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CARDIAC ENERGY METABOLISM AND LIPID PEROXIDATION IN STRESSED MALE AND FEMALE RATS

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Immobilization for 10 min induced a significant increase in cardiac mitochondrial ATPase activity in both male and female rats. Prolonged emotional stress for 7, 14 and 28 days was accompanied by progressive elevation in ATPase activity that was more pronounced in males vs. females. Also, emotional stress induced progressive depression in ATPase response to immobilization. These types of stress were not accompanied by a significant activation of lipid peroxidation in cardiac mitochondria reflecting a high antioxidant reserve in the heart. So, during moderate emotional prolonged stress males demonstrated a much more pronounced elevation in cardiac energy metabolism than females which may result in a more rapid depletion of energy metabolic reserves in the male heart in comparison with the female one.

INTRODUCTION

The great number of stress situation in the life of modern human beings leads to a failure of the adaptation mechanism that results in the development of cardiovascular diseases. It was established that men in comparison with women are more subjected to cardiovascular diseases. Although the potential mechanisms of this phenomenon are not fully understood much efforts has been directed at studying the gender-related particularities in cardiovascular stress reactivity. A marked progress has been achieved in studying the role of lipid peroxidation in the pathogenesis of heart diseases (Singal, Khaper, Palace & Kumar, 1998). *In vitro* studies revealed that free radicals reduce the ability of mitochondria to synthesize ATP, while superoxide dismutase and catalase improve ATP production (Ceconi, Curello, Albertini & Ferrari, 1988; Nohl, Breuinger & Hegner, 1978). It should be noticed that *in vivo* the comparative evaluation of cardiac energy metabolism and lipid peroxidation was done mainly in males that have been subjected to severe and prolonged stress associated with cardiac hypoxia (Jolly, Kane, Bailie, Abrams & Lucchesi, 1984) and chronic pressure overload (Randhawa & Singal, 1992).

This study was aimed at a comparative evaluation of energy metabolism of heart mitochondria and lipid peroxidation in male and female rats subjected to different types of moderate stress.

MATERIALS AND METHODS

Male and female rats were housed under controlled conditions of temperature (22–24°C), humidity (50–70%) and a 12 h light: 12 h darkness cycle. All procedures were perfomed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. In the first set of experiments males and females were subjected to 10 min immobilization. Rats of the second set were subjected to prolonged isolation stress for 7, 14 and 28 days in individual cages in a quiet room. In the third set of experiments the chronically stressed rats were immobilizated for 10 min after 7, 14 and 28 day of social isolation.

The heart was excised immediately after decapitation and placed in a vial containing ice-cold saline. The heart was minced and homogenized using a glass Potter-Elvehjem homogenizer. The suspension of heart mitochondria was obtained by differential centrifugation (Schneider & Hogeboom, 1951). Mitochondrial ATPase activity was estimated by measurement of ATP decomposition. The malondialdehyde (MDA) level was measured by the reaction with thiobarbituric acid. Data were

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calculated as percentages of the relevant control values. Statistical analyses were made by comparing the mean values and tested by the Student's "t" test. The level of significance was set at $P \leq 0.05$.

RESULTS

The basal values of ATPase activity in heart mitochondria did not differ reliably between females and males (0.17 ± 0.02 and 0.16 ± 0.02 μmol P_i/(mg protein × hour), respectively). Immobilization stress for 10 min induced a significant increase in the heart ATPase activity that was similar for females and males, by 265 ± 43% ($P < 0.001$) in females and by 294 ± 49% ($P < 0.001$) in males. Prolonged isolation stress resulted also in a marked elevation of the ATPase activity and these changes were dependent on the gender and duration of isolation (Fig. 1). Thus, ATPase activity increased to the 7th day of stress in females, but to the 14th day in males. Moreover, the stress-induced progressive rise in ATPase activity was significantly greater in males vs. females when measured to the 14th day and especially on the 28th day of isolation. It might therefore be supposed that adaptation to a prolonged psychoemotional stress is characterized by a more pronounced cardiac stress in male organism in comparison with female one.

The result of the second set of experiments revealed a progressive effect of social deprivation on the ATPase response to short-term stress in both males and females. This effect was determined by duration of social stress (Fig. 2). Isolation for 7 days did not change significantly the ATPase reactivity to short-term stress in both males and females. ATPase response to immobilization after 14 days of prolonged stress was nearly 2-fold lower in females and 3-fold lower in males in comparison with immobilization stress alone. After 28 days of prolonged stress the immobilization was not accompanied by essential gain in ATPase activity in cardiac mitochondria in both males and females. Therefore our data indicate that cardiac adaptation to prolonged stress was accompanied by increasing activation of heart energy metabolism that was more pronounced in males than females. Together with increasing elevation of cardiac metabolism, prolonged stress induced a progressive depression of the ATPase response to short-term stress. Perhaps, this fact reflects the significant depletion of heart energy metabolic reserve induced by prolonged stress. On the other hand, the absolute values of ATPase activity during short-term stress were about the same in intact and chronically stressed rats on the 7th, 14th, and 28th days of isolation. It might be supposed that the high level of energy metabolism in cardiac mitochondria induced by chronic stress is sufficient to maintain cardiac activity under conditions of 10 min-immobilization.

We have also studied changes in lipid peroxidation in heart mitochondria during these types of stress. Basal values of MDA in cardiac mitochondria did not differ between females and males (0.064 ± 0.005 and 0.065 ± 0.007 arb. u., respectively). In females short-term immobilization caused a decrease in the MDA level by 22% ($P < 0.05$) reflecting a high myocardial antioxidant reserve. In females prolonged stress was accompanied by an increase in MDA level by 41% ($P < 0.05$) only to the 14th day of isolation. Chronically

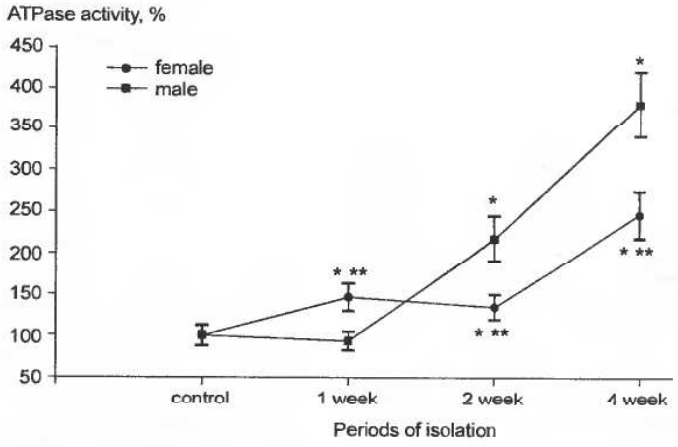

Fig. 1. Responses of cardiac mitochondrial ATPase activity to isolation stress in female and male rats. Significantly different: from control group *$P < 0.05$, from males **$P < 0.05$

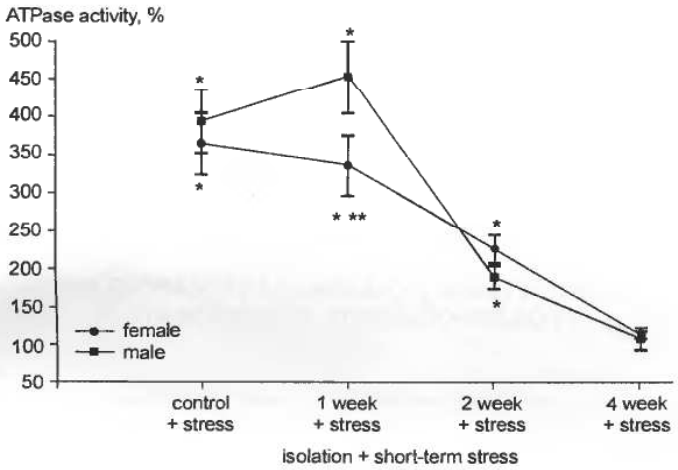

Fig. 2. Responses of cardiac mitochondrial ATPase activity to short-term stress after isolation in female and male rats. Significantly different: from control group *P < 0.05, from males **P < 0.05

stressed females did not reveal the typical decrease in the MDA level during short-term immobilization. As for males, both short-term and prolonged stress and their combination did not change the MDA level in cardiac mitochondria of females.

Therefore the significant activation of cardiac energy metabolism during short-term and prolonged stress that was more pronounced in males vs. females was accompanied by weak changes in lipid peroxidation only in females, but not males. It should be noticed that activation of lipid peroxidation was revealed only in females to the 14th day of isolation. So, we believe that our models of stress were strong enough to cause a significant activation of cardiac energy metabolism but not of lipid peroxidation. Actually, a detectable activation of oxidative stress is usually induced by a more strong and prolonged stress. For example, the decrease in the antioxidant myocardial reserve was demonstrated only after 20 weeks of banding of the ascending aorta, leading to chronic pressure overload (Dhalla & Singal, 1994).

The more pronounced elevation in cardiac energy metabolism in males vs. females during prolonged stress may be explained by the fact that the male organism are much more subjected to hard physical loads in comparison with female ones. Such physical loads have disappeared in the life of modern human beings but the intensity of psychoemotional load has sharply increased. Our results suggest that the greater cardiac energy metabolism elevations during emotional stress in males vs. females may result in a more rapid depletion of cardiac energy metabolic reserves in the male.

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