cl ions in high concentrations.

The effect of salt and pyrophosphate upon the structure of other potential candidates such as Z and M line is not yet known. The increase accumulation of ^{22}Na in contraction at the level of contractile proteins leads to the increase in electric charge on contractile filaments increasing their rigidity. The increase of fixed charges is due to mobilization of cations which are binding at the surface of myosin filaments. The increase in the ionic strength determines the reduction in thickness of double diffusion layer and the repulsive forces appear by redistribution of ions in diffusion layer close to another filament.

In 37 months old rat skeletal muscle it was pointed out a reduction in ^{22}Na uptake in striated muscle in contraction state (Figure 1).

In conditions in which the splitting capacity of ATP is retained in old rat skeletal muscle, the energy liberated by splitting of ATP is used for movement of sub fragment 2 at an angle of 45 degree on actin filament. It is possible that in aging skeletal muscle the presence of myosin with modified features to obstruct the coupling of myosin head at a 45 angle degree with actin and therefore, it is possible not to be achieved a long range electrostatic repulsive force between the filaments.

Aging evolves with a reduction in contraction due to number of fixed charges at the level of contractile proteins, accounted for an increase in superficial charges due to lability of polar groups of contractile proteins with modified features.

Our previous studies pointed out an increase in $C_{14}H_3$-COONa in cardiac muscle of young rats versus old rats.

In Figure 2 there are presented the results of ^{22}Na uptake in young and old heart muscle in contraction, relaxation and rigor.

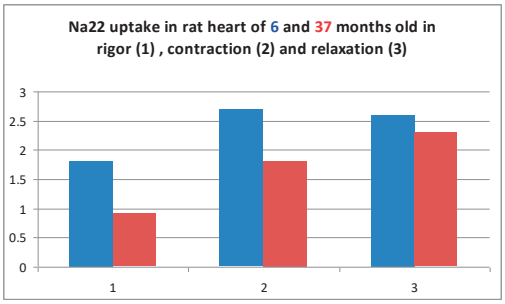

Figure 2. The uptake of ^{22}Na in young and old heart muscle in contraction, relaxation and rigor

In contraction there is an increase uptake of ^{22}Na in young rat heart muscle than in old rat heart muscle. The increase number of negative charges on contractile filaments explain the increase uptake of ^{22}Na in contraction due to excess accumulation of ^{22}Na within the contractile apparatus.

During aging there is a constant reduction of negative charges population in contraction due to the increase degree of dissociable electronegative groups at the level of proteins with modified features accumulated during aging.

Dragomir (1980) studies concerning the measurement of ^{22}Na in frog heart muscle in the presence of Deuterium oxide revealed modifications in ionic behavior of muscle suggesting the intervention of long range action force during contraction state. The water absorbed at the molecular level is decisive for ionic behavior and the functional state of cardiac muscle and this may explain the alterations in ^{22}Na accumulation in the presence of heavy water.

Our results concerning the ionic behavior of contractile apparatus from rat sartorius and papillary ventricular heart muscle in contraction, relaxation and rigor pointed out an increase in ^{22}Na uptake in contraction state in 6 months old rats which is accounted for the presence of an increased quantity of negative charges at the level of contractile filaments.

In 37 months old rat sartorius and papillary ventricle muscle there is a reduction in ^{22}Na uptake in contraction as an expression of changes in negative charges density at the level of contractile filaments implicated in muscle contraction phenomenon, according to the swelling theory.

Contraction and Relaxation in the presence of increased quantity of ^{22}Na are correlated with a reduction in muscle hydration in case of aging muscle, contraction being a function of ions binding to the proteins sites, those sites being important in achieving the hydration state of proteins.

CONCLUSIONS

During aging there is a reduction of negative charges population due to the high degree of dissociable electronegative groups at the level of proteins with modified features accumulated during aging.

Our ^{22}Na uptake studies pointed out another structural aspect of functional implication of contractile proteins with modified features from 37 months old skeletal and cardiac muscle and the data concerning the charges distribution at the level of contractile proteins in Contraction and Relaxation which bring new arguments in the favor of swelling theory(lateral expansion of filaments network) of muscle contraction in skeletal and cardiac muscle during aging process.

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