The Role of Age-Related Factors in the Effect of Long-Term Perfusion on the Contractility of Isolated Heart

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Experiments on isolated hearts of mature and senescent rats showed that in mature rats the heart contractility decreased to a greater extent during the initial hours of a long-term perfusion, but in mature heart tolerance to perfusion was higher. Creatine phosphokinase activity drastically increased in the perfusate of senescent hearts and changed insignificantly in the perfusate of mature hearts. In mature rats a long-term perfusion was accompanied by activation of Na,K-ATPase, which was prevented by actinomycin D. Transfer of perfusate in the donor-recipient double-heart experiments with prior administration of actinomycin D showed that long-term perfusion induces the synthesis of a regulator factor (inverter) in the heart of mature rats which modulates myocardial contractility. This regulator peptide contributes to adaptive potency of the heart in mature animals.

Key Words: *isolated heart; long-term perfusion; contractility; creatine phosphokinase; Na,K-ATPase*

Changes in cardiac contractility during aging were studied in detail in humans and animals [1,4]. They are related to age-dependent changes in the heart and modifications of neurohumoral regulation. This explains the interest of researches in the nature of contractility changes in isolated heart deprived of extracardiac influences. Special attention was focused on the age-associated changes in cardiac activity in hypoxia. A model based on a long-term perfusion of isolated heart, which is inevitably accompanied by ischemia, has been developed [4]. Our aim was to study the age-related changes in cardiac activity under conditions of long-term perfusion and to elucidate the nature of these changes with the use of the donor-recipient double-heart system.

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MATERIALS AND METHODS

Experiments were carried out on isolated hearts of mature (6-8-month-old) and senescent (26-28-month-old) male Wistar rats using a modified method [7]. Coronary perfusion was performed under constant pressure by the Langendorff technique. In some rats, actinomycin D (a blocker of protein synthesis at the transcription level) was injected intraperitoneally in a dose of 50 μg/kg 40 min prior to experiments. A Nihon Kohden polygraph was used to record the cardiogram, left ventricular pressure P, and its first derivative *dP/dt*. These data were used to calculate heart rate (HR) and myocardium mechanical stress described by the following parameters of myocardial contractility: maximum pressure developed by the myocardium P_{max}, rising (*+dP/dt*) and falling (*-dP/dt*) rates of intraventricular pressure. Coronary blood flow was measured by the volume of perfusate collected for 1 min.

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TABLE 1. Changes in CPK Activity in Perfusate during Long-Term Perfusion (mU/g×min, $M \pm m$)

Rats	Perfusion duration, min		
	30	90	150
Mature	17.2±4.1	22.2±4.7	25.3±2.4
Senescent	31.5±6.8	80.9±6.4	104.8±23.4

In addition, the "time-tension" index (P_{max}×intraventricular pressure×HR) corresponding to oxygen consumption rate (OCR) in the myocardium and the coefficient of myocardial hypoxia (Q/OCR) were calculated. The adaptation period for isolated heart was 15-30 min. The heart was perfused for 4 h. In the double heart system the running off perfusate was collected from the 30th till the 90th min of the perfusion period, aerated, and transferred to the other heart.

The cardiomyocyte plasma membranes were isolated as described elsewhere [2], and the protein concentration in the sample was measured by the method of Bradford [6]. Na,K-ATPase activity was determined as the difference between the total and Mg-dependent ATPase activities [9]. The concentration of inorganic phosphorus was measured by a previously described method [8].

The activity of creatine phosphokinase (CPK) was determined in the perfusate using a NAC Activated CPK Test standard kit (Kone). The results were statistically analyzed by nonparametric one-factor dispersion analysis (ANOVA software).

RESULTS

The initial HR and the indices of contractile activity of the mature and senescent hearts were consistent with the literature data [1,4]. They show that aging modifies cardiac contractility in a heterochronous way, the diastolic phase being the most vulnerable stage in this process.

Cardiac activity changed in mature and senescent rats after a 4-h perfusion (Fig. 1): HR, force and rate of cardiac contractions, and coronary blood flow decreased. Our findings indicate that long-term perfusion of isolated heart disturbs function and causes hypoxic contracture of I-II functional degree [1].

Under these conditions the decrease in cardiac contractility in senescent rats was smaller than that in the mature rats. This was observed after 3 h of perfusion (Fig. 1). Then P_{max} and the rate parameters began to decrease drastically in senescent rats, while in mature rats they were relatively stable. Both OCR and Q/OCR varied correspondingly. For example, after 220-240-min perfusion OCR de-

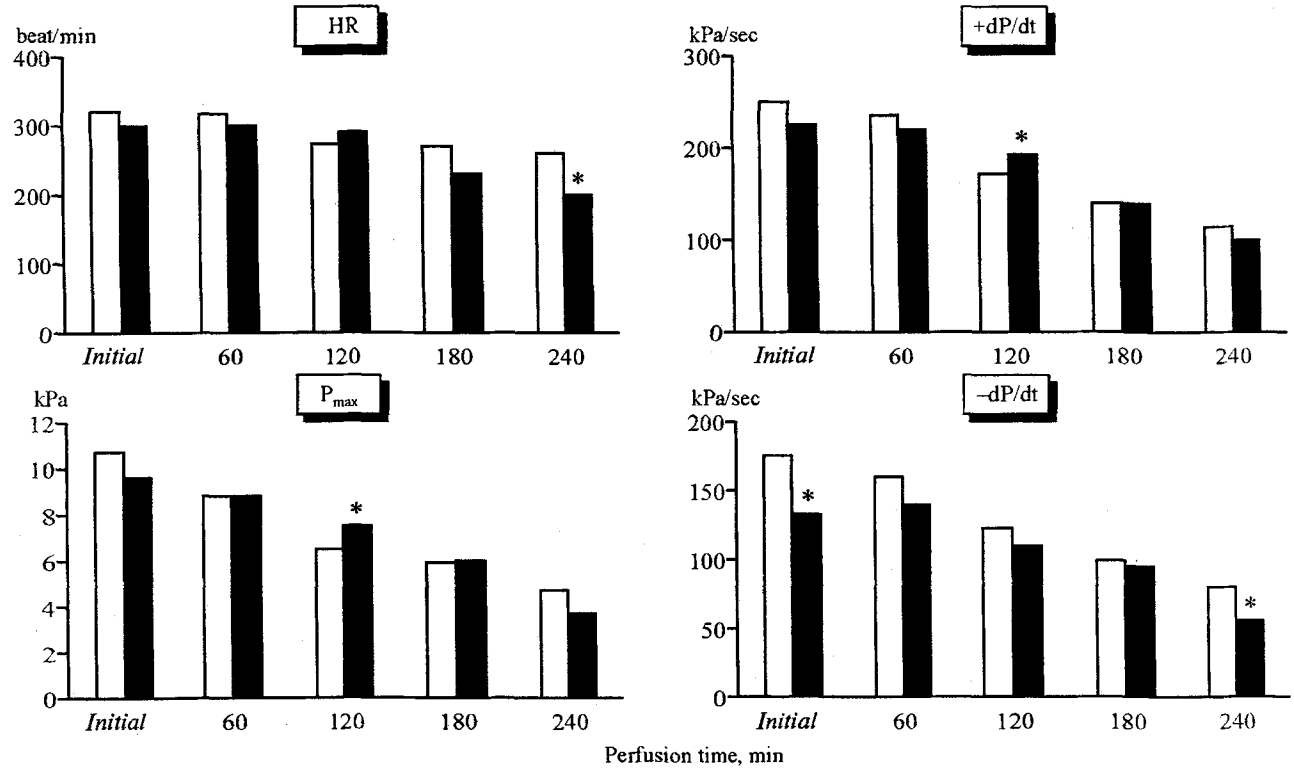

Fig. 1. Changes of isolated heart contractility in mature (open bars) and senescent (solid bars) rats during a long-term perfusion. *$p<0.05$ in comparison with mature rats.

creased by 61% in mature rats, and by 70% in senescent rats. The Q/OCR index in mature rats increased 1.6-fold by the end of perfusion, while the corresponding increase in senescent rats was 1.8-fold. The cardiac tolerance tests showed that isolated mature hearts beat in a steady-state mode during 7-8-h perfusion, while the senescent hearts stopped beating by the 5th hour of perfusion.

The release of CPK by the myocardium has been considered as an objective index of changes in the heart [5]. Perfusate CPK activities are significantly different in mature and senescent hearts. Table 1 shows that the enzyme activity increases considerably in the perfusate of senescent hearts during a long-term perfusion (by 232%) while in mature rats it increases by 47%. Thus, a long-term perfusion decreases the contractility of isolated heart more rapidly in ma-

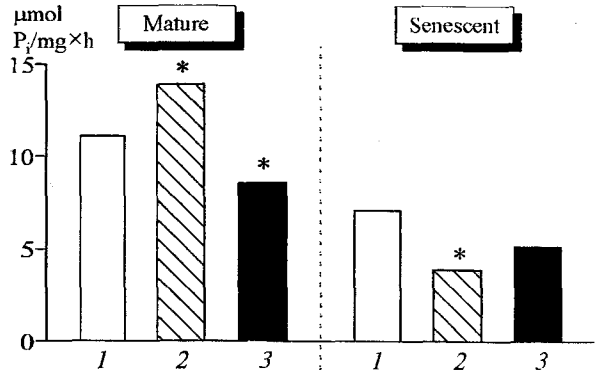

Fig. 2. Activity of Na,K-ATPase in the cardiomyocyte plasma membranes from rats of various age during a long-term perfusion. *1*) initial data (15-20 min of perfusion), *2*) long-term perfusion (90-110 min of perfusion), *3*) long-term perfusion with actinomycin D pretreatment. *$p<0.05$ in comparison with initial data.

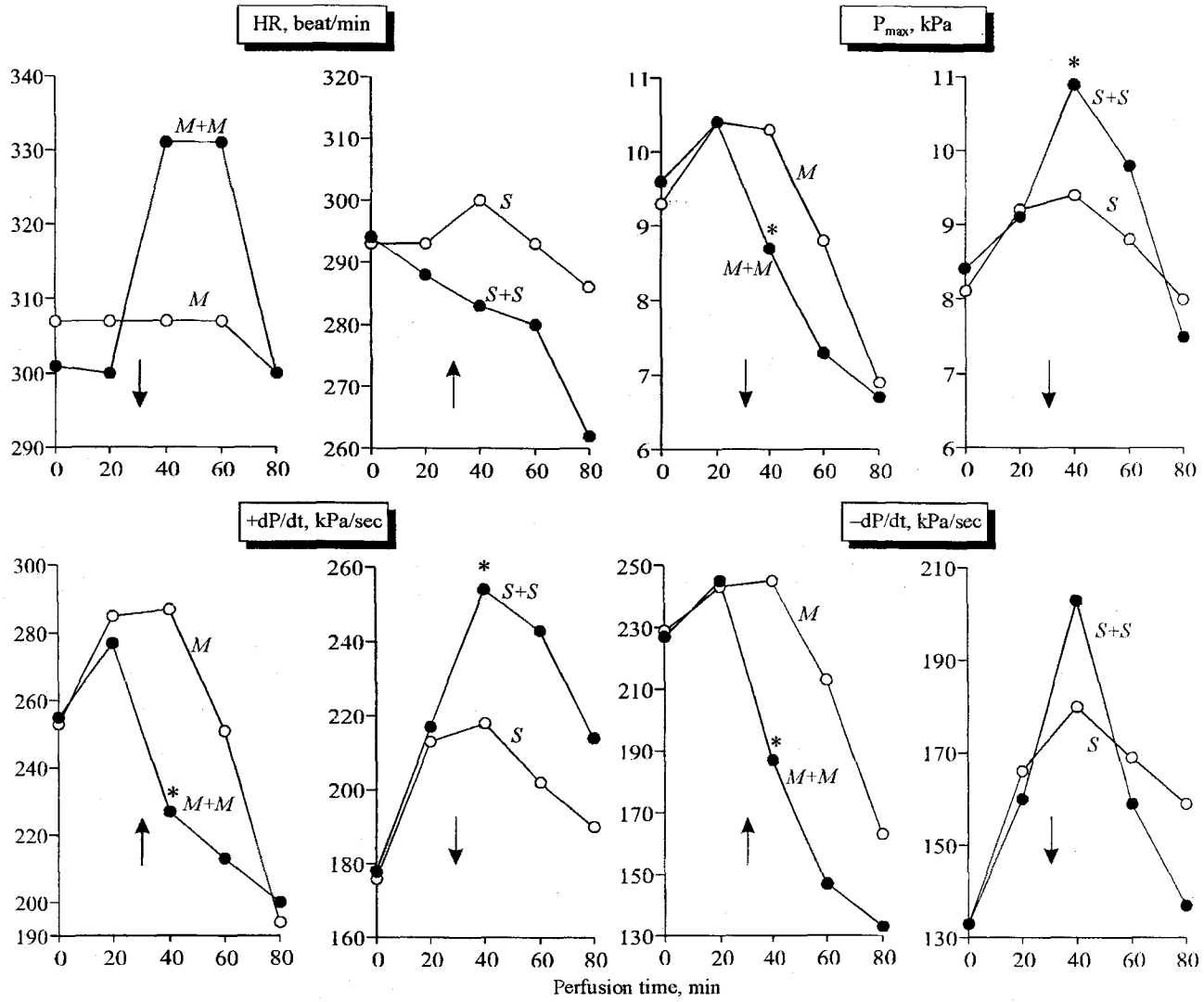

Fig. 3. Effect of perfusate from isolated donor heart on the contractility of recipient heart from the rat of the respective age. *M*) control perfusion of mature hearts; *M+M*) perfusion of mature hearts by perfusate taken from a mature heart; *S*) control perfusion of senescent hearts; *S+S*) perfusion of senescent hearts by perfusate from a senescent heart. *$p<0.05$ in comparison with the control.

ture than in senescent rats. At the same time, cardiac tolerance and ability to maintain a relatively high level of contractile activity is stronger in mature rats.

The study of Na,K-ATPase activity in the fraction of isolated plasma membranes of cardiomyocytes showed that under a long-term perfusion, this enzyme activity increased by 25% in mature rats, while it decreased by 45% in senescent rats. Activation of Na,K-ATPase was prevented by actinomycin D (a blocker of protein synthesis at the transcription level, 50 mg/kg 40 min before perfusion, Fig. 2).

An important role in the age-related differences in contractility of isolated heart may be attributed to modification of the state of the plasma membrane. A new class of substances (inverters) was discovered which are synthesized under the control of genome and regulate the plasma membrane state [3]. It can be suggested that the hypoxia-induced changes in cardiac contractility in mature and senescent rats are determined by the synthesis of substances which attenuate heart sensitivity to hypoxia.

To check up this hypothesis the following donor-recipient double-heart systems were used: mature-mature rats and senescent-senescent rats. The perfusates from the donor hearts of mature and senescent rats affected the contractility of the recipient heart in a different way. The "mature" perfusate decreased, while the "senescent" perfusate increased the contractility of the recipient heart of the corresponding age (Fig. 3). Some factor (peptide inverter) is probably synthesized in the hearts of mature rats which is transferred to the recipient heart, where it modifies the contractility. Presumably, activation of adrenergic system in isolated senescent heart [4] is responsible for activation of the recipient heart by perfusate from a senescent donor heart.

Blockade of protein synthesis by actinomycin D prevented the contractility decrease in the mature heart, which otherwise occurred during the first 3 h of perfusion, although the contractility then decreased. In senescent rats, pretreatment with actinomycin D virtually did not affect myocardial contractility. These data suggest that the mature heart produces a peptide factor that decreases myocardial contractility. This is the factor that is transferred by perfusate and affects the recipient heart.

These findings were further substantiated in double-heart experiments with administration of actinomycin D. Perfusate from mature donor heart taken from a rat pretreated with actinomycin D 40 min prior to heart isolation did not produce the corresponding effect on mature recipient heart. By contrast, perfusate from the senescent hearts pretreated with actinomycin D produced just the same effect on the contractility of senescent recipient heart as in the control experiments without the blocker.

These data show that under unfavorable conditions (a long-term perfusion) cardiomyocytes of mature rats produce a factor that moderates cardiac contractility. The synthesis of this factor is suppressed by the transcription inhibitor actinomycin D, which attests to the peptide nature of this factor. Under certain conditions, synthesis of this factor may play an important adaptive role, protecting the heart from overloading and exhaustion. Aging decreases synthesis of this factor, which may be one of the reasons of low tolerance of the heart and of severe disturbances in its performance under hypoxia.

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