Exercise modulates antioxidant enzyme gene expression in rat myocardium and liver

D. O. WILSON1 AND P. JOHNSON1,2
1Department of Chemistry and Biochemistry and 2Department of Biomedical Sciences, Ohio University, Athens, Ohio 45701

Wilson DO and Johnson P. Exercise modulates antioxidant enzyme gene expression in rat myocardium and liver. J Appl Physiol 88: 1791–1796, 2000.—Our previous studies have shown that exercise caused changes in the tissue activities of the antioxidant enzymes glutathione peroxidase, superoxide dismutase, and catalase in spontaneously hypertensive (SH) and Wistar-Kyoto (WKY) rats. To determine whether the changes observed were due to changes in mRNA levels of the enzymes, levels of tissue mRNA were determined by quantitative RNAse protection assay. Comparisons of tissue enzyme activities and mRNA levels in sedentary and exercised animals showed that, in some cases (e.g., glutathione peroxidase in SH and WKY myocardium), parallel changes in enzyme activity and mRNA levels occurred, whereas in other cases (e.g., catalase in SH and WKY liver), nonparallel changes were found. Exercise of hypertensive rats altered antioxidant enzyme mRNA levels to those seen in normotensive animals in some, but not all, cases. The results suggest that transcriptional control over changes in exercise-related antioxidant enzyme activities is operative in some cases, although in other cases posttranscriptional regulatory mechanisms may exist.

glutathione peroxidase; catalase; Cu/Sn superoxide dismutase; mRNA; spontaneously hypertensive rat

CHRONIC HYPERTENSION IS KNOWN to cause a variety of changes in myocardium, including the increased production of reactive oxygen species (ROS) (1, 28), which have been implicated in the pathway leading to coronary heart disease (8, 29). One method of treatment of hypertension is a regular aerobic exercise program, and it has been shown that exercising individuals have both diminished hypertension and a lower rate of coronary heart disease compared with sedentary hypertensive individuals (9). However, exercise for hypertensive subjects is not without risk because it can increase aerobic metabolism, leading to an elevation in the levels of tissue ROS, and ROS elevation resulting from hypertension and exercise can cause cell damage by modification of molecules, including DNA, membrane lipids, and proteins (23, 24).

To protect against this increase in ROS due to hypertension or exercise, a possible tissue response is an increase in the activity of a group of enzymes generally referred to as antioxidant enzymes, which protect the cells from ROS-generated damage (11, 21). However, exercise has been shown to alter antioxidant enzyme tissue activities in a variety of different ways depending on the type of exercise program used, the amount of exercise, and the existence of a resting period between the exercise regimen and the tissue collection. Some examples of such variation in the response of the specific tissue enzyme activities to exercise are seen from studies on superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) in rat liver and myocardium, in which increases, decreases, and unaltered tissue activities for each of these enzymes have been reported using different exercise regimens and resting periods (10, 13, 14, 26). Such variable responses to exercise have also been observed in other species, including humans, in whom it has been shown that different exercise training programs can lead to different antioxidant enzyme tissue activity changes and different levels of postexercise oxidative stress (2, 19).

Previous studies in our laboratory have examined the long-term effects of exercise on tissue antioxidant enzyme-specific activities in normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SH) rats (13). It was found that exercise training did not cause a significant change in the total SOD activity in the myocardium but did cause decreases in total SOD activity in the liver and decreases in catalase and GPX activities in both the myocardium and liver (13). To determine whether the regulation of these activities is under transcriptional and/or posttranscriptional controls, in the present studies the mRNA levels for the antioxidant enzymes Cu/Zn SOD, catalase, and GPX were determined in myocardium and liver and compared with changes in the tissue-specific activities of these enzymes. Our studies show that both transcriptional and posttranscriptional regulation may occur in particular cases but that transcriptional regulation appears to be the predominant mechanism.

METHODS

Materials. WKY (normotensive) male rats were obtained from Charles River Laboratories (Wilmington, MA), and the SH (genetically hypertensive) male rats were obtained from Harlan Sprague Dawley (Indianapolis, IN) at an age of 11–12 wk. Tri reagent and high-salt precipitation solution were obtained from Molecular Research Center (Cincinnati, OH). The Maxiscript in vitro transcription kit, the RNase protection assay (RPA) II kit, and pTRI-β-actin-125-rat (a linearized
plasmid containing partial sequence of rat β-actin cDNA) were bought from Ambion (Austin, TX). The restriction enzymes Sal I, EcoR I, and Pst I were purchased from New England Biolabs (Beverly, MA). The T4 DNA ligase, restriction enzymes Bgl II and Sma I, pGEM marker, and lambda marker were purchased from Promega Biotech (Madison, WI). Proteinase K, T4 DNA polymerase, 50- and 25-bp DNA ladders, and 2′-0-methylseleno-3′-triphosphate were obtained from Gibco BRL (Gaithersburg, MD). Radiochemicals ([α-32P]CTP and [α-32P]dATP) were purchased from Du Pont-NEN (Boston, MA). Plasmids containing the cDNAs used to synthesize the probes were generously provided by Dr. Y.-S. Ho (LK440-GPX, glutathione peroxidase), Dr. T. Hashimoto (pRCA38-CCAT, catalase), and Dr. L. B. Clerch (a pGEM-blue plasmid containing the 5′ Pst I fragment of the rat Cu/Zn SOD sequence).

Animal care, exercise program, and tissue preparation. The experiments involving rats were approved by and were in conformity with the guidelines of the Ohio University Institutional Animal Care and Use Committee. The exercise program was the same as one used previously that caused a significant change in the specific activities of the antioxidant enzymes (13). In summary, the rats were exercised 5 out of 7 days/wk as follows: 15-min duration, 10 m/min (belt speed), 0° for week 1; 30 min, 10 m/min, 0° for week 2; 30 min, 10 m/min, 5° for week 3; 30 min, 20 m/min, 5° for week 4; 45 min, 20 m/min, 5° for week 5; and 60 min, 20 m/min, 5° for weeks 6–10. Rats in the control (sedentary) group were removed from their cages once a day for 5 days/wk and placed on the stationary treadmill for 15 min to account for handling stress. After a 1-wk detraining sedentary period, the systolic blood pressures of the rats were determined by the tail-cuff technique, and the body weights were measured. The animals were euthanized by CO2-induced asphyxiation, and the outer wall of the left ventricle, remainder of the heart, liver, and quadriceps femoris were immediately removed and washed with cold phosphate buffer to remove erythrocytes (13). The tissues were cut into 100- to 300-mg portions and stored separately at −80°C in 1.5-ml centrifuge tubes.

Preparation of tissue extract and SOD enzyme assay. Homogenates of the tissues were prepared in 1.0 ml of phosphate buffer per 100 mg of tissue using a Powergen 125 homogenizer as described previously (3, 13). The samples were spun at maximum speed at 4°C in a Marathon microcentrifuge, and the supernatants were determined by use of the Bio-Rad Bradford protein assay kit. SOD activity was measured by the method of Bradbeer et al. (1) with the use of homogenate supernatant in a final assay volume of 500 μl. Triplicate samples and appropriate control samples were spun at maximum speed at 4°C in a Marathon microcentrifuge.

Preparation of mRNA probes for the antioxidant enzymes. Standard procedures were used for mini-preparations and large-scale plasmid preparations (22). Plasmids containing cDNA inserts of GPX, catalase, and Cu/Zn SOD were linearized with the use of Sal I, Bgl II, and Sma I before transcription (18).

For transcription, the Ambion MAXi transcription kit was used with [α-32P]CTP as the radiolabeled nucleotide. Transcription of the commercial linearized plasmid containing partial rat β-actin cDNA sequence was performed according to the MAXi script protocol, and, by using T7 RNA polymerase, a 190-base full-length probe was obtained. T7 RNA polymerase was used for transcription of the catalase and GPX cDNAs, and SP6 RNA polymerase was used for transcription of the Cu/Zn SOD cDNA. Labelled RNA probes were purified on an 12% urea-acrylamide gel, and the eluted probes were used within 3 days of preparation.

Preparation of 32P-labeled DNA standard ladder. Both 25- and 50-bp DNA ladders for gel electrophoresis calibration were prepared using the standard protocol of the Gibco BRL kit with dATP as the 32P-labeled deoxyribonucleotide. Ladder preparations were used within 2 wk of their preparation.

RPA and gel analysis. For quantitative analysis of mRNA levels by RPA (17), the recommended procedures of the RPA II kit were followed using 10-μg samples of total RNA. The optimized amounts of each probe were previously determined to be 1 × 10^6 cpm catalase and 1 × 10^5 cpm β-actin, 2 × 10^6 cpm Cu/Zn SOD, and 6 × 10^5 cpm β-actin, 8 × 10^6 cpm GPX and 8 × 10^5 cpm β-actin in the myocardium, and 1 × 10^5 cpm GPX and 1 × 10^5 cpm β-actin in the liver (18). After electrophoresis of triplicate samples and appropriate control samples, the gels were mounted onto filter paper and dried and exposed to Fuji RX Medical X-ray Film at −80°C for up to 2 days using an intensifying screen. Developed films were then scanned using a Bio-Rad GS-670 densitometer and the ratio of antioxidant mRNA probe to β-actin probe intensity was calculated. Figure IA shows a typical autoradiograph of Cu/Zn-SOD mRNA analysis from myocardium, and Fig. IB shows a typical autoradiograph of catalase mRNA analysis from liver.

Statistical methods. The mean values (± SD) for the ratios of the antioxidant mRNA probe to the β-actin mRNA probe were obtained from four separate animals with triplicate RPA analyses of each preparation. Minitab 12.22 was used for one-way ANOVA of the data to determine whether significant differences between the mean data existed at the 95% confidence level (P < 0.05).

RESULTS

Effects of exercise and hypertension on systolic blood pressures. There was no significant change in blood pressure due to exercise for WKY rats (116 ± 10 and 118 ± 12 Torr, respectively, for sedentary and exercised animals), but there was a significant increase for the SH rats (185 ± 3 and 202 ± 10 Torr, respectively, for sedentary and exercised animals). The small but significant increase in blood pressure in the SH rats is in agreement with earlier observations of this phenomenon (13, 27) and may reflect an anticipatory response to exercise by SH animals in the exercised group (5, 15).
In some cases, no significant changes in myocardial antioxidant enzymes due to exercise and hypertension. Autoradiographs in myocardium, there are several *-actin mRNA levels obtained by the scanning of RPA mRNA expressed in myocardium and liver relative to tissue-specific enzyme activities.

**DISCUSSION**

As shown in Table 2, exercise in either WKY or SH rats, or hypertension alone in some cases, caused significant changes in antioxidant enzyme mRNA expression. These changes often, but not always, paralleled the changes seen in antioxidant enzyme tissue-specific activities (Fig. 2). Examples of parallelism between changes in enzyme activity and mRNA levels include the changes in GPX enzyme activity and mRNA for GPX between the WKY exercised and SH exercised rats. The liver mRNA levels showed significant changes in comparison with the WKY sedentary control in all cases except for Cu/Zn SOD in the sedentary SH rats. The mRNA levels for Cu/Zn SOD and GPX showed decreases due to exercise, whereas catalase mRNA levels increased as a result of exercise and hypertension.

**Table 1. Superoxide dismutase tissue enzyme activities for WKY and SH rats in myocardium and liver**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Exercise Status</th>
<th>Enzyme Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mn SOD</td>
</tr>
<tr>
<td><strong>Myocardium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>Sedentary</td>
<td>1.14 ± 0.09</td>
</tr>
<tr>
<td>WKY</td>
<td>Exercised</td>
<td>1.16 ± 0.15</td>
</tr>
<tr>
<td>SH</td>
<td>Sedentary</td>
<td>1.15 ± 0.10</td>
</tr>
<tr>
<td>SH</td>
<td>Exercised</td>
<td>0.98 ± 0.06</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>Sedentary</td>
<td>1.51 ± 0.70</td>
</tr>
<tr>
<td>WKY</td>
<td>Exercised</td>
<td>2.12 ± 0.23</td>
</tr>
<tr>
<td>SH</td>
<td>Sedentary</td>
<td>3.05 ± 1.62</td>
</tr>
<tr>
<td>SH</td>
<td>Exercised</td>
<td>1.42 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SD in units per mg of homogenate protein as defined by the assay procedure in METHODS. Assays were performed on tissue from 8 different animals in each group. SOP, superoxide dismutase. *Significant difference (P < 0.05) between the values for Wistarkyoto (WKY) and spontaneously hypertensive (SH) rats. †Significant difference (P < 0.05) between the sedentary and exercised value.

**Table 2. Relative mRNA levels for myocardial and liver antioxidant enzymes in WKY and SH rats**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Exercise Status</th>
<th>Cu/Zn SOD</th>
<th>Catalase</th>
<th>GPX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myocardium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>Sedentary</td>
<td>0.438 ± 0.135</td>
<td>0.182 ± 0.096</td>
<td>0.492 ± 0.184</td>
</tr>
<tr>
<td>WKY</td>
<td>Exercised</td>
<td>0.380 ± 0.175</td>
<td>0.058 ± 0.005†</td>
<td>0.179 ± 0.056†</td>
</tr>
<tr>
<td>SH</td>
<td>Sedentary</td>
<td>1.328 ± 0.048B</td>
<td>0.080 ± 0.029†</td>
<td>2.819 ± 1.037*</td>
</tr>
<tr>
<td>SH</td>
<td>Exercised</td>
<td>0.778 ± 0.111†</td>
<td>0.422 ± 0.269†</td>
<td>0.142 ± 0.027†</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>Sedentary</td>
<td>1.139 ± 0.581</td>
<td>0.582 ± 0.170</td>
<td>1.778 ± 0.974</td>
</tr>
<tr>
<td>WKY</td>
<td>Exercised</td>
<td>0.822 ± 0.254†</td>
<td>0.911 ± 0.079†</td>
<td>0.761 ± 0.124†</td>
</tr>
<tr>
<td>SH</td>
<td>Sedentary</td>
<td>1.005 ± 0.128</td>
<td>0.767 ± 0.052*</td>
<td>0.754 ± 0.141*</td>
</tr>
<tr>
<td>SH</td>
<td>Exercised</td>
<td>0.634 ± 0.143†</td>
<td>1.397 ± 0.488†</td>
<td>0.442 ± 0.117†</td>
</tr>
</tbody>
</table>

Values are means ± SD for experiments done in triplicate on 4 animals per tissue group. mRNA levels were calculated relative to the β-actin mRNA levels (normalized value of 1.000). *Significant difference (P < 0.05) between the SHR and the WKY value. †Significant difference (P < 0.05) between the exercise and the sedentary value.
Fig. 2. Myocardial and liver antioxidant enzyme relative activities and mRNA levels of exercised and sedentary SH rats (SHR) and WKY rats. Values (means ± SD) for relative tissue enzyme-specific activities and mRNA levels (based on WKY sedentary values of 100%) are shown for each enzyme in SHR rats and exercised WKY rats. *Significant difference from the normotensive rats, \( P < 0.05 \). †Significant difference from the sedentary rats, \( P < 0.05 \). Data for mRNA levels are calculated from results in Table 2, and data for tissue enzyme-specific activities are calculated from previous work [catalase and glutathione peroxidase (GPX)] (13) and from Table 1 (Cu/Zn SOD).
levels that decrease in the myocardium and liver of both WKY and SH rats as a result of exercise. Of the five cases of nonparallelism seen in Fig. 2, four of these involved catalase, with one showing a decrease in the mRNA level and an increase in the enzyme activity and the other three showing increases in mRNA levels and decreases in enzyme activity. Independently of exercise, hypertension was also found to affect myocardial mRNA levels; the Cu/Zn SOD and GPX mRNA levels were higher in SH than in WKY rats, and the enzyme activities of GPX and catalase also increased. Thus, although not all of the antioxidant enzymes showed elevated expression as a result of hypertension, the general trend was an increase in antioxidant enzyme expression as a result of hypertension.

If exercise training were to provide a beneficial effect to tissues in terms of protection against oxidative damage directly through alteration of antioxidant enzyme expression, a relationship in exercise might exist between increases in antioxidant enzyme expression and decreases in tissue oxidative damage. In fact, this simple relationship does not appear to exist, given that in the myocardium of exercised WKY rats, increases in lipid peroxidation (13) are associated with decreases in antioxidant enzyme expression, whereas in liver of the same animals, both lipid peroxidation and antioxidant enzyme expression levels are decreased. These results therefore do not provide clear evidence for a beneficial effect of exercise in which elevations of antioxidant enzyme expression would decrease tissue oxidative damage, and it seems likely that other factors must be involved in mitigating levels of tissue oxidative damage during exercise.

Of particular interest is the comparison of mRNA levels in exercised SH rats to those in WKY rats to examine whether exercise in SH rats changed expression levels to normotensive levels. Although not all mRNA levels were changed in this fashion, our results show that exercise was partially effective in this regard by decreasing myocardial mRNA levels for Cu/Zn SOD and GPX to values that were even lower than those in the sedentary animals. For the catalase mRNA level in myocardium, exercise caused an increase in the mRNA level toward the WKY level, but the attained level of mRNA in the myocardium of the SH exercised rats was actually higher than that in the WKY animals. These results indicate that exercise in SH rats does not have the same effect on antioxidant enzyme expression as treatment of SH rats with antihypertensive drugs, in which it was found that, with three different drugs, enzyme expression was altered to normotensive levels (18). Because exercise (13), unlike antihypertensive drug treatment (3), does not return SH rat blood pressure to the normotensive level, it is possible that the differences in antioxidant enzyme expression seen as a result of exercise and antihypertensive drug treatment relate to the different effects of these treatments on blood pressure.

Parallel changes for mRNA levels and tissue-specific activities that are strongly suggestive of exercise- and/or hypertension-induced transcriptional control were seen in all sedentary-vs.-exercised comparisons for GPX. Other parallel changes also suggestive of transcriptional control were seen for sedentary-vs.-exercise comparisons for myocardial catalase and liver Cu/Zn SOD of WKY rats and the normotensive-vs.-hypertensive comparison for liver catalase in sedentary animals. The changes in antioxidant enzyme tissue-specific activities that do not correspond to changes in their mRNA levels (e.g., catalase in the myocardium for both SH rats and catalase in the liver for the exercised WKY and SH rats) could be due to in vivo posttranscriptional regulation, given that previous work has shown increases in antioxidant mRNA levels due to binding of stabilizing proteins (6, 7) and increases in antioxidant enzyme activity without a corresponding increase in enzyme protein (4, 12). However, such changes could also be the result of the in vitro instability of mRNA, although there are several cases (namely, for catalase in liver of exercised WKY and SH rats and in myocardium of exercised SH animals) in which mRNA levels were elevated in comparison to enzyme activity levels.

Our results differ in some instances from those reported in two previous studies on the effects of exercise on rat antioxidant enzyme mRNA levels (10, 26). One of these differences is in the results for Cu/Zn SOD mRNA levels, as our studies showed either a decrease or no change with exercise whereas both the previous studies showed increases with exercise. Additionally, for catalase, we found a decrease in the mRNA levels in the normotensive rats and an increase in the hypertensive rats due to exercise, whereas the previous study found an increase in catalase mRNA levels due to exercise (26). A third difference is that GPX mRNA levels in our study were found to be decreased in both myocardium and liver as a result of exercise, whereas one of the earlier studies (26) reported an increase in the GPX mRNA level in the myocardium due to exercise, and the other study (10) found no change in the GPX mRNA level in the myocardium and a significant decrease in the liver.

These differences may be related to different experimental factors in the studies, which include the type of rats used, the duration of resting period allowed after the last exercise training period, the myocardial tissue analyzed, and the method of mRNA quantitation used. The two previous studies used either male Fischer 344 or female Sprague-Dawley rats, whereas our studies used male WKY and SH rats, and it is possible that there may be genetic differences in how the different animals respond to exercise. With the shorter term (not more than 24 h) resting periods used in the previous studies, there may be short-term changes in antioxidant enzyme expression that are different from the longer-term changes that were detected by our experimental protocol in which a 7-day resting period was used. Another possible reason for differences in results is that our studies used the outer wall of the left ventricle, rather than mixed myocardium, and it is known that the left ventricle undergoes changes that are different from those in other regions of the heart (16, 25). A final possible reason for the differences in the...
data is that our study used the RPA for mRNA analysis, whereas both previous studies used Northern blot hybridization, a technique that is far less specific and sensitive than RPA (17, 20).

In conclusion, our results show that exercise or hypertension caused changes in antioxidant enzyme mRNA levels in the myocardium and the liver that may or may not parallel changes in the tissue-specific enzyme activities. Exercise of hypertensive rats did not return mRNA levels to those seen in normotensive animals in all cases, although GPX and Cu/Zn SOD levels in myocardium were moved much closer to normotensive levels. Those cases in which the changes in mRNA levels paralleled the changes in the enzyme activities are strongly suggestive of transcriptional regulation of the antioxidant enzymes, and in those cases in which there was not a parallel change in mRNA levels and enzyme activities, it would seem probable that there is posttranscriptional regulation of the antioxidant enzymes by one or more different mechanisms.

We thank Dr. L. B. Clerch (Georgetown University, Washington, DC), Dr. T. Hashimoto (Shinshu University School of Medicine, Matsumoto, Japan), and Dr. Y.-S. Ho (Wayne State University, MI) for generously providing the CDNA clones used in these studies.

Address for reprint requests and other correspondence: P. J. Johnson, Dept. of Chemistry and Biochemistry, Clippinger Laboratories, Ohio Univ, Athens, OH 45701 (E-mail: johnsonp@ohio.edu).

Received 27 October 1999; accepted in final form 20 January 2000.

REFERENCES


