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Age-Related Deterioration of Contractile Activity of Actomyosin Complex in Rat Gastrointestinal Smooth Muscle

Kateryna S. Zelenska, Nataliya E. Nurishchenko, Tetyana V. Beregova, Olga V. Shelyuk, Yuliya V. Tseysler

Educational and Scientific Centre "Institute of Biology", Taras Shevchenko National University of Kyiv, Kyiv 03127, Ukraine

E-mail: czelenska@gmail.com, nnurish@mail.ru

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It was found, that in rat senile age group, a decrease in the ATPase activity of actomyosin leads to deterioration of motility of stomach and colon with aging. White outbred rats aged 3 and 22-24 months were used. An amount of cleavage inorganic phosphate from ATP by myosin active centers was quantified to define the ATPase activity of actomyosin. It was established that Mg^{2+}, Ca^{2+}, K^{+}-ATP-hydrolase activity of actomyosin in smooth muscle of rat stomach decreases as well as in colon. The observed deterioration of contractile activity of actomyosin complex can be explained by revealed oxidative modification of contractile proteins.

1. Introduction

Muscle contraction is provided by the ATPase activity of a complex of specific contractile proteins actin and myosin included in muscle cells. The basic structural unit of muscle, providing a contraction on the molecular level, is actomyosin - a complex of contractile proteins. To perform the mechanical work, muscle uses energy that is released during hydrolytic cleavage of ATP of myosin active centers. Therefore ATFase activity is its main functional characteristics of the muscle contractile ability [1]. Many papers are devoted to investigations of changes in the ATPase activity of actomyosin complex of skeletal muscle under the influence of various extrinsic factors, pathology and treatment whereas numerous processes in smooth muscle require more detailed study at the physiological and biochemical levels. Changes in functional characteristics of contractile proteins with aging identified for skeletal and cardiac muscles [2-5]. The effect of aging is also revealed in an inhibition of signal transduction pathways during the muscle contractile response [6, 7]. Gastrointestinal tract is one of the organ systems, which is inherent to increase the frequency of pathologies with aging, but only a few details regarding changes in smooth muscle contractile apparatus with aging are presented in the papers [6, 8]. Study of aging effect on gastrointestinal motility is limited to a few clinical studies on humans. A significant effect of aging on motility of the gastrointestinal tract in rats is manifested in slow emptying of the stomach and the large intestine and faeces formation [9].

Among the numerous factors affecting the functional activity of the contractile proteins and leading to its structural and functional changes, the phenomenon of age-related destruction of proteins due to oxidative stress by free radicals is one of the major ones causing deterioration in muscle contractile function [10, 11]. Mechanisms of lipid peroxidation and their role in the functioning of organism cells are well known and widely investigated. More and more information appears on what reactive oxygen species can cause the oxidative destruction and modification of not only lipids, but also proteins. Some works are presented data on oxidative modification of proteins in conditions of

various pathologies and the formation of additional carbonyl groups in lateral chains of amino acids shown [12, 13]. However, in the literature, there is no information about changes in the structure of contractile proteins under the action of free radicals, excessive number of which are produced with aging, and that can explain deterioration in the functioning of contractile proteins.
In this work, the effect of aging in contractile function of rat gastrointestinal smooth muscles has been studied. As is known, to perform mechanical work, muscle uses energy which is released during hydrolytic cleavage of ATP in myosin active centers. Thus, the ATP-hydrolase activity of actomyosin was investigated.

2. Experimental Details

20 white outbred rats of different age groups were used for the experiments. The control first group of rats consisted of 10 individuals aged 3 months (weight of 130-160 g). The second group of rats contained also 10 individuals but aged 22-24 months (weight of 390-450 g). Both groups were similar in terms of vivarium maintenance.
The animals were killed by an overdose of anesthetic. Actomyosin was prepared from smooth muscle, pre-peeled of the mucous membrane, using Sobieszek modified technique [14]. All manipulations were conducted at a temperature of 0-4°C. The muscle tissue was preserved in liquid nitrogen at a temperature of −195°C. ATPase reaction of actomyosin was initiated by ATP introduction and stopped by adding of 20 % trichloroacetic acid at a temperature of 37°C.
Experimental data were processed by methods of variation statistics using the program OriginPro 8. The affiliation of sampling to normally distributed general populations was verified using Shapiro-Wilk test.

2.1 Determination of ATPase activity of actomyosin in smooth muscles of the stomach and colon

The ATPase activity was determined by the number of nanomolar of inorganic phosphate, which was produced by its cleavage from ATP by myosin active centers in the incubation medium in terms of milligrams of protein per minute. The total volume of the sample was 1.8 ml. Number of inorganic phosphate (P_i) was determined by Fiske-Subbarow method [15]. The solution optical density was measured at 720 nm. The ATP-hydrolase activity of actomyosin is determined by the following formula:

$$P_i = \frac{(D - D_0) \cdot N \cdot V_{total}}{t_{incub} \cdot V_{pr} \cdot V_{sn} \cdot C_{pr}},$$

where D is the optical density of the sample, D_0 is the optical density of control, V_{total} is the total volume in which color the reaction is carried out, $\mathrm{t_{incub}}$ is the incubation time of the protein with ATP, V_{pr} is the volume protein that is added to the sample, V_{sn} is the volume sampled supernatant after centrifugation of samples, C_{pr} is the concentration of protein that is added, N is the calibration factor.

2.2 Evaluation of carbonyl groups content in actomyosin

The degree of oxidative damage of the actomyosin protein complex has been determined by the content of 2,4-dinitrophenylhydrazone (DNPH), formed in the interaction of oxidized amino acid residues with 2,4-dinitrophenylhydrazyne [16]. The solution optical density was registered at 370 nm on a spectrophotometer SF-26. The number of carbonyl groups determined by the formula [10]:

$$K = \frac{(D - D_0) \cdot V}{22000 \cdot C_{pr}},$$

where D is the optical density of the sample, D_0 is the optical density of control, V is the final volume of the sample, 22000 is a molar extinction coefficient for 2,4-DNPH derivatives, C_{pr} is the concentration of protein that is added.

3. Results and Discussion

Experiments show that the ATP-hydrolase activity of actomyosin is reduced in gastric smooth muscle of senile group of rats. With aging, Mg^{2+}, Ca^{2+}-ATP-hydrolase activity of actomyosin in rat gastric smooth muscle reduces by 23% and K^+-ATP-hydrolase activity – by 53% (Fig.1).

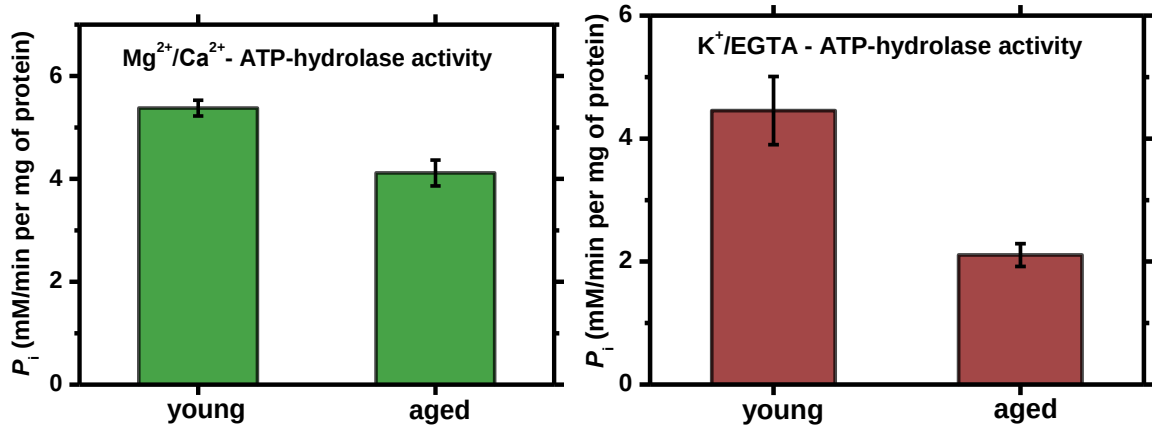

Fig.1. ATP-hydrolase activity of actomyosin in gastric smooth muscles of young and aged rats.

The ATP-hydrolase activity of smooth muscle of colon also decreased with aging, and more significantly than in stomach. Mg^{2+}, Ca^{2+}-ATP-hydrolase activity of actomyosin in smooth muscle of rat colon decreases by 70% and K^+-ATP-hydrolase activity – by 55% (Fig.2).

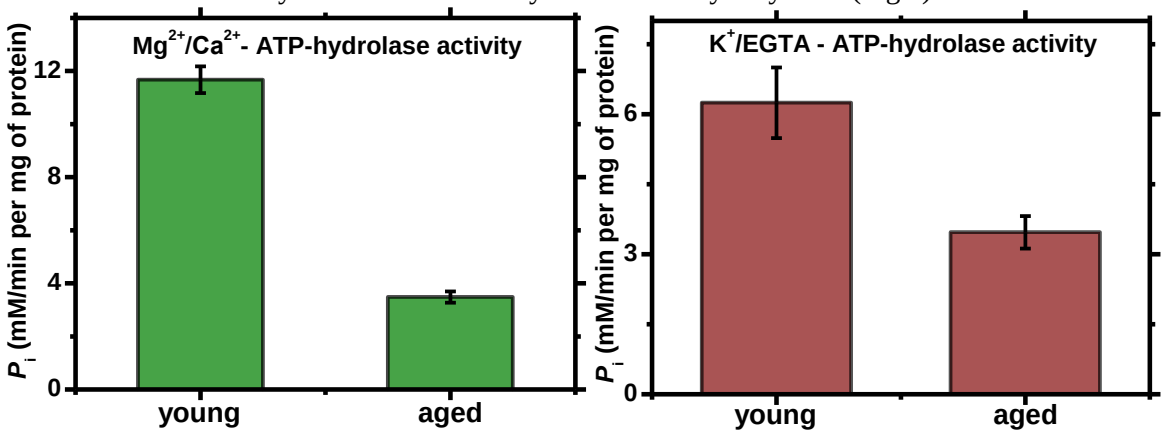

Fig. 2. ATP-hydrolase activity of actomyosin in colon smooth muscles of young and aged rats.

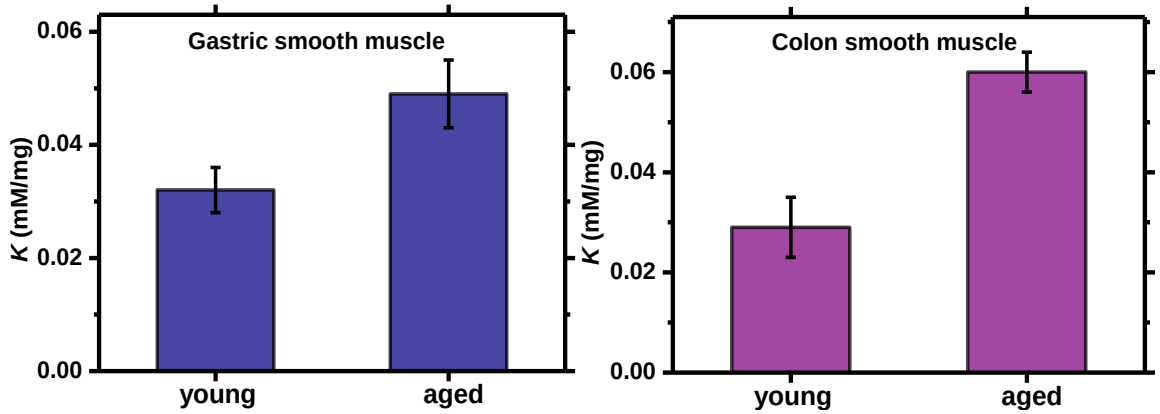

Fig. 3. The number of carbonyl groups in actomyosin complex in gastric and colon smooth muscles of young and aged rats.

It was shown that the quantity of carbonyl groups in the protein complex of actomyosin in gastric smooth muscle of old rats increases by 65,3% and in colon – by 48,8% towards young animals (Fig.3). The revealed oxidative modification of contractile proteins affects their function, namely at ATPase activity that was observed in the experiment.

4. Conclusion

It was established that the contractile function of stomach and colon smooth muscles was reduced with aging. It was accompanied by a decrease in the ATPase activity of actomyosin of the investigated gastrointestinal smooth muscles of aged rats. It was also shown that the oxidative modification degree of the contractile protein complex increased. Age-related reduction of smooth muscle contractility was attributed to essential oxidation of actomyosin due to increased production of free radicals with aging. It can be assumed that oxidative modification causes decline in the ATPase activity and inhibition of the contractile activity of smooth muscles of rat stomach and colon.

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