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THE MANAGEMENT OF WATER STATE IN GLYCERINATED RAT HEART THE ROLE OF 1H NMR SPECTROSCOPY

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Abstract

Ln order to obtain new data concerning muscle contraction at the molecular level, the interrelation water-contractile proteins has been investigated by means of 1 H NMR Spectroscopy. Rat heart muscle from 6 and 37 months old rats has been used for proton transverse relaxation time measurements in Ri, Co and Re. at different [ATP].The distribution of negative charges in contraction and relaxation has been measured by exposing glycerinated muscle from 6 to 37 months old rats to different [Mn+2].Our data have pointed out the existence of two proton relaxation tinies:T2s and 121 accounted for two water compartments. The modification in water state are related with modifications in contractile activity. The elongation od proton transverse relaxation times is associated with a decrease in the degree of water molecules aggregation.T2s and T2l are correlated with a reduction in muscle hydration, contraction being a function of ions binding to the protein sites. These sites are implicated in determination of protein hydration state.

Key words**:** *1 H NMR, aging, glycerinated muscle, contraction, relaxation, rigor*

INTRODUCTION

Literature data [1] concerning muscular contraction phenomenon, have pointed out the appearance of long range repulsive forces within contraction state which tend to repell the myofilaments one from each other.These forces are converted into active shortening tension through passive intervention of transverse myosin crossbridges with an oblique orientation between myofilaments [2].The repulsive forces which take place during contraction are the consequence of the increase in the electric charge of myofilaments[3].

In order to obtain new data concerning muscle contraction at the molecular level,the polar groups from the contractile proteins have been investigated by means of 1 H NMR Spectroscopy, to test the water state from the close proximity of myofllaments in different experimental conditions: in contraction, relaxation and rigor from heart muscle of Wistar rat.

1H NMIR is a very useful method in biology because we can obtain very important data about mobility of some groups at the level of protein molecules,which provide informations about conformation changes which result from the chemical modifications.

The aim of our study was related with:

1.The investigation of proton transverse relaxation times of water from glycerinated muscle in Ri,Re,Co at different ATP concentrations.

2.The distribution of charges in Contraction and relaxation by exposing glycerinated heart muscle from 6 and 37 months old rats to different [Mn+2], by means of 1 H NMR spectroscopy.

MATERIAL AND METHOD

Heart muscle from 6 and 37 months old rat has been used for 1 H NMR studies; the animals have been killed by cervical dislocation and muscle samples from sartorius muscle have been processed for NMR measurements as fresh and glycerinated biological samples according with the published technique [4].Glycerinated heart muscle fragments of 2 cm long have ben washed for 15 minutes in

bidistiled water and then dried on filter paper.The next step was the placement of biological sample m pH 7.2 in order to assure the ionic equilibrium for one hour according with the published method[5]. After preincubation, the muscle fragments have ben incubated for 10 minutes in Ri ,after that have been removed and have been dried on filter paper,and then introduced in special test tubes for reading the relaxation times in an Aremi'78 1 H NMR Spectrometer in impulses of a fixed frequency of 25 MHz.

Alter the readings have been done,the tissue fragments have been introduced in relaxation solutions(0.5mM ATP) for 10 minutes.Then the Spectrograph readings have been recorded, taking care for pH of solution to be the same.

Then, the biological fragments have been introduced in Re solution 2(1mM ATP);the recordings of proton relaxing times have been done ,following another 10 minutes incubation in relaxing solution 3(2mM ATP), followed by readings at Spectrometer. Tissue fragments then have been washed ,preincubated in media without ATP for ionic equilibration and after that have been introduced in contraction media 1(0.5mM ATP), 2(lmM ATP), 3(2mM ATP), according with the protocol used for recording proton relaxation times in RI and Re state.

The composition of RI, Co and Re media has been the same as the incubation media used for optical microscopy studies[6].

Alter recording the values of proton transverse relaxation times, the muscle fragments have been weighted successively for a few days until a constant weight has been achieved, in order to estimate the dry/wet weight ratio.

The method for processing data concerning the proton transverse relaxation times has been previously described [7].

RESULTS AND DISCUSSIONS

By optical measurements done with ML4 optical microscope on sarcomere lengths in contraction state, a decrease in the active shortening capacity of sarcomeres from 1 .46u in 6 months old rats to 1 .67u in 37 months old rats for 0.5mM[ATP] has ben recorded as we For 1 mM ATP, sarcomere length recorded in young rats was 1 .67u and for 37 months old 1 .92u.Concerning 2mM ATP concentration,the mean value of sarcomere length was 1.6 u for young muscle and 1.88 for old muscle.The increase in arcomere length with ageing,is significant from statistical point of view for the three ATP concentrations.

The low milk offer has obliged Romania to import milk for assuring raw material for milk processing industry.

Table 1. Relationship between sarcomere length in heart muscle of young and old rats in Contraction state and T2s and T2l values at three [ATP].

	Heart 6 months old rat			Hear t 37 months old rat		
	T2s (ms)	T2l (ms)	Sarc.length(u)	T2s (ms)	T2l(ms)	Sarc.length(u)
Co(0.5mM)	34	230	1.74	47	280	2.01
Co.(1mM0	33	180	1.72	45	270	1.74
Co(2mM)	30	138	1.70	46	278	1.72

Table 2. Mean values of sarcomere length(u) in Heart muscle from rats of different ages in relaxation state [ATP]

	6 months	37 months
0.5mM	X=2.36+/-0.02	X=2.22+/-0.01
1mM	X= 2.42+/-0.02	X2.24+/-0.0l
2mM	X =2.228+/-0.02	X=2.26+/-0.02

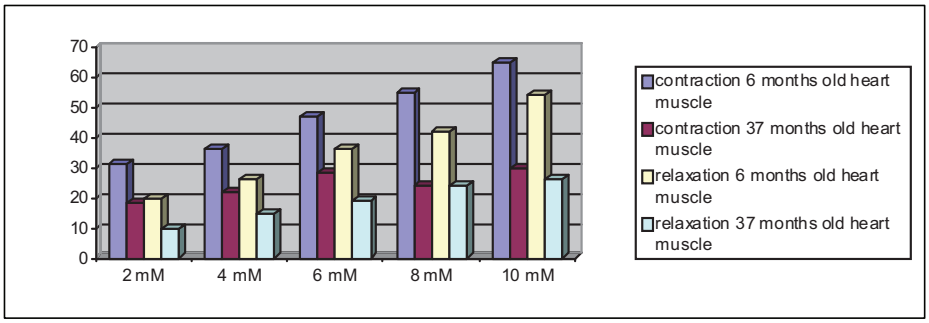

Fig. 2. There is a reduction in relaxation capacity of sarcomeres from old rat accompanied by a lengthening of T2s and T2l proton relaxation times.

Table 3. Proton relaxation times T2s in Contraction in the presence of Mn+2 from Heart muscle from 6 and 37 months old rats

	Heart of 6 month old rat		Heart of 37 months old rat	
-[Mn+2]	T2s	T2l	Ts2	T2l
2mM	50	100	99	150
4mM	65	150	96	160

There is an decrease in T2s proton transverse relaxation times in young rat versus old rat, as an expression of a decrease with aging in the active shortening capacity of sarcomeres.
Relationship between sarcomere length in Heart muscle of young and old rats in relaxation state and T2s and T21 values at three different [ATP].
Our studies concerning ionic charges distribution in Contraction and Relaxation using glycerinated skeletal muscle from young and old rats exposed to different concentrations of [Mn+2] have pointed out an increase in T2s in contraction in old rats.
As it can be seen, the elongation of proton transverse relaxation times is proportional with the concentration of.Mn+2 are accommodated supplementary in Contraction at the level of Concerning relaxation for all threeATP concentrations, an age dependent reduction of sarcomere length without statistical significance has been recorded. contractile proteins due to their fixation at the level of negative charges on contractile protein filaments.
In ageing muscle there is an elongation of proton transverse relaxation times T2s and T2l both for
Contraction state in the presence of an increased quantity of Mn+2.T2s and T21 are correlated with a reduction of muscle hydration in case of old muscle,contraction being a function of ions binding to the proteic sites;these sites being important in determination of hydration of proteins.

Table .4. Proton transverse relaxation times from 6 and 37 months old rats at different Mn+ concentrations

[Mn+2]	6 months old heart		37 months old heart	
	T2s	T2l	T2s	T2l
2mM	20	130	30	160
4mM	16	149	37	180

According to Elliott studies[8] the long range of charge is achieved only if subfragment 2 of myosin tail which carries aproximatively 1/3 of negative charge of molecule,has been tdted at a *45* degree towards the filament skeleton.
C.T. Dragomir [9] has studied the level of fixed charge in rabbit muscle,and he concluded that the level of fixed charges increases with the external electrolyte. For example, in the presence of 100 mM KC1 the concentration of fixed charges is aproximatively 75 mM for psoas muscle in Rigor.
1H NMR studies in presence of Mn+ related with negative charge density in heart muscle from 6 and 37 months old rats have revealed an elongation of T2s and T2l as a function of [Mn+],this being more reduced in Co than in Re which accounts for suplimentary accumulation of Mn+ in Co at the level of contractile proteins negatively charged.

CONCLUSIONS

Our data have pointed out the existence in glycerinated rat heart muscle of two proton relaxation times:T2s and T2l accounted for two water compartments. The modifications in water state are related with modifications in contractile activity.
The elongation of proton transverse relaxation times is associated with a decrease in the degree of water molecules aggregation.
T2s and T21 are correlated with a reduction in muscular hydration, contraction being a function of ions binding to the protein sites. These sites are implicated in determination of protein hydration.

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