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Effects of Antihypertensive Drugs on Rat Tissue Antioxidant Enzyme Activities and Lipid Peroxidation Levels

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ABSTRACT. The effects of three commonly used antihypertensive agents (captopril, hydralazine, and terazosin) on tissue antioxidant enzymes and lipid peroxidation in spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY rats) were studied by analysis of antioxidant enzyme specific activities and lipid peroxidation levels in control and drug-treated animals. In the myocardium, changes in some of the enzyme activities between normotensive WKY and hypertensive SHR rats were mitigated by treatment of the SHR rats with an antihypertensive drug. Thus, all three drugs caused significant increases in myocardial Cu/Zn superoxide dismutase (up to 133% of SHR control activity) and decreases in glutathione peroxidase (down to 59% of SHR control activity) to values that were closer to those in untreated WKY rats. Captopril also increased Mn superoxide dismutase activity, and hydralazine and terazosin decreased catalase activity towards untreated WKY values. Hydralazine was the only drug to alter the lipid peroxidation level in the myocardium of SHR rats (a 28% decrease), but in WKY rats all three drugs caused significant decreases in myocardial lipid peroxidation levels. In WKY rats, none of the drugs affected myocardial Mn and Cu/Zn superoxide dismutase activities, although glutathione peroxidase activity was decreased by hydralazine and terazosin treatment and catalase activity was increased by captopril treatment. Enzyme activity changes in liver and skeletal muscle indicated that such changes were often tissue specific. No pattern was found for coordinated changes in antioxidant enzyme expression as a result of the drug treatments, and the changes in antioxidant enzyme specific activities did not correlate generally with changes in lipid peroxidation levels. BIOCHEM PHARMACOL **54**;1:133–141, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. SHR rat; captopril; hydralazine; terazosin; antioxidant enzymes; myocardium; lipid peroxidation

Aerobic organisms employ a battery of defense mechanisms to prevent or mitigate tissue oxidative damage caused by the generation of reactive oxygen species, such as the hydroxyl radical, which results primarily from the use of O_2 as a terminal electron acceptor in metabolism [1]. A major type of defense in living systems against oxidative damage is the use of "antioxidant" enzymes (notably superoxide dismutase, glutathione peroxidase, and catalase) to convert reactive oxygen species into non-toxic compounds, and the tissue activities of these enzymes have been reported to be changed in response to stresses such as hyperbaric oxygen and exercise [2, 3]. Such changes appear to be the result of alterations in transcriptional rates of the structural genes of the antioxidant enzymes [4] or the stabilization of the mRNA for the antioxidant enzyme [5].

In addition to other types of stress, hypertension may also compromise the antioxidant defense mechanisms of the cardiovascular system and the myocardium in particular. Examples of such effects of hypertension include the elevation of oxidative damage in the myocardium of hypertensive animals [6, 7], the higher susceptibility of the hypertensive myocardium to post-ischemic reperfusion injury [8], and changes in the activities of tissue antioxidant enzymes (catalase, glutathione peroxidase, and superoxide dismutase) of hypertensive rats [9].

Control of hypertension by traditional antihypertensive drugs such as diuretics, β-blockers, and direct-acting peripheral vasodilators sometimes has undesirable side-effects [10, 11]. For this reason, other antihypertensive agents including centrally acting agents, calcium-channel blockers, selective α_1-adrenergic blockers, and ACE† inhibitors have been developed [12], which mitigate or do not have some of these side-effects [13, 14]. The objective of this study was to determine if commonly used antihypertensive agents could induce changes in the tissue activities of antioxidant enzymes that might mitigate the effects that

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† *Abbreviations:* ACE, angiotensin-converting enzyme; SHR, spontaneously hypertensive rats; TBARS, thiobarbituric acid reactive substances; and WKY, Wistar-Kyoto.

hypertension has on antioxidant defense mechanisms. This is a potentially important consideration given that a major type of defense in living systems against oxidative damage is the use of antioxidant enzymes to convert reactive oxygen species into non-toxic compounds.

For these studies, we selected three antihypertensive agents based on their different modes of action, their frequency of current use, and, in two cases, on prior reports of their effects on tissue antioxidant metabolism. The first of the agents chosen for this study was the ACE inhibitor captopril, which is a relatively modern antihypertensive agent of widespread use that has cardioprotective effects including reduction of left ventricular hypertrophy [15], utility in the treatment of congestive heart failure [16], mitigation of oxidative damage associated with post-ischemic reperfusion [17], and reduction of the levels of serum lipid peroxidation products in survivors of acute myocardial infarction [18]. Some of these advantages have been ascribed to the antioxidant properties of captopril [19], and captopril has been shown to alter serum glutathione levels and serum glutathione peroxidase activities in humans [20] and to elevate glutathione peroxidase and superoxide dismutase activities in mouse liver [21]. The second agent chosen for this study was hydralazine, a direct-acting peripheral vasodilator that has been a widely used antihypertensive agent for over 30 years [11]. Its use, however, is, not without some potentially serious side-effects *in vivo* [11, 22, 23], and it has also been reported to cause *in vitro* changes in antioxidant enzyme activities and to increase the levels of lipid peroxidation in cultured fibroblasts [24]. The third antihypertensive agent selected for our studies was terazosin [25], a recently introduced selective α_1-blocker that has been shown to alleviate left ventricular hypertrophy and to decrease serum triglyceride, cholesterol, and low density lipoprotein levels [26]. No previous reports have appeared concerning its effects on antioxidant metabolite levels or enzyme activities.

For the experiments described in this paper, spontaneously hypertensive (SHR) and normotensive (WKY) rats were treated with the antihypertensive agents under conditions that reduced blood pressure, and the levels of tissue oxidative damage and antioxidant enzyme activities in the treated animals were then compared with those levels and activities in untreated animals. The direct effects of the antihypertensive agents on the tissue enzymes were also investigated to determine if tissue enzyme activity changes could be the result of a direct interaction between the agent and the enzyme [21]. Although the principal interest in our studies was on drug-related changes in the myocardium, two other tissues (a white anaerobic skeletal muscle and liver) were also examined to determine if the drug-related changes in myocardium were unique for this tissue. Our results have shown that all three of the antihypertensive agents tested caused significant changes in some of the tissue antioxidant enzyme activities and also in some of the tissue lipid peroxidation levels, and that the enzyme activity changes were most likely caused by changes in the levels of expression of these enzymes.

MATERIALS AND METHODS

Materials

Captopril (Capoten, Capozide) and terazosin (Hytrin) were obtained from Bristol–Myers Squibb, Princeton, NJ, and Abbott Laboratories, Abbott Park, IL, respectively. Hydralazine (Apresoline), reagents, and enzymes for antioxidant enzyme assays were purchased from the Sigma Chemical Co., St. Louis, MO.

Animals

WKY (normotensive) and SHR (spontaneously hypertensive) male rats (*Rattus norvegicus*) were obtained from Harlan Sprague Dawley, Indianapolis, IN, at an age of 11–12 weeks.

Drug Administration Protocols and Animal Care

The experiments involving rats, conducted in this study, were approved by and were in conformity with the guidelines of the Ohio University IACUC. Each drug was administered to separate groups of six WKY and SHR rats over a 12-week period via drinking water, and two groups of six WKY and SHR rats were used as no-drug controls. Each animal was housed in a separate cage and was fed a standard rat diet and allowed access to water *ad lib*. The drugs were given at the following approximate doses: captopril, 50 mg/kg body wt/day [27]; terazosin, 45 mg/kg/day [28]; hydralazine, 15 mg/kg/day [29]. Drug delivery was calculated based on measurements of daily consumption of drinking water and weekly measurements of body weight. At the end of the 12-week period, body weights were measured, and systolic blood pressures were determined by the tail-cuff technique.

Tissue Collection and Homogenate Preparation

The animals were euthanized by CO_2-induced asphyxiation 24 hr after cessation of the last drug treatment, and selected tissues (septum-free outer wall of the left ventricle, mixed myocardium consisting of the right ventricle and the inner wall of the left ventricle, superficial vastus lateralis muscle of the quadriceps femoris, and liver) were removed and prepared as described previously [9, 30]. The chilled exsanguinated tissues were then cut into approximately 50- to 100-mg portions on ice and stored separately at $-70°$ in plastic vials. Homogenates from these samples were prepared after the addition of 1.0 mL phosphate buffer per 100 mg of tissue as described previously [9], with the exception that homogenization was performed in a PowerGen 125 tissue homogenizer. Protein concentrations of the supernatant preparations were measured by the Bio-Rad Bradford protein assay kit.

Enzyme Activity Assays

Superoxide dismutase activity was measured by the cytochrome *c* reduction procedure [31] using 3- to 200-μL aliquots of homogenate supernatant in a final assay volume of 3.0 mL. Assays were performed on supernatant samples preincubated in 1 mmol/L cyanide for 2 hr at 37° to measure Mn superoxide dismutase activity and on supernatant samples preincubated without cyanide to measure both Cu/Zn and Mn superoxide dismutase activities [32]. The unit of enzyme activity is the amount of enzyme that gave 50% inhibition of the control rate of cytochrome *c* reduction. The assays for catalase and glutathione peroxidase were performed by the respective methods of Aebi [33] and Flohe and Gunzler [34]. Assays on a given sample were performed in triplicate, and enzyme specific activities (using the standard definition of an enzyme activity unit from the method for the assay) were expressed in units of activity per milligram of protein in the tissue extracts as described previously [9]. In muscle, possible hypertension-related changes in extracellular non-myocytic protein content have been reported to be primarily in Type I and Type III collagens [35]. These proteins would not be solubilized in our extraction procedure and, therefore, should not affect the calculation of intracellular myocytic specific activities of the antioxidant enzymes.

Measurement of Tissue Lipid Peroxidation Levels

Estimates of tissue lipid peroxidation levels were performed by the TBARS procedure [36] on 50- to 200-μL aliquots of homogenate supernatants in triplicate as previously described, and values are expressed as femtomoles of malonaldehyde per milligram of protein in the tissue extract [9]. Although this method is an indirect measure of lipid peroxidation which is susceptible to interference by endogenous and exogenous substances, it was selected for use because of its frequency of use in many other studies, and because no single analytical method gives a completely satisfactory measurement of tissue lipid peroxidation levels [1]. For these reasons, the values of TBARS analyses should be regarded as an index rather than as an absolute measure of total tissue lipid peroxide levels [37].

Statistical Analysis

Initial assessment of TBARS and antioxidant enzyme activity data was made on a group-by-drug basis using two-way ANOVA (drug treatment df = 3,33; rat type df = 1,33; and drug-by-rat interaction df = 3,33). One-way ANOVA was used to detect significant differences between individual sets of the TBARS level and antioxidant enzyme activity data, and also to analyze blood pressure and body weight data. Correlations between changes in tissue TBARS levels and antioxidant enzyme activities were calculated as Pearson product-moments (r), and the statistical significance of the r value was evaluated from the P value at df = 11. A matched-pairs t-test was used to analyze data in the experiments on the effects of antioxidant drugs on the TBARS standard curve. In all of these analyses, values for $P > 0.05$ were taken to indicate no significant difference.

TABLE 1. Effects of antihypertensive drug administration on blood pressure and body weight in SHR and WKY rats

Drug	Rat	Blood pressure (torr)	Body weight (g)
None			
	SHR	240 ± 13	416 ± 20
	WKY	182 ± 6	383 ± 25
Captopril			
	SHR	203 ± 15*	400 ± 9
	WKY	159 ± 4*	346 ± 16†
Hydralazine			
	SHR	118 ± 10*	385 ± 16†
	WKY	124 ± 8*	365 ± 24
Terazosin			
	SHR	136 ± 8*	427 ± 18
	WKY	132 ± 12*	413 ± 21

Values are means ± SD, N = 6 for each group.
* Significantly different from control group values ($P < 0.005$).
† Significantly different from no-drug control group values (P between 0.05 and 0.005).

RESULTS

Effects of Drug Regimens on Rat Systolic Blood Pressure and Body Weight

Table 1 summarizes the effects of drug administration on systolic blood pressure and body weight of SHR and WKY rats relative to control rat groups that were not drug treated. All three drugs caused blood pressure to be lowered significantly, with hydralazine causing the most pronounced effect in both SHR and WKY rats. With the exceptions of captopril in WKY rats and hydralazine in SHR rats, no significant differences were found between the body weights of untreated and drug-treated rats at the end of the drug treatment period, and all of the animals in each group showed no obvious health abnormalities. These observations show that for each drug used, the dosage and duration of the regimen were effective in obtaining a blood pressure decrease without severely compromising the health of the drug-treated animals.

Effects of Drug Administration on Antioxidant Enzymes and TBARS Levels in Rat Tissues

Table 2 and 3, respectively, show the results from SHR and WKY rats of the effects of antihypertensive drugs on tissue antioxidant enzyme activities and TBARS levels following the drug administration regimens. In addition to data shown in Tables 2 and 3 for the outer wall of the left ventricle, determinations were also made on the mixed myocardium from the inner wall of the left ventricle and the complete right ventricle, but these data are not shown

TABLE 2. Effects of antihypertensive drug administration on tissue antioxidant enzyme specific activities and TBARS levels in SHR rats

Parameter	Tissue	Enzyme activity/TBARS level after treatment of SHR rats			
		Captopril	Hydralazine	Terazosin	No drug
Mn-SOD	OWLV	4.32 ± 0.48	3.48 ± 0.60	2.88 ± 0.56*	3.77 ± 0.52†
	QF	0.68 ± 0.15	0.37 ± 0.12	0.38 ± 0.04*	1.00 ± 0.52
	Liver	1.15 ± 0.14‡	1.78 ± 0.17	1.52 ± 0.29*	1.98 ± 0.27
Cu/Zn-SOD	OWLV	12.9 ± 1.7*	12.9 ± 1.8*	13.4 ± 1.8*	10.1 ± 1.8†
	QF	5.35 ± 1.2	5.52 ± 1.2	5.23 ± 1.2	6.35 ± 1.8
	Liver	11.6 ± 1.7‡	17.6 ± 0.5‡	16.1 ± 0.6	15.3 ± 1.1
GPX	OWLV	187 ± 20‡	140 ± 8‡	163 ± 9‡	238 ± 15†
	QF	22.8 ± 3.3	24.7 ± 1.7	25.0 ± 3.6	25.2 ± 4.3†
	Liver	288 ± 6‡	339 ± 19*	460 ± 34‡	375 ± 21†
CAT	OWLV	28.0 ± 1.8	22.2 ± 1.2‡	21.2 ± 3.0‡	28.6 ± 3.3§
	QF	3.37 ± 0.42	2.98 ± 0.41	3.98 ± 0.32‡	3.12 ± 0.47
	Liver	362 ± 18‡	570 ± 70	534 ± 42	501 ± 46
TBARS	OWLV	239 ± 19	164 ± 16‡	196 ± 38	229 ± 36§
	QF	350 ± 63	401 ± 60	385 ± 36	335 ± 67
	Liver	390 ± 40	389 ± 34	536 ± 83*	400 ± 50§

Enzyme specific activities and TBARS levels in the outer wall of the left ventricle (OWLV), quadriceps femoris (QF), and liver are expressed as the means ± SD in units per mg of homogenate protein as defined in the assay procedure described in Materials and Methods. Values were obtained from assays on 6 different animals in each group. For clarity, mean values are simplified to three and SD values to two, significant figures. The abbreviations used for enzymes in the parameters column are: SOD, superoxide dismutase; GPX, glutathione peroxidase, and CAT, catalase. Values shown for CAT are actual × 10^3.

* Statistically significant difference between a drug-treated data set and the corresponding no-drug control set, P between 0.05 and 0.005.

† Statistically significant difference between SHR no-drug control and WKY no-drug control (see Table 3) data set, P between 0.05 and 0.005.

‡ Statistically significant difference between a drug-treated data set and the corresponding no-drug control set, $P < 0.005$.

§ Statistically significant difference between SHR no-drug control and WKY no-drug control (see Table 3) data set, $P < 0.005$.

in Tables 2 and 3 as they closely paralleled the data obtained for the outer wall of the left ventricle. The results of two-way ANOVA on the data on a group-by-drug basis are shown in Table 4, and this table shows that there were significant drug-by-rat type interactive effects, particularly in myocardium and liver. Because this analysis showed that the drugs have specific effects on different rat types and in different tissues, further analysis of the data was performed by one-way ANOVA between individual data sets of no-drug control and drug-treated groups, and the results of these analyses are shown in Tables 2 and 3.

Table 2 shows that Mn-dependent superoxide dismutase

TABLE 3. Effects of antihypertensive drug administration on tissue antioxidant enzyme specific activities and TBARS levels in WKY rats

Parameter	Tissue	Enzyme activity/TBARS level after treatment of WKY rats			
		Captopril	Hydralazine	Terazosin	No drug
Mn-SOD	OWLV	4.43 ± 0.48	4.33 ± 0.29	4.08 ± 0.45	4.52 ± 0.29
	QF	0.73 ± 0.15	0.35 ± 0.18	0.37 ± 0.05	0.85 ± 0.21
	Liver	1.56 ± 0.35	1.53 ± 0.26	1.61 ± 0.19	1.63 ± 0.33
Cu/Zn-SOD	OWLV	12.1 ± 2.1	13.2 ± 1.3	14.7 ± 1.5	13.9 ± 2.0
	QF	5.60 ± 0.56	5.85 ± 0.85	6.05 ± 0.87	6.57 ± 0.41
	Liver	14.8 ± 2.6	14.3 ± 1.1	21.4 ± 1.8*	15.2 ± 1.5
GPX	OWLV	169 ± 12	115 ± 9*	143 ± 11*	175 ± 15
	QF	20.9 ± 2.7	16.3 ± 2.4	21.3 ± 3.8	19.2 ± 4.1
	Liver	485 ± 32*	486 ± 25*	592 ± 24*	555 ± 7
CAT	OWLV	20.8 ± 1.9†	15.9 ± 1.6	17.4 ± 2.6	18.1 ± 1.3
	QF	2.35 ± 0.46*	2.83 ± 0.30†	2.50 ± 0.39*	3.32 ± 0.31
	Liver	419 ± 27*	477 ± 40	557 ± 47*	469 ± 16
TBARS	OWLV	272 ± 31†	183 ± 24*	228 ± 42*	337 ± 52
	QF	322 ± 55	274 ± 65	370 ± 86	354 ± 76
	Liver	218 ± 28†	237 ± 26	264 ± 29	251 ± 16

Enzyme specific activities and TBARS levels in the outer wall of the left ventricle (OWLV), quadriceps femoris (QF), and liver are expressed as the means ± SD in units per mg of homogenate protein as defined in the assay procedure described in Materials and Methods. For clarity, mean values are simplified to three, and SD values to two, significant figures. The abbreviations used for enzymes in the parameter column are: SOD, superoxide dismutase; GPX, glutathione peroxidase; and CAT, catalase. Values shown for CAT are actual × 10^3. Values were obtained from assays on 6 different animals in each group.

*, † Statistically significant differences between a drug-treated data set and the corresponding no-drug control set: * $P < 0.005$, and †P between 0.05 and 0.005.

TABLE 4. Results of two-way ANOVA on the effects of antihypertensive drug administration on rat tissue antioxidant enzyme specific activities and TBARS levels

		P value from two-way ANOVA		
Parameter	Tissue	Drug treatment*	Rat type†	Interaction‡
Mn-SOD	OWLV	<0.005	<0.005	NSD§
	QF	<0.005	NSD	NSD
	Liver	<0.005	NSD	0.05–0.005
Cu/Zn-SOD	OWLV	NSD	0.05–0.005	0.05–0.005
	QF	NSD	NSD	NSD
	Liver	<0.005	0.05–0.005	<0.005
GPX	OWLV	<0.005	<0.005	<0.005
	QF	NSD	<0.005	NSD
	Liver	<0.005	<0.005	0.05–0.005
CAT	OWLV	<0.005	<0.005	0.05–0.005
	QF	NSD	<0.005	<0.005
	Liver	<0.005	NSD	<0.005
TBARS	OWLV	<0.005	<0.005	0.05–0.005
	QF	NSD	NSD	NSD
	Liver	<0.005	<0.005	0.05–0.005

The two-way ANOVA was performed on data sets shown in Tables 2 and 3. The abbreviations used for enzymes in the parameter column are: SOD, superoxide dismutase; GPX glutathione peroxidase; and CAT, catalase. The abbreviations for tissues are: OWLV, outer wall of the left ventricle; and QF, quadriceps femoris.

* The drug main effect included three drug treatments (captopril, hydralazine, and terazosin) and a no-drug control.

† The rat type main effect consisted of the SHR and WKY rat types.

‡ Drug-by-rat type interaction.

§ NSD indicates no significant difference ($P > 0.05$).

activity was not affected by captopril or hydralazine treatment in either myocardium or skeletal muscle, whereas terazosin was found to decrease Mn-dependent superoxide dismutase activity in the muscle tissues and liver. In contrast, it was found that Cu/Zn-dependent superoxide dismutase activity was elevated in the myocardium by all three drugs (to between 128 and 133% of control activity), whereas no changes occurred in the skeletal muscle and different effects were found in liver for each drug. In the case of glutathione peroxidase, all three drugs decreased myocardial activities (down to between 59 and 68% of control activity) but again had no effect on skeletal muscle activity and mixed effects on liver activities, with either decreases (with captopril and hydralazine) or an increase (with terazosin) being observed. For catalase, both hydralazine and terazosin decreased myocardial activity (to 78 and 74%, respectively, of control activity), but captopril had no significant effect. In the case of skeletal muscle catalase, neither captopril nor hydralazine had an effect on activity, whereas terazosin was found to increase the catalase activity to 128% of control activity. Only captopril was found to cause a change in liver catalase activity, where a decrease to 72% of the control activity occurred. With respect to the effects of drug treatment on tissue TBARS levels, only hydralazine caused a significant change in myocardial TBARS levels (a decrease to 72% of the control level), and the only other change in TBARS levels was found to be caused in liver by terazosin, where an increase to 134% of the control level occurred.

Analysis of the no-drug control SHR data in Table 2 and the no-drug control WKY data in Table 3 confirmed the conclusions of our earlier studies [9] that hypertension *per se* affects tissue antioxidant enzyme activities and TBARS levels. Thus, Table 2 shows that significant differences were found between the specific activity levels for all four myocardial enzymes, for the glutathione peroxidase activities in liver and quadriceps femoris, and for the TBARS levels in myocardium and liver.

To determine if the drug-related changes in antioxidant enzyme activities and TBARS levels that were found in SHR rats also occurred in normotensive rats, a parallel series of studies was performed on WKY rats using the same drug regimens. The results of these studies (Table 3) demonstrated that the responses to drug administration in the WKY normotensive rats were rather different from those observed in the SHR rats, although similar changes in glutathione peroxidase activities were seen in myocardium and liver of both animal groups. Both groups showed no drug-related changes in skeletal muscle glutathione peroxidase activity, and captopril had no significant effect on this enzyme activity in the myocardium of WKY rats.

With Mn-dependent superoxide dismutase, it was found that tissue activities were unaffected by drug administration in WKY rats, which contrasts with the effects that terazosin and captopril were found to have in the SHR rats. For Cu/Zn-dependent superoxide dismutase in WKY rats, in contrast to the increases in myocardial activities observed in SHR rats, all three drugs had no effects on myocardial activity. Differences in the effects of all three drugs on liver Cu/Zn-dependent superoxide dismutase activity were also found with SHR and WKY rats, with terazosin causing an increase in WKY rats, whereas captopril and hydralazine had no effect on activity. Catalase activities also were changed in different ways in the WKY rats, with none of the drugs causing decreases in myocardial activities but causing decreases in skeletal muscle activities (to between 71 and 81% of control activity) in contrast to the results seen with SHR rats.

Myocardial TBARS levels were found to be decreased by all three drugs in WKY rats (to between 54 and 81% of control levels), whereas only hydralazine was found to cause a decrease in SHR rats. Differences between SHR and WKY rats were also noted in the effects of captopril and terazosin on liver TBARS levels, although no changes in skeletal muscle TBARS levels were found to be caused by any of the drugs in either animal group.

Effects of Drugs on Antioxidant Enzyme Assays and TBARS Determinations

Experiments were conducted to determine if the changes in tissue antioxidant enzyme activities and TBARS levels were the results of direct effects of the drugs on the enzymes or interference with the TBARS assay. The concentration of drugs in the assays for these experiments was between 50 and 100 μmol/L to approximate possible *in situ* concentra-

TABLE 5. Effects of antihypertensive drugs on the *in vitro* activities of antioxidant enzymes

Enzyme	Enzyme activity in the absence or presence of an antihypertensive drug			
	No drug	Captopril	Hydralazine	Terazosin
Mn SOD				
	0.648 ± 0.021	0.645 ± 0.030		
	0.496 ± 0.012		0.500 ± 0.134	
	0.490 ± 0.027			0.503 ± 0.029
Cu/Zn SOD				
	0.837 ± 0.051	0.832 ± 0.022		
	0.818 ± 0.048		0.812 ± 0.030	
	0.805 ± 0.112			0.759 ± 0.050
GPX				
	7.16 ± 0.25	6.20 ± 0.51*		
	3.45 ± 0.21		3.58 ± 0.21	
	5.24 ± 0.06			5.37 ± 0.08
CAT				
	2.31 ± 0.12	2.42 ± 0.10		
	2.10 ± 0.06		2.05 ± 0.07	
	1.52 ± 0.07			1.54 ± 0.056

Mean enzyme activities (± SD) from triplicate assays are expressed as activity units as defined in Materials and Methods for each enzyme assay procedure. Separate no-drug controls were used for each drug in each enzyme assay, and the control activities for each enzyme set are not identical because different tissue preparations were used in each experiment. Captopril and hydralazine were used at 100 μmol/L in each assay, and terazosin was used at 100 μmol/L for the superoxide dismutase (SOD) and glutathione peroxidase (GPX) assays, and at 50 μmol/L for the catalase (CAT) assay.

* Significantly lower value ($P = 0.002$) than the corresponding control value, whereas all other values were not significantly different from their respective control values.

tions in experimental animals based on daily drug administration in relation to body weight and on the quoted half-lives of these drugs in human and animal tissues [38].

To study the direct effect of the drugs on the antioxidant enzymes, assays of each antioxidant enzyme were performed on myocardial preparations from animals that had not been treated with drugs, and the enzyme activities measured in the presence of added drug were compared with control (no drug addition) activities. For the studies on catalase activity, a concentration of 50 μmol/L was used because the high absorbance of terazosin at 100 μmol/L prevented accurate measurement of catalase activity.

The results shown in Table 5 indicate that none of the drugs had a significant effect on Mn superoxide dismutase, Cu/Zn superoxide dismutase and catalase activities. In the case of glutathione peroxidase, hydralazine and terazosin also had no significant effect on activity, whereas captopril had a statistically significant inhibitory effect (activity in the presence of the drug was 87% of control activity, $P = 0.002$). However, it appears unlikely that observed decreases in tissue glutathione peroxidase activities could have been caused by an inhibitory effect of captopril on the enzyme *in situ* as a 24-hr period was allowed to elapse between final drug administration and tissue collection, during which time it has been reported that 95% of ingested captopril is excreted [38]. Furthermore, in certain cases (myocardium of WKY rats and in the quadriceps femoris of SHR and WKY rats), no decrease in glutathione peroxidase activity was caused by captopril administration, indicating that the drug must have been cleared from the tissue by the time of tissue collection.

To determine if the drugs interfered with the TBARS determinations, standard curves for the TBARS determination using 1,1,3,3-tetraethoxypropane were determined in the presence of each drug (100 μmol/L) and compared with the standard curve obtained in the absence of each drug. The results shown in Fig. 1 indicate that under the conditions of the TBARS assay, none of the drugs significantly affected the assay results within the range of the standard curve (matched-pairs *t*-test *P* values for captopril, hydralazine and terazosin compared with no-drug control were 0.94, 0.082, and 0.64, respectively). These results indicated that TBARS measurements on tissue preparations from drug-treated animals were not directly affected by the possible presence of a drug in the tissue preparation.

DISCUSSION

We have found that, under conditions where captopril, hydralazine, and terazosin lowered blood pressures in SHR and WKY rats, there were significant changes in the specific activities of antioxidant enzymes and in the levels of lipid peroxidation that were both tissue and rat-strain specific. The aerobic tissues (myocardium and liver) generally showed more pronounced changes than the anaerobic skeletal muscle, which suggests that the aerobic tissues may have more flexible control mechanisms for regulating the expression of antioxidant enzymes than does an anaerobic tissue. For SHR rats, the antihypertensive drugs caused more changes in antioxidant enzyme activities than in WKY rats, with 21 out of 36 measured enzyme activities significantly altered (Table 2) in comparison to 16 out of 36

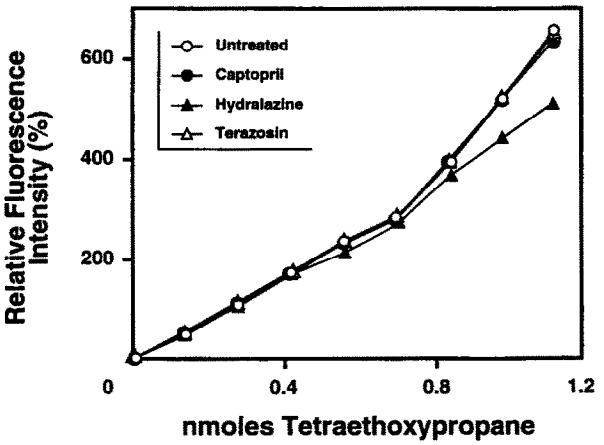

FIG. 1. Effects of antihypertensive drugs on the TBARS assay standard curve. Standard curves using tetraethoxypropane were generated in the absence ("untreated") and presence of antihypertensive drugs by the procedure of Yagi [36]. Each drug was present in the assay at a concentration of 100 μmol/L, and the amount of tetraethoxypropane per assay was varied between 0 and 1.1 nmol. All determinations were performed in triplicate, and the graph shows mean values of the triplicate assays. The excitation and emission wavelengths used were 515 and 553 nm, respectively, and results are expressed as percent relative fluorescence compared with samples containing no tetraethoxypropane.

measured activities that were changed in WKY rats (Table 3). It therefore appears that, in SHR rats, the mechanisms that control expression of the antioxidant enzymes are more sensitive to modulation by antihypertensive drugs. It is possible that this increased sensitivity may be related to the altered levels of antioxidant enzyme expression in SHR and WKY rats which we had described in an earlier study [9], and which are again seen in the "no drug" control data in Tables 2 and 3, where significant differences were found for all four myocardial enzyme activities and for glutathione peroxidase activity in liver and skeletal muscle activities between SHR and WKY rats.

The changes in rat tissue enzyme activities caused by drug administration ranged from an increase to 141% of control activity (Table 3, terazosin effect on liver Cu/Zn superoxide dismutase) to a decrease to 38% of control activity (Table 2, terazosin effect on quadriceps femoris Mn superoxide dismutase). These changes are quantitatively similar to those observed in earlier studies on the effects of exercise and aging on antioxidant enzyme activities [e.g. Refs. 9 and 39–41]. Although relatively small, such activity changes could be critical in antioxidant defense mechanisms, given previous evidence indicating that these enzyme activities may be present in tissues such as muscle at rate-limiting levels [42].

Previous studies have shown that changes in tissue antioxidant enzyme activities in response to internal or external stimuli do not always parallel each other. Thus, in response to a 90-min exercise regimen [40], total superoxide dismutase activity in rat soleus muscle was increased by 41%, whereas glutathione peroxidase activity was increased only by 8% and catalase activity was decreased to 71% of control activity. In hyperoxia over a 5-day period [43], rat lung mRNA levels for Mn and Cu/Zn superoxide dismutases were increased to quite different extents (to 267 and 122%, respectively, of control levels), and glutathione peroxidase activity was decreased only marginally (to 92% of control levels), whereas catalase was decreased to a much greater extent (to 63% of control levels). Studies on the 1- to 5-day post-natal development of rat lung [44] also showed a lack of coordinated changes in these enzyme activities with only catalase activity being changed significantly (increased). Our studies provide further evidence that antioxidant enzyme expression is not coordinately regulated as has been suggested previously [43, 44], as antihypertensive drugs often caused both increases and decreases in different antioxidant enzyme activities in the same tissue. Examples of a lack of coordinated changes in the present study were seen in both SHR and WKY rat tissues, but in SHR rats, there was a higher incidence of multiple activity changes within a tissue in response to drug treatment (see Tables 2 and 3). Thus, in SHR rats, treatment with each of the three antihypertensive drugs gave different non-coordinated patterns of activity changes for all four myocardial antioxidant enzymes, and other examples of non-coordinated changes were seen in skeletal muscle in response to treatment with terazosin and in liver following treatment with hydralazine or terazosin. The only case of a statistically significant coordinated change in more than two tissue enzyme activities was in liver of captopril-treated SHR rats, in which case there were positive correlations between the decreases in all four antioxidant enzyme activities (all r values > 0.71, all P values < 0.01). These results suggest that each antioxidant enzyme has its tissue activity regulated by unique mechanisms that are responsive to a wide variety of stimuli. The complexity of this situation is further emphasized as the regulation of expression of an antioxidant enzyme in different tissues can be affected differently by the same antihypertensive drug. Examples of such differences were seen with the effects of captopril on tissue Cu/Zn superoxide dismutases in SHR rats, with captopril on catalase in WKY rats, and with terazosin on glutathione peroxidases and catalase in both SHR and WKY rats.

As can be seen from data in Tables 2 and 3, treatment of SHR rats with an antihypertensive drug significantly altered myocardial antioxidant enzyme specific activities, in some cases to values that were not significantly different from those observed in untreated normotensive WKY rats. Examples of such specific activity changes in drug-treated SHR rats were seen as a result of captopril treatment for both superoxide dismutases and glutathione peroxidase, and with terazosin treatment for Cu/Zn superoxide dismutase and glutathione peroxidase. In the case of hydralazine treatment, Cu/Zn superoxide dismutase activity was elevated to the untreated WKY rat value, whereas the glutathione peroxidase activity was decreased to a level below the untreated WKY rat activity. Although none of

the drug treatments caused the myocardial catalase activities to decrease to the untreated WKY rat activities, both hydralazine and terazosin did cause significant decreases in myocardial catalase activities ($P = 0.0012$ and 0.0023, respectively), which brought these values closer to the untreated WKY rat value than to the untreated SHR rat value. The significance of these changes is not yet clear, but they may be beneficial in the myocardium and could be related to the lower levels of lipid peroxidation that are found in the myocardium of drug-treated SHR rats compared with those in untreated WKY rats (Tables 2 and 3). However, in the liver of SHR rats (Table 2), the level of lipid peroxidation was elevated significantly by terazosin treatment and was about twice that observed in untreated WKY rats, despite a decrease in Mn superoxide dismutase and an increase in glutathione peroxidase activities to values that were closer to those in untreated WKY rats. These results show that the response to antihypertensive drug treatment is tissue specific, and comparison of our results of the effects of captopril on liver antioxidant enzymes with those of a previous study [21] on mouse liver enzymes shows that the effects of antihypertensive drugs on antioxidant enzymes are also species specific.

Analysis of the data for myocardium in Tables 2 and 3 demonstrates that a simple inverse relationship between lipid peroxidation levels and antioxidant enzyme activities did not always occur. Thus, although the decrease in the TBARS level observed between non-drug-treated WKY and SHR rats was found to correlate negatively with increases in glutathione peroxidase ($r = -0.72$, $P < 0.01$) and catalase ($r = -0.71$, $P < 0.01$) activities, there was a positive correlation between the TBARS level decrease and the decrease in Mn superoxide dismutase activity ($r = 0.56$, $P < 0.05$). In hydralazine-treated SHR rats, this pattern of correlations was reversed, with the decrease in TBARS levels in the drug-treated rats showing a negative correlation with the increase in Cu/Zn superoxide dismutase activity ($r = -0.67$, $P < 0.02$), but having positive correlations with decreases in glutathione peroxidase ($r = 0.89$, $P < 0.001$) and catalase ($r = 0.71$, $P < 0.01$) activities. Hydralazine-treated SHR rats also showed a positive correlation ($r = 0.83$, $P < 0.001$) between the decreases in TBARS level and glutathione peroxidase activity. In addition to such positive correlations in myocardium, positive correlations were also seen in liver between TBARS levels and glutathione peroxidase activity ($r = 0.74$, $P < 0.01$) in terazosin-treated SHR rats, and between TBARS levels and glutathione peroxidase ($r = 0.69$, $P < 0.01$) and catalase ($r = 0.58$, $P < 0.05$) activities in captopril-treated WKY rats. The reasons why there is not a simple inverse relationship between tissue lipid peroxidation levels and the antioxidant enzyme activities measured in these studies are unclear, but may be related to changes in tissue concentrations of antioxidant metabolites (e.g. ascorbic acid) and in the specific activities of other enzymes involved in antioxidant metabolism, which could be caused by hypertension or treatment with antihypertensive drugs.

In summary, these studies demonstrated that the three antihypertensive drugs can affect the activities of rat tissue antioxidant enzymes, and, in myocardium, the changes in some of the enzyme activities observed between normotensive WKY and hypertensive SHR rats were mitigated by treatment of the SHR rats with an antihypertensive drug. Data obtained from myocardium, skeletal muscle, and liver indicated that drug effects on tissue antioxidant enzyme activities are tissue specific and more pronounced in SHR rats than in WKY rats. The results indicate that the changes are caused by drug effects on transcriptional, translational, or post-translational control mechanisms, and add to the list of proteins whose expression can be altered by hydralazine [29, 45] and captopril [29, 46]. These studies do not provide evidence for drug-related coordinate regulation of different enzymes in the same tissue or of the same enzyme in different tissues. Experiments in our laboratory are in progress to investigate the precise mechanisms for changes in antioxidant enzyme expression as a result of hypertension and for these drug-related effects. Of particular interest will be the question of whether these drugs operate by affecting the same or different regulatory mechanisms for the expression of an antioxidant enzyme gene.

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References

1. Halliwell B and Gutteridge JMC, *Free Radicals in Biology and Medicine*, 2nd Edn. Oxford University Press, Oxford, 1989.
2. Wispe JR, Warner BB, Clark JC, Dey CR, Neuman J, Glasser SW, Crapo JD, Chang LY and Whitsett JA, Human Mn-superoxide dismutase in pulmonary epithelial cells of transgenic mice confers protection from oxygen injury. *J Biol Chem* **267:** 23937–23941, 1992.
3. Jaruga P, Zastawny TH, Skokowski J, Dizdaroglu M and Olinski R, Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer. *FEBS Lett* **341:** 59–64, 1994.
4. Quinlan T, Spivack S and Mossman BT, Regulation of antioxidant enzymes in lung after oxidant injury. *Environ Health Perspect* **102 (Suppl 2):** 79–87, 1994.
5. Clerch LB, A 3′ untranslated region of catalase mRNA composed of a stem-loop and dinucleotide repeat elements binds to a 69-kDa redox-sensitive protein. *Arch Biochem Biophys* **317:** 267–274, 1995.
6. Ito H, Torii M and Suzuki T, A comparative study on defense systems for lipid peroxidation by free radicals in spontaneously hypertensive and normotensive rat myocardium. *Comp Biochem Physiol* **103B:** 37–40, 1992.
7. Uotila J, Tuimala R, Pykko K and Ahotupa M, Pregnancy-induced hypertension is associated with changes in maternal and umbilical blood antioxidants. *Gynecol Obstet Invest* **6:** 153–157, 1993.
8. Turrens JF, Thornton J, Barnard ML, Snyder S, Liu G and

Downey JM, Protection from reperfusion injury by preconditioning hearts does not involve increased antioxidant defenses. *Am J Physiol* **62:** H585–H589, 1992.
9. Hong H and Johnson P, Antioxidant enzyme activities and lipid peroxidation levels in exercised and hypertensive rat tissues. *Int J Biochem Cell Biol* **27:** 923–931, 1995.
10. Schoenberger JA, Antihypertensive drug therapy and coronary heart disease risk. *J Fam Pract* **36:** 70–84, 1992.
11. Lemke LE and McQueen CA, Acetylation and its role in the mutagenicity of the antihypertensive agent hydralazine. *Drug Metab Dispos* **23:** 559–565, 1995.
12. van Zwieten PA, New avenues in antihypertensive drug treatment. *Clin Exp Hypertens* **15:** 1205–1220, 1993.
13. Mira ML, Silva MM, Queiroz MJ and Manso CF, Angiotensin converting enzyme inhibitors as oxygen free radical scavengers. *Free Radic Res Commun* **19:** 173–181, 1993.
14. Aruoma OJ, Characterization of drugs as antioxidant prophylactics. *Free Radic Biol Med* **20:** 675–705, 1996.
15. Vulpis V, Roncali L, Bertossi M, Nico B, Seccia T, Ricci S and Pirrelli A, Left-ventricular hypertrophy in the spontaneously hypertensive rat: Effect of ACE inhibitors on ultrastructural morphology. *Cardiology* **84:** 14–24, 1994.
16. Takeda N, Tanamura A, Iwai T, Kato M, Noma K and Nagano M, Beneficial effect of ACE inhibitor in congestive heart failure. *Mol Cell Biochem* **129:** 139–143, 1993.
17. Engelman RM, Rousou JA, Iyengar J and Das DK, Captopril, an ACE inhibitor for optimizing reperfusion after acute myocardial infarction. *Ann Thorac Surg* **52:** 918–926, 1991.
18. Sogaard P, Klausen IC, Rungby J, Faergeman O and Thygesen K, Lipoprotein(a) and oxygen free radicals in survivors of acute myocardial infarction: Effects of captopril. *Cardiology* **87:** 18–22, 1996.
19. Lapenna D and Cuccurullo F, Captopril: Does it act as a direct antioxidant by free radical scavenging in humans? *Free Radic Biol Med* **18:** 377–378, 1995.
20. Schneider R, Iscovitz H, Ilan Z, Bernstein K, Gros M and Iaina A, Oxygen free radical scavenger system intermediates in essential hypertensive patients before and immediately after sublingual captopril administration. *Isr J Med Sci* **26:** 491–495, 1990.
21. de Cavanagh EMV, Inserra F, Ferder L, Romano L, Ercole L and Fraga CG, Superoxide dismutase and glutathione peroxidase activities are increased by enalapril and captopril in mouse liver. *FEBS Lett* **361:** 22–24, 1995.
22. Glowacki A, Kucharz EJ and Olczyk K, Glycosaminoglycan–collagen interactions *in vitro* in the hydralazine-induced collagen-like syndrome. *Acta Biochim Pol* **40:** 182–185, 1993.
23. Martelli A, Allavena A, Campart GB, Canonero R, Ghia M, Mattioli F, Mereto E, Robbiano L and Brambilla G, *In vitro* and *in vivo* testing of hydralazine genotoxicity. *J Pharmacol Exp Ther* **273:** 113–120, 1995.
24. Weglarz L, Drodz M, Wardas M and Goss M, Activities of antioxidant enzymes in fibroblasts cultured *in vitro* in the presence of hydralazine. *Biomed Biochim Acta* **48:** 489–493, 1989.
25. Cohen J, Long-term efficacy and safety of terazosin. *J Clin Pharmacol* **33:** 272–278, 1993.
26. Somberg J, Ehrenpreis S, Shroff G and Koshla S, Risk factors not addressed in antihypertensive therapy based solely on blood pressure control. *J Clin Pharmacol* **33:** 279–285, 1993.
27. Mukherjee D and Sen S, Collagen phenotypes during development and regression of myocardial hypertrophy in spontaneously hypertensive rats. *Circ Res* **67:** 1474–1480, 1990.
28. Jonsson JR, Head RJ and Frewin DB, Effect of α_1-adrenoceptor blockade on the development of hypertension in the spontaneously hypertensive rat. *Eur J Pharmacol* **211:** 263–268, 1992.
29. Alden SM, Pierce RA, Tozzi CA, Mackenzie JW, Deak SB and Boyd CD, Changes in aortic levels of tropoelastin mRNA following treatment of rats with the antihypertensive drugs captopril and hydralazine. *J Surg Res* **51:** 491–494, 1991.
30. Criswell D, Powers S, Dodd S, Lawler J, Edwards W, Renshler K and Grinton S, High intensity training-induced changes in skeletal muscle antioxidant enzyme activity. *Med Sci Sports Exerc* **25:** 1135–1140, 1993.
31. Crapo DJ, McCord JM and Fridovich I, Preparation and assay of superoxide dismutase. *Methods Enzymol* **53:** 382–393, 1978.
32. Paoletti F and Mocali A, Determination of superoxide dismutase activity by purely chemical system based on NAD(P)H oxidation. *Methods Enzymol* **186:** 209–220, 1990.
33. Aebi H, Catalase *in vitro*. *Methods Enzymol* **105:** 121–126, 1984.
34. Flohe L and Gunzler WF, Assays of glutathione peroxidase. *Methods Enzymol* **105:** 114–121, 1984.
35. Philips N, Bashey RI and Jimenez SA, Collagen and fibronectin expression in cardiac fibroblasts from hypertensive rats. *Cardiovasc Res* **28:** 1342–1347, 1994.
36. Yagi K, A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem Med* **15:** 212–216, 1976.
37. Draper HH and Hadley M, Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol* **186:** 421–431, 1990.
38. Schumacher MM, *Physicians' Desk Reference*, 47th Edn. Medical Economics Data, Montvale, NJ, 1993.
39. Hammeren J, Powers S, Lawler J, Criswell D, Martin D, Lowenthal D and Pollock M, Exercise training-induced alterations in skeletal muscle oxidative and antioxidant enzyme activity in senescent rats. *Int J Sports Med* **13:** 412–416, 1992.
40. Powers SK, Criswell D, Lawler J, Ji LL, Martin D, Herb RA and Dudley GA, Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. *Am J Physiol* **266:** R375–R380, 1994.
41. Leeuwenburgh C, Fiebig R, Chandwaney R and Ji LL, Aging and exercise in skeletal muscle: Responses of glutathione and antioxidant enzyme systems. *Am J Physiol* **267:** R439–R445, 1994.
42. Wohaieb SA and Godin DV, Starvation-related alterations in free radical tissue defense mechanisms in rats. *Diabetes* **36:** 169–173, 1987.
43. Ho Y-S, Dey MS and Crapo JD, Antioxidant enzyme expression in rat lungs during hyperoxia. *Am J Physiol* **270:** L810–L818, 1996.
44. Chen Y and Frank L, Differential gene expression of antioxidant enzymes in the perinatal rat lung. *Pediatr Res* **34:** 27–31, 1993.
45. Iwai N, Hanai K, Tooyama I, Kitamura Y and Kinoshita M, Regulation of neuronal nitric oxide synthase in rat adrenal medulla. *Hypertension* **25:** 431–436, 1995.
46. Katayama S, Abe M, Inaba M, Itabashi A and Ishii J, Effects of captopril or nitrendipine in left ventricular collagen or laminin B2 gene expression. *Jpn Heart J* **34:** 773–783, 1993.