MECHANISM OF SELF-REGULATION AND OF THE INOTROPIC RESPONSE OF THE MYOCARDIUM IN AGING RATS

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Investigations on man and laboratory animals have shown that the contractility of the myocardium decreases during aging [3, 6, 8, 9, 11], which is expressed primarily as a decrease in the maximal rates of rise of pressure in the ventricles of the intact heart, the development of tension and shortening in isolated preparations of the myocardium, and an increase in the temporal characteristics of contraction.

The aim of this investigation was to study the effect of aging on regulation of isotonic contraction of heart muscle, effected by pre- and afterloading, by the frequency of contractions, and also by certain inotropic factors such as paired stimulation, extracellular calcium concentration, and hypothermia.

EXPERIMENTAL METHOD

Experiments were carried out on isolated papillary muscles of the heart from mature (8-10 months) and old (24-26 months) rats. Under thiopental-sodium anesthesia (80 mg/kg) the heart was removed from the animal and transferred into oxygenated Krebs–Henseleit solution (in mM): NaCl - 120, KCl - 4.8, $NaHCO_3$ - 25, KH_2PO_4 - 1.2, $MgSO_4$ - 1.25, $CaCl_2$ - 2.5, glucose – 8.6. The posterior papillary muscle was excised and placed in a constant-temperature glass chamber with a capacity of 100 ml, through which a solution of the same composition flowed continuously at a temperature of 30°C. The perfusion solution was saturated with a gas mixture (95% O_2 + 5% CO_2) and its pH was maintained at 7.4. The experimental situation was such that work of the muscle could be investigated under isotonic conditions. The muscle preparations were stimulated by above-threshold square pulses with a duration of 5 msec and a frequency of 0.3 Hz. Before the beginning of the experiments the muscle contracted for 1 h. By varying the preload from 0.05 to 1.0 g, and measuring the length of the muscle (L) with a micrometer, the extensibility of the muscle and dependence of the parameters of isotonic contraction on length [2] were investigated. The value of L_{max} was then determined – the length of muscle at which the amplitude of shortening was maximum, and the force – velocity curve was plotted by the method of afterloading contractions. The effect of inotropic factors was studied at L_{max}. The frequency of contractions was increased from 0.1 to 4 Hz. Paired stimulation was applied with the second pulse following from 40 to 1000 msec after the first. The extracellular calcium concentration was increased from 0.5 to 10 mM by replacing the solution in the chamber. The effect of hypothermia was investigated between 30 and 15°C. The degree of shortening was expressed in muscle length (m.l.), i.e., the ratio of the absolute values of shortening and the length of the muscle, in percent, and the maximal rates of contraction and relaxation were determined in units of muscle length per second (m.l./sec) by dividing the absolute value of velocity by the length of the muscle. The effectiveness of heterometric regulation was estimated from the ratio of the increase in amplitude of shortening to the increase in length of the muscle [5]. The significance of differences was established by Student's t test.

EXPERIMENTAL RESULTS

Lengthening of the papillary muscles in response to an increase in the stretching load was reduced in old animals with all the loads tested (Fig. 1a). This means that equal preloading causes less stretching of the myocardium and, correspondingly, less potentiation of cardiac contractions in old than in mature animals. With the same initial length of the mus-

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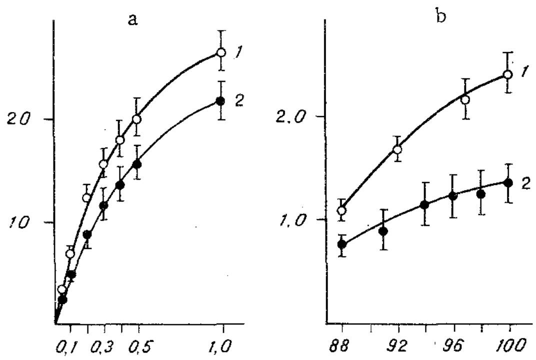

Fig. 1

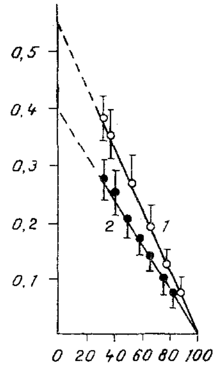

Fig. 2

Fig. 1. Extensibility (a) and dependence of shortening on length (b) in papillary muscles of mature (1) and old (2) rats. Abscissa: a) load (in g); b) length of muscle (in percent of L_{max}); ordinate: a) change in length of muscle (in percent); b) amount of shortening (in m.l.).

Fig. 2. Force—velocity curve for papillary muscles of mature (1) and old (2) rats. Abscissa, normalized force (total isotonic load/total isometric load, in percent); ordinate, velocity of shortening (in m.l./sec).

cles L_{max} for mature animals was reached after preloading of 0.3 g, compared with 0.5 g in old animals, evidence of an increase in the passive ridigity of the myocardium during aging.

The curve showing the dependence of the amount of shortening on length of the muscle for the old myocardium is shifted downward (Fig. 1b). Similar changes were found during analysis of the velocities of shortening and lengthening as functions of muscle length. The efficiency of the Starling mechanism during stretching of the papillary muscle from 0.88 L_{max} to L_{max} in mature animals was 2.4, whereas in old animals it was only 1.0. These results are evidence of weakening of heterometric regulation of the biomechanical function of the myocardium during aging.

Investigation of the force—velocity of shortening relationship showed that the corresponding curve for the myocardium of old animals was shifted downward and the velocity of

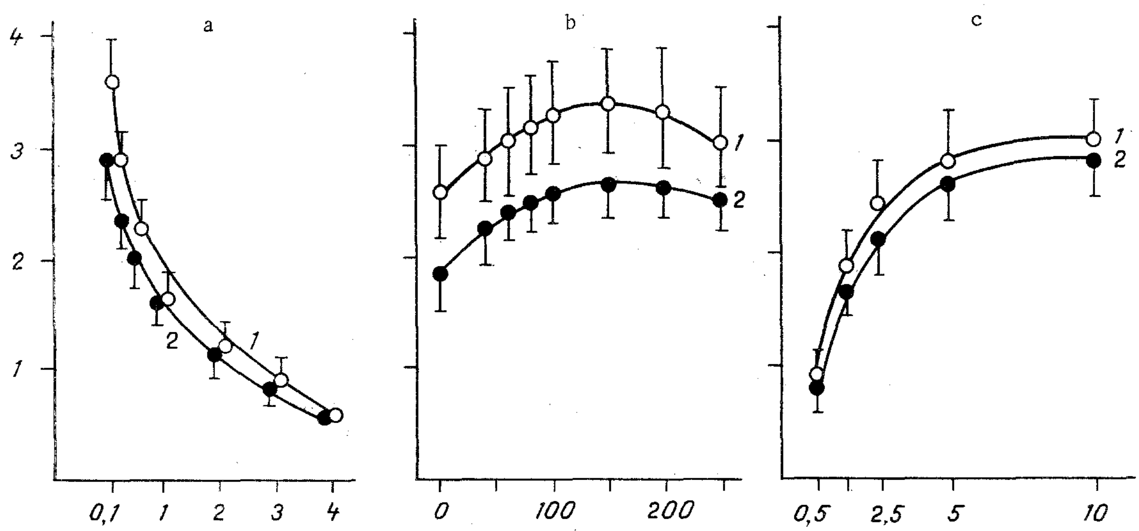

Fig. 3. Effect of frequency of contractions (a), of paired stimulation (b), and of Ca^{++} concentration in solution (c) on amount of shortening of papillary muscles of mature (1) and old (2) rats. Abscissa: a) frequency of stimulation (in Hz); b) interval between pulses (in msec); c) Ca^{++} concentration in solution (in mM); ordinate: amount of shortening (in m.l.).

TABLE 1. Biomechanical Parameters of Myocardium of Mature (A) and Old (B) Rats at Different Temperatures

Temperature, °C	Amount of shortening, m.l.		Velocity of shortening, m.l./sec		Velocity of lengthening, m.l./sec	
	A	B	A	B	A	B
30	3,28±0,39	2,26±0,36*	0,391±0,045	0,25±0,039*	0,253±0,032	0,148±0,025*
25	3,16±0,64	2,26±0,55	0,244±0,049	0,128±0,032*	0,173±0,032	0,087±0,026*
20	2,16±0,45	2,25±0,75	0,118±0,022	0,092±0,031	0,083±0,017	0,072±0,028
15	1,79±0,46	1,94±0,73	0,064±0,016	0,055±0,021	0,044±0,015	0,041±0,019

Legend. *) Differences between mature and old animals are significant.

contraction with zero load (V_{max}) was reduced (Fig. 2). These changes suggest that the intrinsic contractility of the heart muscle diminishes during aging.

The rat myocardium is characterized by negative chronoinotropic coupling [13, 14]. A similar relationship was obtained in the present investigations under isotonic conditions of contraction. An increase in the frequency of stimulation from 0.1 to 4 Hz led to a decrease in the amount of shortening and in the velocity of contraction and relaxation, the degree of which in the myocardium of the mature and old animals did not differ significantly (Fig. 3a).

Stimulation by pairs of pulses gave an equal effect of potentiation of contraction in both age groups. The greatest amount of rate of change in length in the myocardium of the mature and old rabbits were recorded when the interval between pulses was 150-170 msec (Fig. 3b). Investigation of the effect of external calcium on contractile function also showed that the increase in amount and velocity of shortening of the papillary muscles accompanying an increase in calcium ion concentration did not differ with age (Fig. 3c).

Changes in the biomechanics of the myocardium in response to an increase in the frequency of contractions, to stimulation by paired pulses, and to an increase in the external calcium concentration are linked with modification of calcium transport, more especially through the sarcolemma. On this basis it can be posulated that Ca^{++} transport through the cardiomyocyte plasma membrane is unchanged during aging.

Unlike the inotropic effects of a high frequency, of paired stimulation, and of external calcium, changes in the biomechanical parameters of the myocardium during hypothermia showed well-marked age differences. The amount of shortening with a fall of temperature to 15°C was reduced in the mature animals, whereas in the old rats it was virtually unchanged. Age differences in the velocities of shortening and lengthening, which existed at 30°C, disappeared when the temperature was lowered, so that the degree of their decrease in the myocardium of mature rats was considerably greater (Table 1). Values of Q_{10} for the velocity of contraction within the temperature range from 25 to 15°C were 4.0 in the myocardium of mature rats and 2.1 in that of old rats; the corresponding figures for the velocity of relaxation were 4.3 and 2.2.

Lowering the temperature inhibits the rate of Ca^{++} uptake by the sarcoplasmic reticulum [1], which plays the principal role in the rat myocardium in extraction of Ca^{++} from the sarcoplasm. Thus the considerably smaller changes in the biomechanical parameters during hypothermia in the old myocardium may be associated with a lower rate of Ca^{++} uptake by the sarcoplasmic reticulum and its smaller Ca^{++}-accumulating capacity. Other investigations also have yielded evidence of a decrease in the velocity of calcium transport in the reticulum [4, 7, 12].

The results indicate a decrease in the intrinsic contractility of the myocardium and weakening of its heterometric regulation during aging. One of the mechanisms of the changes observed in the biomechanical function of heart muscle is a change in calcium transport in the sarcoplasmic reticulum. The electrogenic transport of these ions through the sarcolemma is not reduced. On the contrary, it may undergo a compensatory increase [10].

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ROLE OF HYDROPHOBIC COMPONENTS OF THE PLASMA MEMBRANE IN THE RESPONSE OF FROG URINARY BLADDER CELLS TO ANTIDIURETIC HORMONE

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Interaction between antidiuretic hormone (ADH) and its receptor located in the basal plasma membrane of the cell is accompanied by cyclic AMP (cAMP) formation and culminates in molecular restructuring of the apical plasma membrane and increased permeability for water [8]. The role of hydrophobic membrane components in realization of the effect of ADH has not hitherto been studied, although the state of the hormone receptor, of adenylate cyclase, and also reorganization of the water channels and the formation of aggregates of particles in the apical membrane could be dependent on lipids. It has recently been shown that exogenous lipids can potentiate the action of ADH [5].

The aim of this investigation was to study the functional role of hydrophobic interaction in the plasma membranes of epithelial cells in response to ADH and to an increase in the flow of water along the osmotic gradient. For this purpose, the detergent Triton X-100 was applied to the isolated frog urinary bladder and changes in water transport in response to ADH were recorded.

EXPERIMENTAL METHOD

The investigation was conducted on spring frogs (*Rana temporaria* L.). Water transport was studied in the isolated urinary bladder by a gravimetric method [3]. Ringer's solution diluted 1:10 with water was introduced into the bladder from the side of the mucous membrane, and undiluted Ringer's solution was applied from the side of the serous membrane. The following reagents were used: Triton X-100 (Ferak, West Berlin), theophylline (Richter, Hungary), forskolin (Calbiochem, USA), and cAMP (Reanal, Hungary); the source of the ADH was pituitrin P (Kaunas Endocrine Preparations Factory). The sodium and potassium concentrations were measured on a Zeiss III frame photometer in an air-propane flame.

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