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Exercise Preconditioning against Hydrogen Peroxide-Induced Oxidative Damage in Proteins of Rat Myocardium

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Both regular physical exercise and low levels of H_2O_2 administration result in increased resistance to oxidative stress. We measured the accumulation of reactive carbonyl derivatives and the activities of proteasome complex and DT–diaphorase in cardiac muscle of trained and untrained rats after chronic i.p. administration of 1 ml *t*-butyl H_2O_2 (1 mmol/kg for 3 weeks every second day). Twenty-four rats were randomly assigned to a control group administered with saline, control administered with H_2O_2, and exercised administered either saline or H_2O_2. The activity of DT–diaphorase significantly increased in H_2O_2 administered and exercised groups, indicating that an increase in H_2O_2 levels stimulate the activity of this enzyme. The cardiac muscle of H_2O_2 administered nonexercised animals accumulated significantly more carbonyl than control group ($P < 0.05$). The exercise and H_2O_2 administration resulted in less oxidatively modified protein than found in nonexercised groups ($P < 0.05$). The peptide-like activity of proteasome complex was induced by the treatment of H_2O_2 and exercise and exercise potentiate the effect of H_2O_2. On the other hand, the chymotrypsin-like and trypsin-like activities were stimulated only by physical training and H_2O_2 administration. The data suggest that chronic administration of H_2O_2 after exercise training decreases the accumulation of carbonyl groups below the steady-state level and induces the activity of proteasome and DT–diaphorase. Hence, the stimulating effect of physical exercise on free radical generation is an important phenomenon of the exercise-induced adaptation process since it increases resistance to oxidative stress. Regular exercise training is a valuable physiological means of preconditioning the myocardium to prolonged oxidative stress. © 2000 Academic Press

Cardiovascular and related diseases such as hypercholesterolemia, hypertension, and diabetes mellitus are often causative factors of myocardial infarction. The inability of myocardium to maintain physiological function during prolonged ischemia is partly due to the limited adaptability of this tissue. Regular physical exercise, which increases the numerical density and lumenal surface of the myocardial capillaries, not only improves the efficiency of the oxygenation of the myocardium, but probably, due to the tachycardia-induced adaptation, also has preconditioning effects to oxidative stress (1, 2). It has been postulated that rigorous physical exercise results in increased formation of reactive oxygen species (ROS)[1] and these radicals might be stimulators of antioxidant enzymes in cardiac muscle (3, 4). Indeed, a significant increase in the activity of antioxidant enzymes in cardiac muscle has been observed after exercise training (4, 5). Moreover, it has also been shown that swimming training hypertrophied cardiac muscle is more resistant to ischemia-reperfusion induced oxidative stress than normal heart (6). Hence, it appears that heart trained by regular exercise, is more resistant to ROS induced damage.

It has also been demonstrated that administration of low levels of H_2O_2 results in increased resistance to oxidative stress (7). Most previous studies measured lipid peroxidation as a marker of oxidative damage following the administration of H_2O_2. However, it was reported that H_2O_2 alone does not induce lipid peroxidation (8) but could induce oxidative damage of proteins (9). Recently we have shown that lipid peroxida-

[1] Abbreviations used: ROS, reactive oxygen species; AMC, 7-amino-4-methyl-coumerin; SUC-LLVY-MCA, succinyl–Leu–Leu–Val–Tyr–MCA; MCA, monochloroacetic acid; BOC-LLR-MCA, butyloxycarbonyl–Leu–Arg–Arg–MCA.

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tion and oxidative damage of proteins can occur separately and by different mechanisms (10). The reaction of H_2O_2 with Fe(II)–protein complex results in formation of reactive carbonyl derivatives in the peptide side chain of arginyl, aspartyl, glutamyl, lysyl, prolyl, and threonyl residues. The appearance of carbonyl groups in proteins leads to inactivation of the proteins and increases in the susceptibility proteolytic degradation (11). Accumulation of carbonyl and other oxidatively modified adducts is dependent upon the rate of ROS generation as counteracted by the activity of repair systems, or ROS removal by degradation of the whole molecule, or a combination of these processes (12). Therefore, the activity of proteasome complex plays a regulatory role in the accumulation of oxidative damage. Proteasome complex is responsible for the degradation of oxidatively modified proteins. However, it is also a subject of free radical damage and is inhibited by hydrogen peroxide (13).

The present investigation was designed to study the effects of exercise training-induced adaptation and chronic H_2O_2 administration-induced adaptation on carbobyl group accumulation and activities of proteasome and DT–diaphorase in cardiac muscle of rats. DT–diaphorase is able to catalyze two-electron reduction and to protect against xenobiotics which could generate ROS and therefore can be regarded as an antioxidant enzyme (14, 15). In addition it has preventive effects on mutagenesis and carcinogenesis (14, 16).

METHODS

Rats. Twenty-four young male Wistar rats, 4 weeks old, were used in the study and cared for according to the "Guiding Principles for the Care and Use of Animals". The rats were randomly assigned to control administered with saline, control administered with H_2O_2, and exercised administered either with saline or H_2O_2.

Training protocol. The exercised rats were exposed to swimming exercise five times a week for 9 weeks. Swimming duration was 60 min for 6 weeks and then increased to 90 min for the remaining 3 weeks in water temperature maintained at 32°C. Exercised and control rats were injected intraperitoneally with 1 ml H_2O_2 (*t*-butyl H_2O_2) at a dose of 1 mmol/kg body weight (17) or saline. This administration was carried out for 3 weeks every second day. One day after the last administration the rats were sacrificed and hearts were excised, frozen in liquid nitrogen, and stored at −80°C for later analysis. Subsequently, the samples were homogenized for various biochemical assays.

Assays. The measurement of carbonyl derivatives was done as described earlier (18) by spectrophotometric method. Proteasomes have at least five distinct protease activities (19) and among these, two types of peptidase activities were measured for each fraction of the gradient as described previously (20). These peptidase activities were determined fluorometrically by measuring the release of 7-amino-4-methyl-coumarin (AMC) from the peptides succinyl–Leu–Leu–Val–Tyr–MCA (SUC-LLVY-MCA) and butyloxycarbonyl–Leu–Arg–Arg–MCA (BOC-LRR-MCA) for chymotrypsin-like and trypsin-like activities, respectively.

For the determination of DT–diaphorase activity the Erstner (21) method was used. The assay mixture contained 25 mM Tris–HCl (pH 7.4), 0.3 mM NADH, 0.04 mM 2,6-dichloroindophenol, and 0.2% Tween-20. The reaction was started by the addition of the muscle extracts and was followed by absorbance at 600 nm. To measure the effects of physical exercise, the activity of citrate synthetase was measured as described by Shepherd and Garland (22).

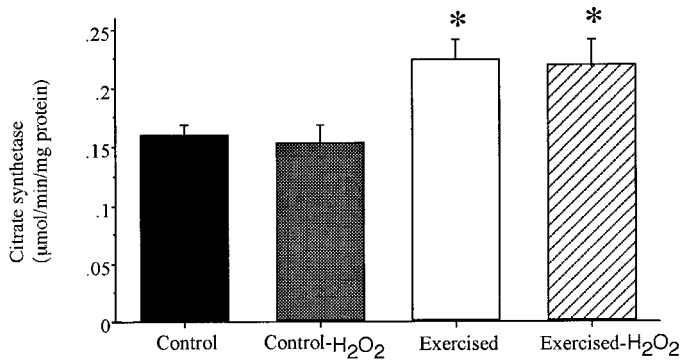

FIG. 1. Nine weeks of swimming increased the activity of citrate synthetase in heart of exercised groups. No alteration was observed in nonexercised animals. Values are means ± SD ($n = 6$).

Statistical analysis. The statistical significance of the data was assessed by ANOVA, followed by Scheffe's post hoc test. When applicable, an unpaired Student *t* test was used. The significance was set at $P < 0.05$.

RESULTS

Nine weeks of swimming resulted in increases in activity of citrate synthetase in exercised animals, demonstrating that this training regime significantly improved the oxidative metabolism of the cardiac muscle (Fig. 1). The activity of the DT–diaphorase increased following the administration of H_2O_2 and exercise, suggesting that an increase in H_2O_2 level stimulates the activity of this enzyme (Fig. 2). The carbonyl group levels of the measured four groups varied significantly (Fig. 3). The cardiac muscle of nonexercised H_2O_2 administered group accumulated significantly more carbonyl derivatives than control group. In addition, the accumulation of carbonylated proteins in exercised and exercised H_2O_2 treated groups was significantly smaller than in nonexercised groups. The peptide-like activity of proteasome complex was induced by the treatment of H_2O_2 and exercise. Moreover, exercise potentiated the effects of chronic H_2O_2 administration. On the other hand, the chymotrypsin-like and trypsin-like activities were stimulated only by physical training and hydrogen peroxide administration (Fig. 4).

DISCUSSION

Regular physical exercise is a well known natural tool that decreases cardiovascular mortality. Exercise-induced bradycardia, increased stroke volume, and cardiac hypertrophy are just some of the factors of the adaptive process of cardiac muscle (23). It has been

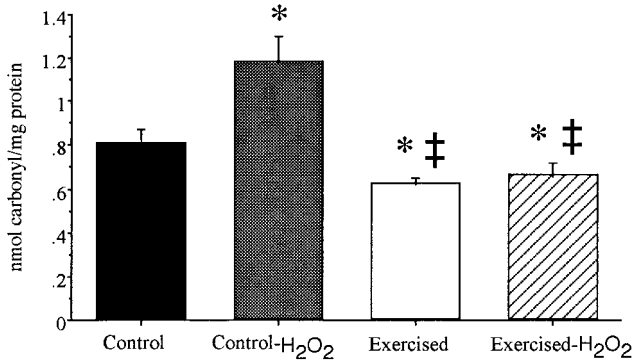

FIG. 2. The accumulation of carbonylated proteins increased in hydrogen peroxide administered nonexercised animals and decreased in exercised groups. Values are means ± SD (n = 6). *P < 0.05 compared with C; ‡P < 0.05 compared with hydrogen peroxide-treated nonexercised animals.

shown that exercise training upregulates the activity of antioxidant enzymes in cardiac muscle (3, 24, 25), resulting in better protection against ROS. It has also been demonstrated that exercise training decreases the steady-state level of lipid peroxidation in heart (25). In addition, it was reported that aortic constriction or swimming training-mediated hypertrophy is associated with increased resistance to ischemia/reperfusion-induced oxidative stress (4). In the present study we showed that regular exercise-evoked adaptation is a preconditioning factor against chronic H_2O_2 administration-induced accumulation of carbonylated proteins. Moreover, the damage was not just absent but the steady-state level of carbonyl derivatives was significantly decreased. It is tempting to suggest that this is due to the increase in the activity of proteasome complex resulting in fast and efficient removal of oxidatively modified proteins. Physical exercise increases the activity of proteasome in skeletal muscle (12) and the data revealed that chronic administration of H_2O_2 also stimulates this enzyme complex. The combined effects of exercise and chronic H_2O_2 administration most probably resulted in faster protein degradation and synthesis and effective elimination of carbonyl derivatives. The antioxidant-repair effects of proteasome were supported by the activity of DT–diaphorase, which was inducible in both hydrogen peroxide-treated groups. We recently showed that exercise training alone induces DT–diaphorase (12) and the present data suggest that chronic administration of H_2O_2 also stimulates this enzyme. However, the stimulating pathway is unknown. A likely possibility is that since DT–diaphorase is a Phase 2 detoxifying antioxidant enzyme like glutathione transferase, epoxide hydrolase, and glucuronosyltransferases (26), the increase in ROS might directly stimulate the activity of the enzyme. The decrease in glutathione level appears to be an inducer of Phase 2 enzymes because glutathione is

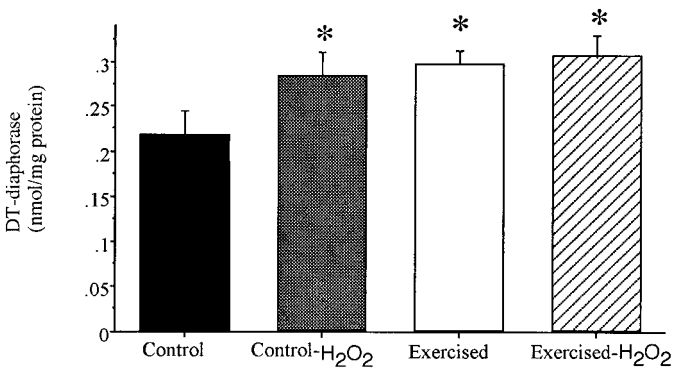

FIG. 3. The activity of DT–diaphorase was increased in by chronic administration of hydrogen peroxide and exercise. Values are means ± SD (n = 6).). *P < 0.05 compared with control.

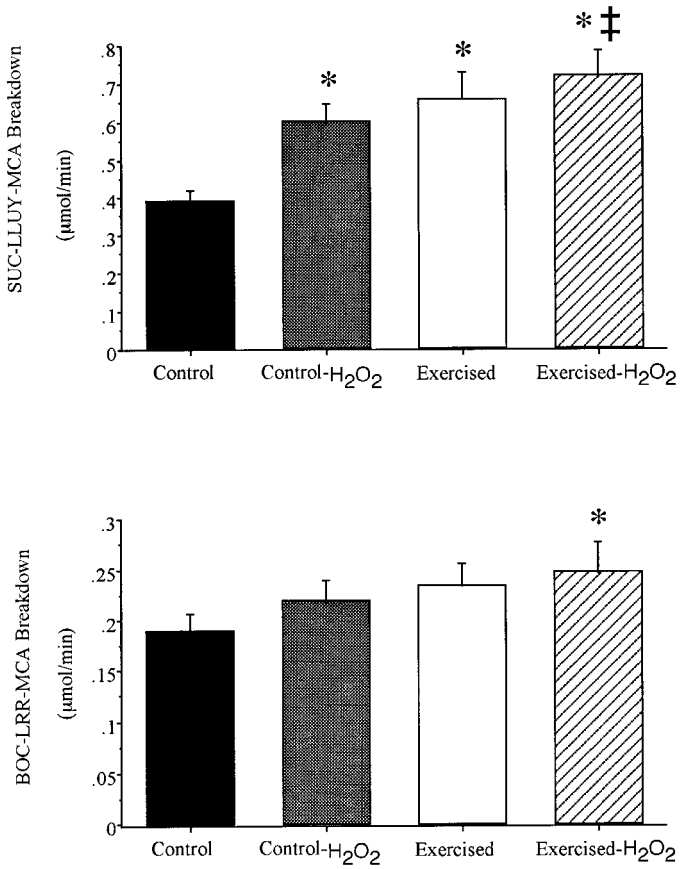

FIG. 4. The peptidase-like activity (breakdown of SUC-LLVY-MCA) of proteasome complex (top); the chymotrypsin-like, trypsin-like activity (breakdown of BOC-LRR-MCA) (bottom). Selective response in proteasome activity is observed to hydrogen peroxide administration and exercise and hydrogen peroxide administration. Exercise training potentiated the effects of hydrogen peroxide treatment. Values are means ± SD (n = 6). *P < 0.05 compared with control; ‡P < 0.05 compared with nonexercised hydrogen peroxide-treated.

a substrate of glutathione peroxidase, which removes hydrogen peroxide.

It is well documented that H_2O_2-mediated oxidative damage to proteins is associated with inactivation of enzyme activity (11, 27, 28). It has also been demonstrated that treatment with H_2O_2 inhibits the activity of proteasome complex (13). In the present study we observed an increase in the activity of proteasome suggesting that either the concentration of H_2O_2 was too low to curb the activity of the enzyme or, due to repeated administration, the activity was stimulated as an adaptive response to prolonged oxidative stress. Indeed, it have been shown that low level treatment with H_2O_2 increases protection against subsequent exposure to higher levels of H_2O_2 (7). Chronic administration of H_2O_2 even potentiated the effects of exercise training on peptidase activity of proteasome complex. The peptidase activity of the proteasome complex was stimulated and the chymotrypsin- and trypsin-like activities were not altered by chronic administration of hydrogen peroxide. Therefore, it is possible that H_2O_2 administration selectively stimulates the different kinds of activity of proteasome complex or some other factors also play a role in the selective stimulation.

The data of the present study revealed that chronic administration of H_2O_2 induces an adaptative response by stimulating the peptidase-like activity of proteasome complex and DT–diaphorase, resulting in the accumulation of carbonylated proteins in the myocardium. On the other hand, chronic administration of H_2O_2 after exercise training decreases the accumulation of carbonyl derivatives to below the steady-state level and induces the activity of proteasome and DT–diaphorase. The stimulating effect of physical exercise on ROS generation is an important phenomenon of the exercise-induced adaptation process since it increases the resistance against prolonged oxidative stress and is a valuable physiological means of preconditioning the myocardium.

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