Invertor-Mediated Modulation of Na,K-ATPase Activity in Myocardial Plasma Membranes in the Emergency Stage of Compensatory Cardiac Hyperfunction in Mature and Senescent Rats

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Myocardial hyperfunction is experimentally modeled by coarctation of the aorta in mature (6-8-month-old) and senescent (26-28-month-old) Wistar rats. During the emergency phase of cardiac hyperfunction Na,K-ATPase activity is shown to rise reliably in mature animals, while in old rats it remains unchanged. Cardiomyocyte cytosol and blood plasma from mature (but not old) rats with experimental coarctation of the aorta activate Na,K-ATPase in membranes from the myocardium of both the mature and old rats. It is assumed that the activation of Na,K-ATPase during the emergency stage of cardiac hyperfunction is mediated through synthesis of specific invertors. In senescent animals the synthesis of invertors probably becomes insufficient, while membrane sensitivity to them is preserved.

Key Words: *cardiomyocytes; coarctation of the aorta; Na,K-ATPase; invertors*

The emergency phase of cardiac hyperfunction caused by coarctation of the aorta (CA) is known to be characterized by a combination of damage to cell structures and their rapid growth — *de novo* formation of membranes, mitochondria, and myofibrils, augmented RNA and protein synthesis, enhanced functioning of myocardial structures, and energy deficiency [3,4,13]. In senescent animals, long-term heart strain shifts the time of development and changes the pattern of compensatory hyperfunction. Previous studies have shown that in senescent animals heart failure may be caused by the type of strain inducing adaptive hyperfunction in young animals [9]. Active ion transport and shifts in Na,K-ATPase activity are considered to play an important role in the mechanism of myocardial hyperfunction. Our previous studies demonstrated that activation of membrane Na,K-ATPase under conditions of enhanced protein synthesis in the cell may be mediated through some cytosol factors regulating its activity, factors that we dubbed "invertors" [6-8,10,12].

The aim of the present study was to compare changes in Na,K-ATPase activity during the emergency stage of compensatory heart hyperfunction in mature and senescent rats.

MATERIALS AND METHODS

The experiments were carried out on mature (6-8-month-old) and senescent (26-28-month-old) Wistar rats. Myocardial hypertrophy was modeled by CA, which narrowed the aorta 2.5-fold [2]. Cardiomyocyte (CM) plasma membranes were isolated as described elsewhere [5], and the protein concentration in the sample was measured after Bradford [11]. Na,K-ATPase activity was determined from the difference between total and Mg-dependent ATPase activities [1,15]. The concentration of inorganic phosphorus was measured by a previously described me-

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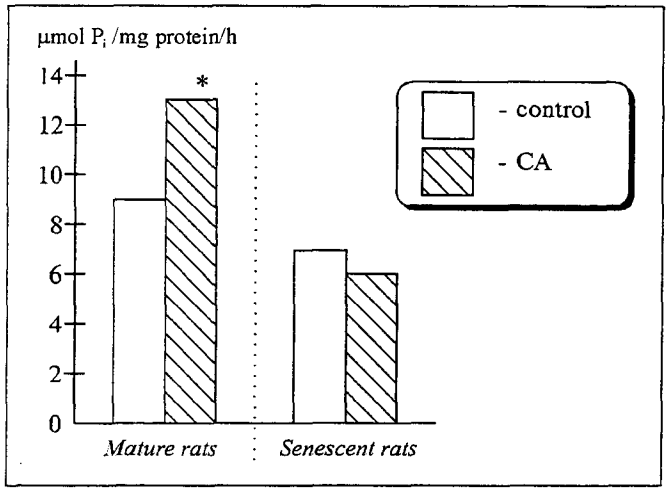

Fig. 1. Effect of coarctation of the aorta (CA) on Na,K−ATPase activity in a fraction of plasma membranes isolated from the myocardium of mature and senescent rats. Here and in Fig. 2: *$p<0.05$ in comparison with the control.

thod [14]. The isolated plasma membranes of CM were incubated *in vitro* with CM cytosol and rat serum: 150 μl CM cytosol or rat serum and an aliquot of CM membranes (70 μg protein/sample) were added to the ATP-free reaction mixture for measurement of Na,K-ATPase or Mg-ATPase activity. The samples were incubated for 40 min in an ice-cold bath with constant shaking, after which ATP was added and ATPase activity was measured as described above.

RESULTS

The experiments showed that on day 6 after CA, Na,K-ATPase activity in CM membranes from mature rats was reliably elevated (13.0±0.6 *vs.* 8.9±0.2 μmol P_i/mg protein/hour in the control, $p<0.05$, Fig. 1), whereas in senescent rats it remained practically unchanged (6.0±0.5 *vs.* 6.7±0.9 μmol P_i/mg protein/hour in intact rats).

As mentioned above, the emergency stage of cardiac hyperfunction is characterized by enhanced protein and RNA synthesis in cardiomyocytes. It may be assumed that activation of Na,K-ATPase after CA is mediated through synthesis of Na,K-ATPase-activating invertors. To verify this assumption we carried out special experiments with "cell hybrids": the isolated plasma CM membranes from intact rats were incubated *in vitro* with CM cytosol from rats after CA. The CM cytosol from mature rats after CA was found to reliably activate Na,K-ATPase in the fraction of isolated plasma membranes from the myocardium of intact mature rats (by 55% on average, Fig. 2), whereas CM cytosol from senescent rats did not activate Na,K-ATPase in membranes from senescent intact animals (Fig. 2). The experiments with heterochronous cell hybrids (plasma membranes and cytosol of CM from animals of different age) showed that CM cytosol from senescent rats after CA reliably activates Na,K-ATPase in the CM membranes from senescent rats (by 48% on average, Fig. 2), while CM cytosol from senescent rats does not activate Na,K-ATPase in CM membranes from mature intact rats (Fig. 2). These data attest to the appearance of certain factors in CM cytosol during the emergency phase of cardiac hyperfunction in mature rats which activate Na,K-ATPase in the plasma membrane. In senescent animals synthesis of Na,K-ATPase-activating factors after CA probably dwindles. However, the ability of the CM plasma membrane to react to the Na,K-ATPase-activating factors is preserved.

The Na,K-ATPase-activating factors enter the blood of the experimental animals. For instance, plasma taken from mature rats during the development of the emergency phase of cardiac hyperfunction activates Na,K-ATPase of CM membranes from mature and senescent intact rats by an average of 47% and 46%, respectively (Fig. 2). The plasma from

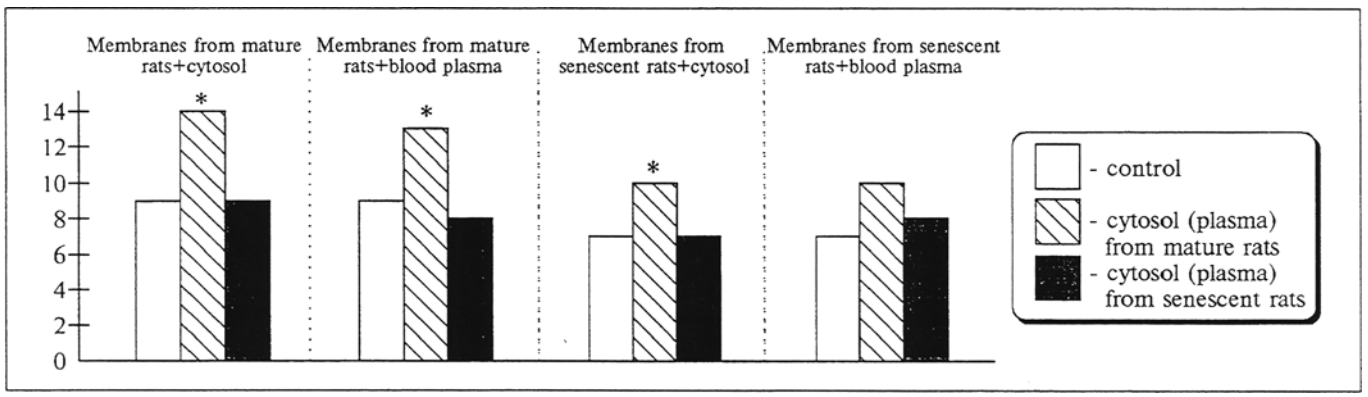

Fig. 2. Effect of cytosol from cardiomyocytes and blood plasma of mature and senescent rats after coarctation of the aorta on Na,K−ATPase activity in the fraction of plasma membranes isolated from the myocardium of rats of different age. Ordinate: μmol P_i/mg protein/h.

old rats taken during the emergency phase of cardiac hyperfunction hardly affects Na,K-ATPase activity in CM membranes of mature and senescent rats.

Thus, in mature animals during the emergency phase of cardiac hyperfunction Na,K-ATPase is reliably activated, while in senescent rats under these conditions the activity of the enzyme remains unchanged. This is probably because during this phase, when protein biosynthesis is being activated, the synthesis of invertors stimulating Na,K-ATPase in mature animals is also activated, whereas in old animals it is inhibited. On the other hand, the sensitivity of the CM plasma membrane to invertors is preserved in senescent rats.

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