Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 2, pp. 355–366, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00117-4

Alteration of Antioxidant Status in Diabetic Rats by Chronic Exposure to Psychological Stressors

PHILIP M. TOLEIKIS AND DAVID V. GODIN[1]

Department of Pharmacology and Therapeutics, The University of British Columbia, Vancouver, B.C. V6T1W5

Received 21 July 1994

TOLEIKIS, P. M. AND D. V. GODIN. *Alteration of antioxidant status in diabetic rats by chronic exposure to psychological stressors.* PHARMACOL BIOCHEM BEHAV **52**(2) 355–366, 1995.—Antioxidant status was measured in heart, liver, kidney, lung, and erythrocytes of 2-week streptozotocin-diabetic male Wistar rats exposed to chronic intermittent psychological stress consisting of 1 h of restraint twice daily for 14 days. Diabetes reduced erythrocyte and heart and liver susceptibility to hydrogen peroxide-induced glutathione depletion. Susceptibility to peroxide-induced thiobarbituric acid reactive substance (TBARS) formation increased in erythrocytes, liver, kidney, and lung but decreased in heart. Significant changes also occurred in glutathione levels (increased in heart and decreased in liver) and in the activities of catalase (reduced in liver and kidney), glutathione reductase (elevated in heart and liver), and glutathione peroxidase (decreased in liver and lung), but not Cu,Zn-superoxide dismutase. Stress potentiated diabetes-associated hyperglycemia and attenuated diabetes-induced hyperlipidemia. In addition, the reduction in peroxide-induced glutathione depletion in heart and liver and the increased TBARS formation in kidney and lung were reversed. Similarly, the diabetes-induced increase in liver glutathione reductase and decreases in liver and lung glutathione peroxidase activities were abolished by stress. Thus, the relative resistance of antioxidant systems to stress can be modified under pathologic conditions in which antioxidant alterations are present.

Antioxidant status Psychological stress Hyperlipidemia Lipid peroxidation Glutathione
Antioxidant enzymes Stress

PSYCHOLOGICAL stress can be described in physiological terms as a metabolic state in which the actions of stress hormones, including glucocorticoids and catecholamines, predominate over insulin (7). Such a metabolic state also characterizes uncontrolled diabetes associated with a reduction in either insulin release (Type I) or in the insulin sensitivity of target tissues (Type II). In addition to a reduction in the action of insulin, changes in catecholamine and glucocorticoid levels have also been noted in diabetes. Thus, elevations in urinary noradrenaline (4) and basal plasma corticosterone have been recorded in diabetic rats (15,37,50), and abnormal patterns of glucocorticoid circadian rhythms have been noted in both diabetic rats (45) and humans (31). Elevated glucocorticoid levels can increase tissue insulin resistance (2,48) in normal individuals, and this effect would likely be magnified in the diabetic state and vice versa.

It can, therefore, be postulated that the effects of emotional stress might exacerbate the metabolic abnormalities associated with the diabetic state (10,15,32). The appearance of diabetes has been shown to follow stressful life events in predisposed humans (51) and diabetes-prone BB Wistar rats (8). Stress is also associated with increased insulin requirements in diabetic patients (17,29). Furthermore, patients under poor glycemic control exposed to acute psychological stressors have been shown to have exaggerated elevations in plasma epinephrine concentration, despite normal basal levels (29). Other studies have demonstrated that the diabetic state is associated with an increased susceptibility to acute stress, as reflected in plasma levels of glucocorticoids or catecholamines. For example, streptozotocin-diabetic rats showed enhanced plasma corticoid levels in response to intraperitoneal cold-water injections (15), and elevated plasma epinephrine and norepinephrine levels associated with an acute period of intermittent foot shock (32).

[1] To whom requests for reprints should be addressed.

Transient alterations in plasma levels of stress hormones in diabetes, although readily measurable, do not provide information on the important cumulative functional consequences of stress, which may have important pathologic implications (58). Stress has a permissive effect on the development of cardiovascular disorders, including myocardial infarction (6,18) and atherosclerosis (24,28), which are major complications of the chronic diabetic state. Reactive oxygen radicals have been implicated in both of the above conditions (20,23), and diabetes is associated with marked alterations in enzymatic (4,44,57) and nonenzymatic antioxidant components (27,44).

Given the putative role of reactive oxygen radicals in the development of diabetes and its associated cardiovascular complications (11), stress could influence the course of their development through effects on antioxidant systems. The aim of the present study was, therefore, to examine the effects of chronic stress on tissue antioxidant status in normal and diabetic animals.

METHOD

Reagents

Streptozotocin (STZ), 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), oxidized and reduced glutathione, nitroblue tetrazolium (NBT), and glutathione reductase were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were reagent grade.

Animal Care

Male Wistar rats, weighing approximately 300 g, were obtained from the University of British Columbia Animal Care breeding colony and housed individually in hanging wire-mesh cages. The housing facility was temperature (22°C) and humidity controlled. The light–dark cycle was kept constant (lights on at 0600 and off at 1800 h). Rats had access to Purina rat chow and water ad lib. Following transfer from the breeding colony, rats were allowed to adapt for 1 week prior to the experimental protocol. The experimental protocol was approved by the University of British Columbia Animal Care Committee.

Induction of Diabetes

Diabetes was induced in halothane-anesthetized rats by a single injection of STZ (60 mg/kg b.wt.), in 0.3 ml sterile saline, via the tail vein. Control animals received the same volume of saline. Each rat was weighed weekly, and its rate of weight gain calculated. Only those STZ-injected animals that had blood glucose values above 300 mg/dl at the end of the study were considered diabetic; results from STZ-injected animals with glucose levels below 300 mg/dl were omitted.

Stress Protocol

Beginning on the 15th day following injection of STZ or saline, an equal number of animals from both groups was either exposed to stress or left in their home cages for the next 14 days. The stress protocol involved exposure, for a 1-h period, to one of seven different restraint stressors twice daily, the first exposure between 0900 and 1200 h, and the second between 1300 and 1600 h. To minimize habituation, the sequence of the stressors was randomized for both the first seven morning and afternoon exposures. The series was repeated during the second week, with the exception that the morning and afternoon sequences were exchanged. The stressors used included: a) towel wrap secured with tape; b) the latter, with animals placed in a supine position; c) restraint in a plastic box with lid; d) restraint in a polyvinylchloride tube closed at either end; e) immobilization on a board with tape; f) the latter, with animals placed in a supine position; and g) restraint in a metal bar cage. Each stressor exposure was conducted in a room remote from the animal facility, and animals exposed to stressors were returned to the animal facility 15 min following stress exposure to minimize disturbance to control animals. Restraint stress was used rather than a stress involving motion, i.e., swimming, to eliminate a possible training effect due to exercise. The stress protocol employed in this study has been shown to produce marked elevations in plasma corticosterone and catecholamine levels as measured through remote sampling using indwelling catheters during the first, seventh and 14th day of the protocol (Toleikis and Godin, unpublished results).

Tissue Isolation and Sample Preparation

At 0700 h on the day following the 4-week experimental period, animals were removed from the animal facility individually and killed within 30 s by decapitation in a room separate from the housing facility. Only two animals were euthanized each morning in rapid succession, to minimize disturbances to glucocorticoid levels known to occur following repeated entry into the animal facilities. Blood was collected in a glass centrifuge tube rinsed with heparin solution and centrifuged at 3,000 × *g* for 5 min at 4°C. Plasma was removed and separate aliquots frozen at −70°C for later determination of glucose, triglyceride, and cholesterol levels. The red cells were then washed twice by centrifugation. Heart, liver, kidney, and lung were excised and placed in 50 mM Tris–0.1 mM ethylendiamine tetraacetic acid (pH 7.6) (homogenizing buffer). These tissues were homogenized (10% w/v) on ice for two 15-s bursts using a Brinkmann Polytron homogenizer at 25% maximal speed.

Tissue and red cell susceptibility to increasing concentrations of H_2O_2, causing depletion of acid-soluble sulfhydryl groups and lipid peroxidation (as determined by TBARS formation in tissues and malondialdehyde MDA formation in erythrocytes), were assayed by procedures described in detail in previous work from our laboratory (54). Antioxidant enzyme activities were measured using well-established published procedures (1,16,19,26,33,46,53,56). Plasma levels of glucose, cholesterol, and triglycerides were determined using kits obtained from Sigma Chemical Co.

Results are presented as the mean ± standard error of the mean. Groups were compared using one-way analysis of variance, followed by Duncan's multiple range test.

RESULTS

Body weights and plasma biochemical composition for control, stressor-exposed, diabetic, and diabetic/stressor-exposed groups are shown in Table 1 and Fig. 1A–C. As expected, both groups of diabetic rats exhibited significant elevations of plasma glucose and gained weight more slowly than the control and stress-exposed groups. Diabetic animals also showed significant increases in plasma cholesterol and triglycerides. Stress alone did not alter the plasma levels of glucose, triglycerides, or cholesterol; however, diabetic rats exposed to the stress protocol exhibited significant increases in plasma glucose levels and significant decreases in plasma cholesterol and triglyceride levels. In fact, the cholesterol and triglyceride

TABLE 1

WEIGHTS OF WISTAR RATS BEFORE INJECTION OF STZ OR VEHICLE AND FOLLOWING EXPOSURE TO A STRESS PROTOCOL (STRESSOR EXPOSED) OR CONTROL CONDITIONS AND PERCENT CHANGE IN WEIGHT

	Control	Stressor Exposed	Diabetic	Diabetic/Stressor Exposed
Initial weight	317 ± 16 (n = 6)	300 ± 9 (n = 6)	333 ± 19 (n = 6)	302 ± 6 (n = 5)
Final weight	467 ± 18 (n = 6)	400 ± 10* (n = 6)	368 ± 19* (n = 6)	345 ± 8* (n = 5)
Percent change	44 ± 5 (n = 6)	33 ± 2 (n = 6)	11 ± 6* (n = 6)	14 ± 2* (n = 5)

Values are mean ± SEM.
*$p < .05$, significantly different from control.

values in the diabetic/stressor-exposed group decreased to levels that were not significantly different from control values.

The diabetic state was associated with changes in several antioxidant components in heart, liver, kidney, and lung tissue, but not erythrocytes. In the heart, glutathione reductase activity and basal glutathione level were elevated; however, glutathione peroxidase and Cu,Zn-superoxide dismutase activities were not altered (Table 2). In liver tissue, glutathione reductase activity was elevated, catalase and glutathione peroxidase activities as well as glutathione levels were reduced, and Cu,Zn-superoxide dismutase activity was unchanged (Table 3). Kidney tissue showed a decrease in catalase activity (Table 4), and lung tissue had reduced glutathione peroxidase activity (Table 5).

The diabetic state was also associated with alterations in susceptibility of erythrocytes and tissue homogenates to in vitro peroxide challenge. Sulfhydryl group depletion was significantly decreased in erythrocytes (Fig. 2A), heart (Fig. 2B), and liver (Fig. 2C), but not kidney or lung (data not shown). On the other hand, lipid peroxide formation in liver (Fig. 3A), kidney (Fig. 3B), lung (Fig. 3C), and erythrocytes (Fig. 4) was significantly increased while peroxide formation was significantly reduced in myocardial tissue (Fig. 3D).

Exposure of nondiabetic rats to the stressor protocol had minimal effects on antioxidant status. The only significant changes noted were a reduction in kidney basal glutathione levels (Table 4) and an increased susceptibility of erythrocytes to H_2O_2-induced sulfhydryl group oxidation (Fig. 2A).

In diabetic rats exposed to the stressor protocol, the increase in liver glutathione reductase (Table 3) and decreases in liver and lung glutathione peroxidase activities (Tables 3 and 4) observed with diabetes alone were abolished. In addition, the diabetes-induced increase in heart basal glutathione level was no longer significant following exposure to the stress protocol. Similarly, the reduction in H_2O_2-induced sulfhydryl group depletion in heart (Fig. 2B) and liver (Fig. 2C) and the elevation in lipid peroxidation in both kidney (Fig. 3B) and lung (Fig. 3C) found in nonstressed diabetic rats was not present in the diabetic stressor-exposed group.

Other diabetes-associated changes, notably the elevated glutathione reductase activity in heart (Table 2), and reduced catalase activity in liver (Table 3) and kidney (Table 4), were not altered by repeated exposure to the stressors. Exposure of diabetic rats to the stress protocol also did not alter the reduction in erythrocyte sulfhydryl group susceptibility to oxidation (Fig. 2A), the increases in MDA formation in erythrocytes (Fig. 4) and TBARS formation in liver (Fig. 3A) or the reduction in myocardial TBARS formation (Fig. 3D) found in the diabetic group alone. Erythrocyte catalase, although not changed by either diabetes or stress alone, was increased in diabetic rats exposed to the stress protocol (Table 6).

DISCUSSION

The aim of the present study was to investigate the independent and synergistic effects of streptozotocin-induced diabetes and chronic intermittent stress on antioxidant status in a number of tissues in rats. Tissues were chosen for analysis because of their association with complications arising from diabetes (heart, kidney), exposure to sustained high oxygen tensions (lung), or as the major site of oxidative metabolism (liver). Erythrocytes were analyzed to assess their potential clinical use as early predictors of antioxidant changes in tissues.

Results from this study demonstrate that the diabetic state is associated with complex alterations in organ antioxidant status. For example, basal glutathione levels were unaltered in erythrocytes and kidney, increased in heart, and decreased in liver. Glutathione reductase activity was unaltered in the kidney, increased in heart and liver, and decreased in the lung. The direction of some alterations is consistent with other published reports, including elevated heart glutathione reductase activity (57) and reductions in liver catalase (57) and glutathione peroxidase (57) activity as well as basal glutathione (34,57), and kidney catalase activity (57); however, there are inconsistencies in the direction of other antioxidant alterations, including liver Cu,Zn-superoxide dismutase activity (12,35,36,57), which may relate to differences in duration of diabetes, rat strain, and gender.

The functional consequences of diabetes-associated antioxidant changes in terms of peroxide-induced glutathione depletion and TBARS formation have not previously been examined. In the present study, erythrocytes and tissue homogenates both showed alterations in susceptibility to in vitro peroxide challenge. In general, most tissues from diabetic rats, including erythrocytes, heart, and liver, but not kidney, exhibited a reduced susceptibility to hydrogen peroxide-induced sulfhydryl group depletion. Previous work in our laboratory has demonstrated reduced susceptibility of erythrocytes from 12-week alloxan or streptozotocin diabetic rats to hydrogen peroxide-induced sulfhydryl group depletion (57). We have shown that this reduced susceptibility is likely related to stimulation of the hexose monophosphate shunt by hyperglycemia,

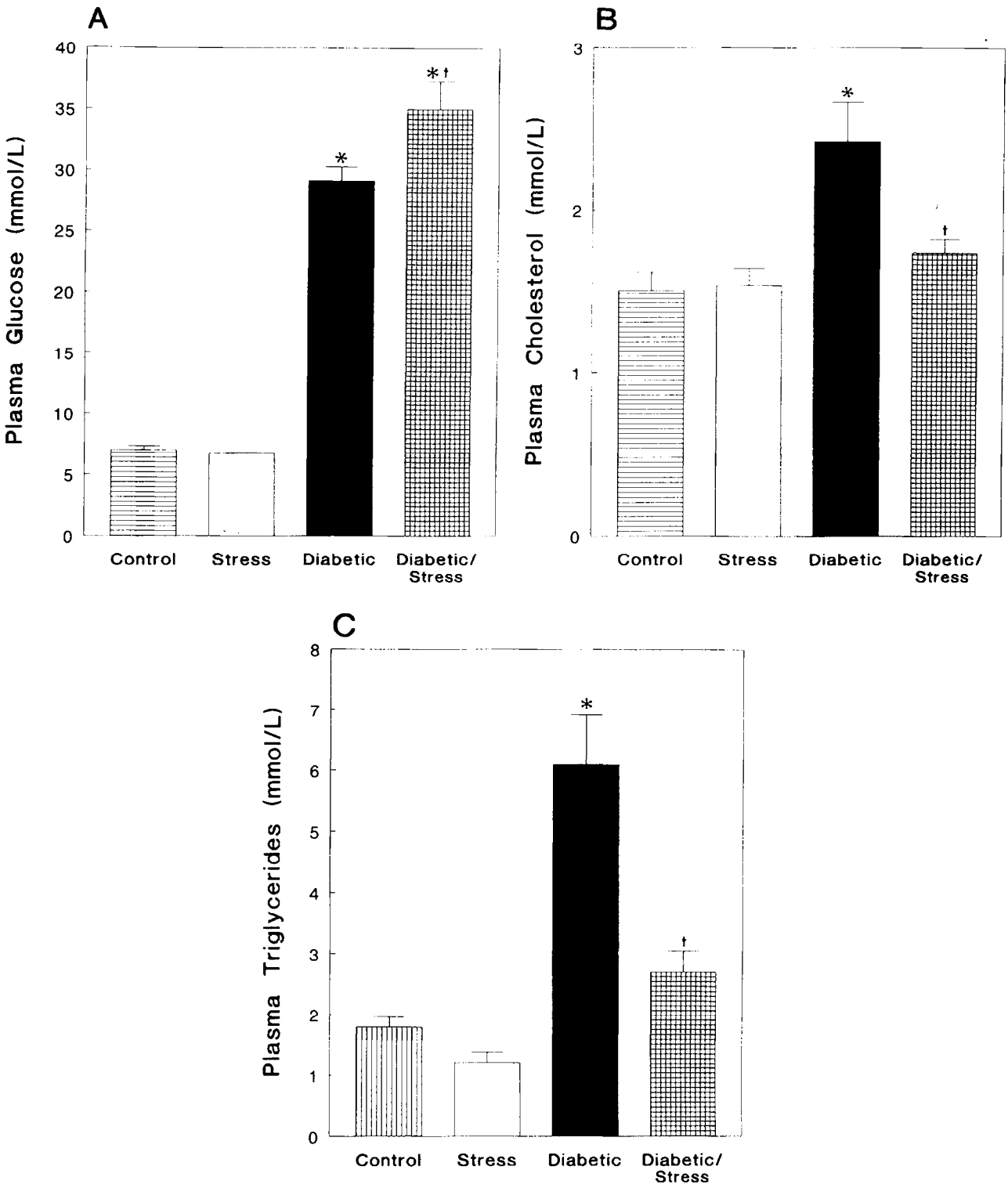

FIG. 1. Plasma glucose (A), cholesterol (B), and triglyceride (C) levels in normal and STZ-treated rats exposed to a stress protocol or control conditions. Values are means ± SEM. $n = 6$ for control, stress, and diabetic groups; $n = 5$ for the diabetic/stress group. Significant difference relative to: control, $^*p < 0.01$, or diabetic $\dagger p < 0.01$.

TABLE 2

ANTIOXIDANT STATUS IN HEARTS FROM NORMAL AND DIABETIC WISTAR RATS EXPOSED TO A STRESS PROTOCOL (STRESSOR EXPOSED) OR CONTROL CONDITIONS

	Heart			
	Control (n = 6)	Stressor Exposed (n = 6)	Diabetic (n = 6)	Diabetic/Stressor Exposed (n = 5)
Catalase (k/mg wet wt)	N/A	N/A	N/A	N/A
Cu, Zn-SOD (U/mg wet wt)	1.09 ± 0.05	0.97 ± 0.04	1.14 ± 0.05	1.23 ± 0.20
GSH-PX (nmol NADPH min^{-1} mg^{-1} wet wt)	6.65 ± 0.78	6.04 ± 0.55	5.54 ± 0.09	5.23 ± 0.41
GSSG-RD (nmol NADPH min^{-1} mg^{-1} wet wt)	0.493 ± 0.010	0.525 ± 0.021	0.611 ± 0.020*	0.574 ± 0.039*
GSH (nmol/mg wet wt)	1.82 ± 0.12	1.78 ± 0.12	2.14 ± 0.11*	2.02 ± 0.08

Values are mean ± SEM.

Cu,Zn-SOD = Cu,Zn-Superoxide dismutase, GSH-PX = Glutathione peroxidase, GSSG-RD = Glutathione reductase, GSH = Glutathione, N/A = Enzyme activity below measurable level.

*$p < 0.01$, significantly different from control.

thereby elevating intracellular availability of NADPH, which may be limiting to the glutathione reductase system under conditions of in vitro oxidative stress (21). The same mechanism may also be applicable to the tissues showing decreased susceptibility to peroxide-induced glutathione depletion. However, the reason for the unaltered susceptibility of kidney to sulfhydryl group depletion is unknown. Conversely, most tissues showed an increased susceptibility to hydrogen peroxide-induced lipid peroxidation including erythrocytes, liver, kidney, and lung. In striking contrast, TBARS formation was reduced in myocardial homogenates. This finding is in agreement with a previously published finding, in 6-week alloxan and streptozotocin diabetic rats, of decreased myocardial susceptibility to lipid peroxidation induced by an in vitro Fe^{2+}/ascorbate free radical generating system (47).

The decrease in myocardial susceptibility to lipid peroxidation may be related to the increases (possibly compensatory) in glutathione levels and glutathione reductase activity to alterations in membrane lipid fatty acid composition (9,22) known to occur in diabetic rats (47) or to alterations in nonenzymatic antioxidants, as discussed below. In some instances, increased susceptibility to lipid peroxidation can be rationalized in terms of impaired enzyme activities. For example, the increased hepatic lipid peroxidation in diabetic rats could be related to decreases in levels of glutathione as well as in the activities of catalase, and glutathione peroxidase. In the kid-

TABLE 3

ANTIOXIDANT STATUS IN LIVER TISSUE FROM NORMAL AND DIABETIC WISTAR RATS EXPOSED TO A STRESS PROTOCOL (STRESSOR EXPOSED) OR CONTROL CONDITIONS

	Liver			
	Control (n = 6)	Stressor Exposed (n = 6)	Diabetic (n = 6)	Diabetic/Stressor Exposed (n = 5)
Catalase (k/mg wet wt)	0.031 ± 0.005	0.028 ± 0.003	0.020 ± 0.003*	0.020 ± 0.004*
Cu, Zn-SOD (U/mg wet wt)	4.75 ± 0.25	4.89 ± 0.40	5.24 ± 0.42	5.56 ± 0.38
GSH-PX (nmol NADPH min^{-1} mg^{-1} wet wt)	17.13 ± 1.53	19.03 ± 0.81	11.46 ± 1.18*	17.28 ± 2.21†
GSSG-RD (nmol NADPH min^{-1} mg^{-1} wet wt)	4.13 ± 0.13	4.03 ± 0.12	5.14 ± 0.54*	4.57 ± 0.26†
GSH (nmol/mg wet wt)	4.82 ± 0.24	4.18 ± 0.24	3.81 ± 0.24*	3.90 ± 0.28*

Values are mean ± SEM.

Cu,Zn-SOD = Cu,Zn-Superoxide dismutase, GSH-PX = Glutathione peroxidase, GSSG-RD = Glutathione reductase, GSH = Glutathione.

*$p < 0.01$, significantly different from control.

†$p < 0.01$, significantly different from diabetic.

TABLE 4

ANTIOXIDANT STATUS IN KIDNEY TISSUE FROM NORMAL AND DIABETIC WISTAR RATS EXPOSED TO A STRESS PROTOCOL (STRESSOR EXPOSED) OR CONTROL CONDITIONS

	Kidney			
	Control (n = 6)	Stressor Exposed (n = 6)	Diabetic (n = 6)	Diabetic/Stressor Exposed (n = 5)
Catalase (k/mg wet wt)	0.0087 ± 0.0008	0.0080 ± 0.0010	0.0044 ± 0.0006*	0.0051 ± 0.0010*
Cu, Zn-SOD (U/mg wet wt)	2.80 ± 0.22	2.66 ± 0.14	2.94 ± 0.19	2.45 ± 0.18
GSH-PX (nmol NADPH min^{-1} mg^{-1} wet wt)	6.25 ± 0.68	5.73 ± 0.52	7.70 ± 0.65	7.37 ± 0.60
GSSG-RD (nmol NADPH min^{-1} mg^{-1} wet wt)	7.54 ± 0.34	7.70 ± 0.33	7.60 ± 0.86	7.02 ± 0.45
GSH (nmol/g wet wt)	2.48 ± 0.18	2.13 ± 0.12*	2.55 ± 0.09	2.37 ± 0.08

Values are mean ± SEM.

Cu,Zn-SOD = Cu,Zn-Superoxide dismutase, GSH-PX = Glutathione peroxidase, GSSG-RD = Glutathione reductase, GSH = Glutathione.

*$p < 0.01$, significantly different from control.

ney, reduced catalase and in the lung reduced glutathione peroxidase activities (in the absence of increases in other enzyme activities) may have contributed to the increased susceptibility of these tissues to lipid peroxidation. However, it is not known to what extent antioxidant enzymes are active in the in vitro peroxidizing systems. Diabetes-related alterations in levels of nonenzymatic antioxidants, such as tocopherol (3,25) and dihydroascorbic acid (41,52), may also play a role.

A major focus of the present study was to determine the extent to which chronic-intermittent stress influences tissue antioxidant status in control and diabetic animals.

In nondiabetic stress-exposed rats, the absence of changes in basal plasma glucose, triglyceride, and cholesterol levels is consistent with the results of previously published studies (14,55). In contrast, Tsopanakis et al. (55) reported a reduced level of serum cholesterol in animals subjected to swimming stress, but the confounding metabolic effects due to exercise cannot be readily dissociated from those of nonexercise related stress factors. For example, while an increase in insulin sensitivity is associated with exercise (5,30,40), an increase in insulin resistance is associated with stress (2,48). Paralleling the findings of the present study, humans exposed to significant psychological stress were found to exhibit no corresponding changes in lipid and lipoprotein levels (42).

Diabetic rats exposed to the stress protocol had elevated basal plasma glucose levels relative to the nonstressed diabetic animals. This elevation might occur through the combined effects of insulin resistance and elevations in corticosterone

TABLE 5

ANTIOXIDANT STATUS IN LUNG TISSUE FROM NORMAL AND DIABETIC WISTAR RATS EXPOSED TO A STRESS PROTOCOL (STRESSOR EXPOSED) OR CONTROL CONDITIONS

	Lung			
	Control (n = 6)	Stressor Exposed (n = 6)	Diabetic (n = 6)	Diabetic/Stressor Exposed (n = 5)
Catalase (k/mg wet wt)	N/A	N/A	N/A	N/A
Cu, Zn-SOD (U/mg wet wt)	0.95 ± 0.13	0.72 ± 0.06	0.72 ± 0.04	0.72 ± 0.08
GSH-PX (nmol NADPH min^{-1} mg^{-1} wet wt)	3.27 ± 0.38	3.17 ± 0.30	1.78 ± 0.17*	2.68 ± 0.14†
GSSG-RD (nmol NADPH min^{-1} mg^{-1} wet wt)	1.62 ± 0.06	1.56 ± 0.05	1.51 ± 0.06	1.58 ± 0.09

Values are mean ± SEM.

Cu,Zn-SOD = Cu,Zn-Superoxide dismutase, GSH-PX = Glutathione peroxidase, GSSG-RD = Glutathione reductase, N/A = Enzyme activity below measurable level.

*$p < 0.01$, significantly different from control.

†$p < 0.01$, significantly different from diabetic.

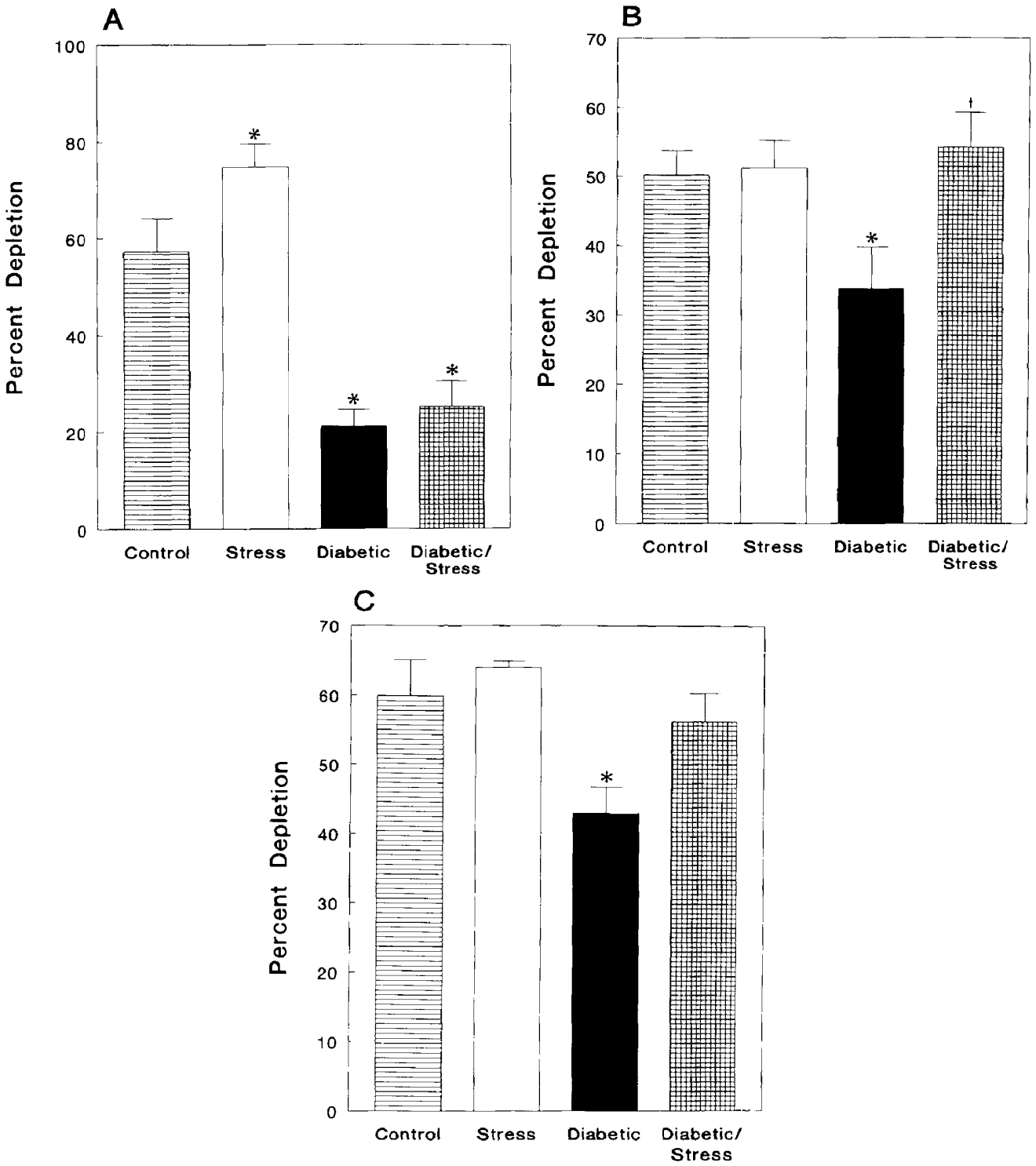

FIG. 2. Percent sulfhydryl group depletion following incubation of H_2O_2 with erythrocytes (A), and myocardial (B), and liver (C) homogenates from normal and STZ-treated rats exposed to a stress protocol or control conditions. H_2O_2 concentrations used were: 0.1 mM (erythrocytes and heart) and 0.25 mM (liver). Values are means ± SEM. $n = 6$ for control, stress, and diabetic groups; $n = 5$ for the diabetic/stress group. Significant difference relative to: control, $^*p < 0.01$ or diabetic $\dagger p < 0.01$.

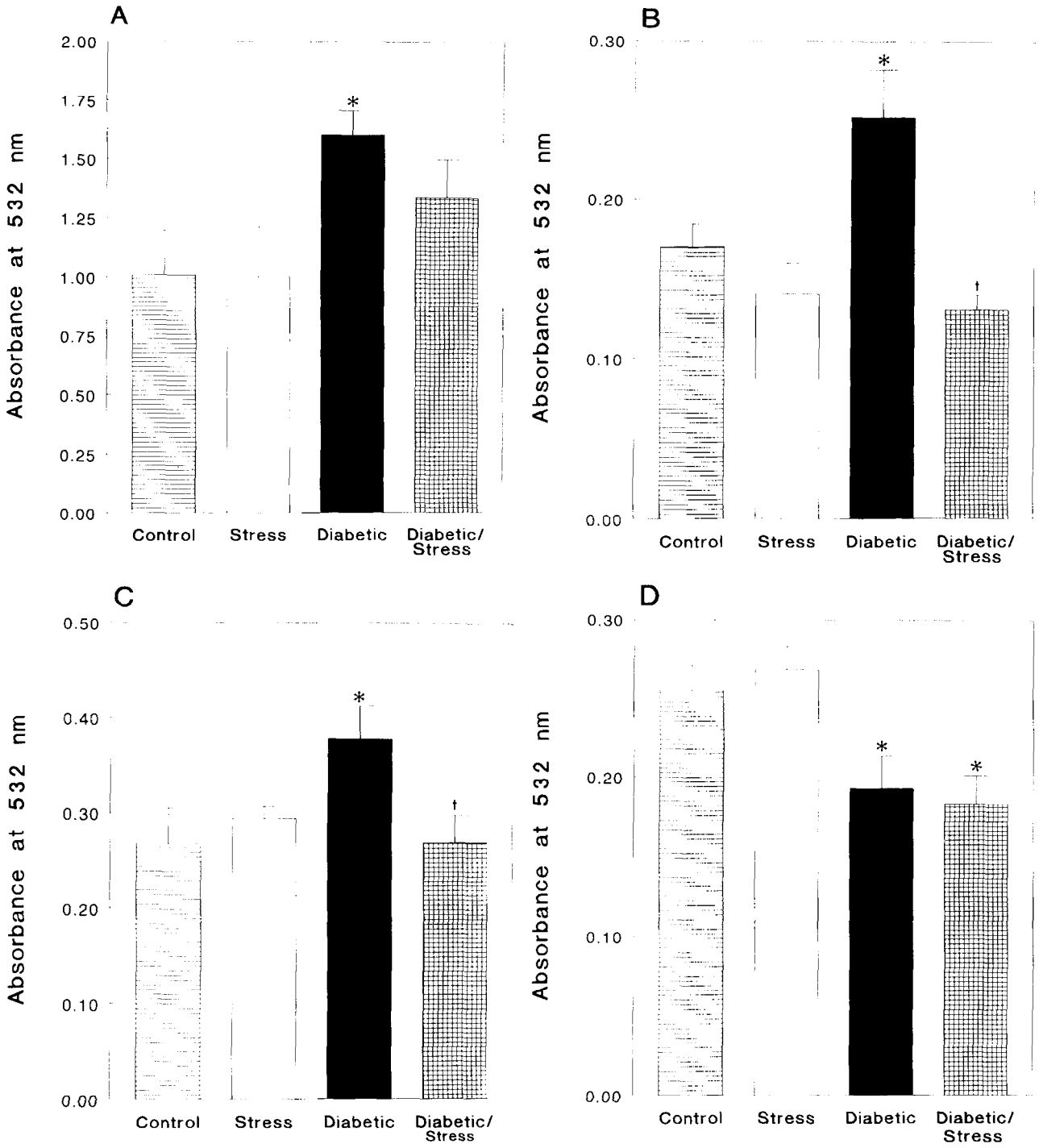

FIG. 3. TBARS formation following incubation of liver (A), kidney (B), lung (C), and heart (D) homogenates with H_2O_2 from normal and STZ-treated rats exposed to a stress protocol or control conditions. H_2O_2 concentrations used were: 20.0 mM (liver), 2.5 mM (kidney), 0.75 mM (lung), and 1.5 mM (heart). Values are means ± SEM. $n = 6$ for control, stress, and diabetic groups; $n = 5$ for the diabetic/stress group. Significant difference relative to: control, $^*p < 0.01$ or diabetic $^\dagger p < 0.01$.

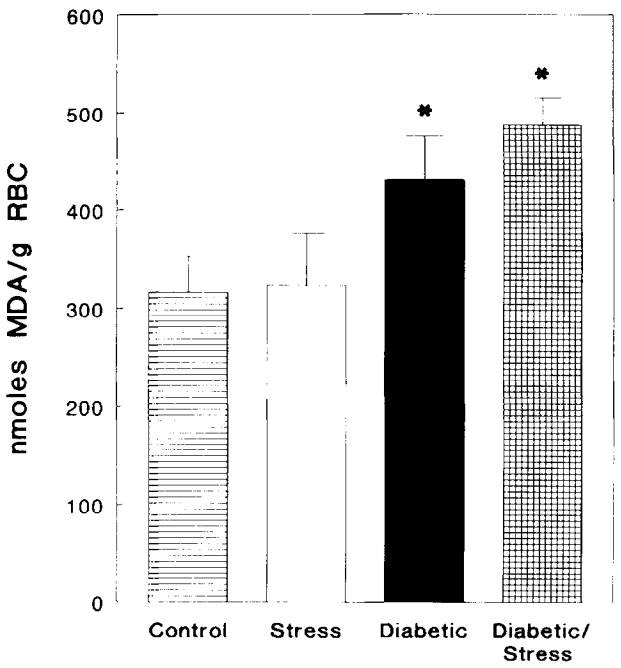

FIG. 4. Malondialdehyde (MDA) formation following incubation of erythrocytes with 2.5 mM H_2O_2 from normal and streptozotocin-treated rats exposed to a stress protocol or control conditions. Values are means ± SEM. n = 6 for control, stress, and diabetic groups; n = 5 for the diabetic/stress group. Significant difference relative to control, $*p < 0.01$.

and epinepherine, promoting gluconeogenesis and liver glycogenolysis, respectively. The observation that the elevations in plasma triglycerides and cholesterol in diabetic animals were lowered to control levels following exposure to chronic stress was unexpected, given the aforementioned absence of changes in plasma levels of these lipids in nondiabetic animals subjected to the same stress protocol. These changes are not a consequence of altered food intake, because the weights of animals in the diabetic-stressed group did not differ from their nonstressed diabetic counterparts. Elevations in triglyceride levels in diabetic rats have, in part, been attributed to a reduction in triglyceride lipase activity associated with an increase in liver lipid peroxides (43). Normalization of this enzyme activity following treatment with tocopherol has been shown to be associated with corresponding decreases in liver peroxide levels and triglycerides (49). It might, therefore, be postulated that stress-induced normalization of triglycerides could be the result of an increase in lipoprotein lipase activity. We have recently studied the effects of our chronic intermittent stress protocol on plasma lipid levels in hyperlipidemia of dietary rather than diabetic origin (54). Stress did not reduce lipid levels in hyperlipidemic animals, suggesting that the normalizing effects of stress on diabetes-induced elevations in plasma lipids is a particular feature of the diabetic state and cannot be solely ascribed to the associated hyperlipidemia per se.

Our findings regarding the effects of stress on antioxidant status reflect a similar complex series of positive and negative alterations, the number of which was greater in diabetic as opposed to nondiabetic rats relative to their respective controls. For example, the only change in nondiabetic rats exposed to chronic intermittent stress was a significant elevation in erythrocyte susceptibility to H_2O_2-induced sulfhydryl group oxidation. This increase in sensitivity was not accompanied by changes in erythrocyte antioxidant enzyme activities or peroxide-induced lipid peroxidation. Oxygen radicals have been shown to induce protein degradation in intact red cells in the absence of lipid peroxidation or detectable membrane damage (13). Thus, increased sulfhydryl group susceptibility to in vitro peroxidation may be an early indicator of stress-induced alterations in free radical mediated processes.

The finding that the chronic-intermittent stress protocol used in this study did not significantly alter antioxidant status in the nondiabetic group was not unexpected. While others (38,39) have found alterations in antioxidant components in nondiseased animals following acute stressor exposure, the nature of the stress required to produce these changes was severe, involving long bouts of hypothermia or unpredictable shock. We question the relevance of an acute stress model that initiates numerous antioxidant alterations in an otherwise

TABLE 6

ANTIOXIDANT STATUS IN PACKED ERYTHROCYTES FROM NORMAL AND DIABETIC WISTAR RATS EXPOSED TO A STRESS PROTOCOL (STRESSOR EXPOSED) OR CONTROL CONDITIONS

	Erythrocytes			
	Control (n = 6)	Stressor Exposed (n = 6)	Diabetic (n = 6)	Diabetic/Stressor Exposed (n = 5)
Catalase (k/mg Hb)	0.0492 ± 0.0020	0.0493 ± 0.0028	0.0447 ± 0.0016	0.0536 ± 0.0027*
Cu, Zn-SOD (U/mg Hb)	4.83 ± 0.31	4.72 ± 0.26	5.18 ± 0.56	4.68 ± 0.42
GSH-PX (nmol NADPH min^{-1} mg^{-1} Hb)	70.39 ± 6.9	66.39 ± 5.56	68.70 ± 8.90	75.02 ± 3.03
GSH (nmol/mg rbc)	1594.80 ± 55.28	1592.19 ± 95.46	1371.51 ± 69.16	1365.42 ± 128.24

Values are mean ± SEM.
Cu,Zn-SOD = Cu,Zn-Superoxide dismutase, GSH-PX = Glutathione peroxidase, GSH = Glutathione.
$*p < 0.01$, significantly different from diabetic.

normal animal, given that endogenous antioxidant mechanisms have presumably evolved to cope with elevated levels of oxidant stress. The chronic intermittent stress protocol in the present study was designed to determine whether subtle alterations in antioxidant status would occur in a disease state with preexisting oxidative stress. Our finding that the stress protocol used in this study resulted in minimal changes in antioxidant status in normal animals but did produce numerous changes in diabetic animals strengthens the suggestion that moderate stress can alter antioxidant status in animals with preexisting pathology.

In all tissues studied, some diabetes-associated changes in antioxidant status reverted to control values in animals exposed to chronic intermittent stress. Whereas antioxidant status was improved in erythrocytes and lung, stress reduced diabetes-associated compensatory changes in the heart. Some changes in functional antioxidant status occurred coincident with changes in antioxidant enzyme activity. These included a reduction in lung susceptibility to peroxide-induced TBARS formation associated with an increase in glutathione peroxidase activity. Other changes, however, such as reduced kidney susceptibility to lipid peroxidation and increased myocardial sensitivity to sulfhydryl group oxidation in diabetic stress-exposed rats, occurred in the absence of detectable antioxidant enzyme changes. The relative increase in liver sensitivity to H_2O_2-induced sulfhydryl group depletion occurred without a concurrent change in basal glutathione level, which remained reduced from normal, but was accompanied by a return of glutathione reductase activity to control from elevated values, while glutathione peroxidase activity, which was reduced in diabetes alone, returned to control values following the stress protocol. Assuming that the concentrations of H_2O_2 used did not cause enzyme inactivation, the decreased ability to generate reduced glutathione may be a factor in the increased susceptibility of the liver to glutathione depletion.

The finding that chronic-intermittent stress normalized a number of features associated with the diabetic state including elevated plasma lipid levels, and a number of antioxidant components and functional measures of antioxidant status suggests the possibility that moderate stress may be of some benefit in diabetes. Although some changes including lowered lipid levels have obvious implications in long-term diabetes, the potential benefits of the positive changes in antioxidant status, including normalized lung and liver glutathione peroxidase activity and improved peroxide-induced suceptibility to TBARS formation in lung and kidney, remain to be studied. In addition, some stress-induced alterations in the antioxidant status of diabetic animals occurred in a negative direction, including liver glutathione reductase activity and heart basal glutathione, as well as liver and heart peroxide-induced susceptibility to sulfhydryl group depletion. Further, following the chronic stress protocol, the diabetic group exhibited elevated resting plasma glucose levels. The situation is, thus, highly complex, and a detailed understanding of the relative benefits or adverse consequences of stress-induced antioxidant changes will require more research on organ function following chronic stress in longer term diabetes, during which the probability of development of secondary complications increases.

In summary, exposure of rats to chronic-intermittent stress in the absence of an underlying disease state was found to have minimal effects on antioxidant status. One altered functional index of antioxidant status, increased sensitivity of erythrocytes to peroxide-induced sulfhydryl group depletion, was noted, and this may be an early indicator of stress-induced disruption of antioxidant capacity. We have confirmed the presence of diabetes-associated changes in antioxidant components, which, for the most part appear to be increased in the myocardium and reduced in the other organs studied. The findings that stressor exposure in diabetic animals exacerbated elevated plasma glucose and reduced the increase in triglyceride and cholesterol levels indicates that chronic stress can modify diabetes-related changes in carbohydrate and lipid metabolism. The latter changes may, in part, be related to the return of several diabetes-associated functional antioxidant indices to control levels. In addition, while some antioxidant enzyme activity changes paralleled the functional changes, others did not, implicating a possible role for nonenzymatic antioxidants in this regard. Thus, chronic-intermittent stressor exposure has multiple effects in diabetes, the cause and consequences of which are currently under further investigation in our laboratory.

ACKNOWLEDGEMENTS

We wish to express our gratitude to the British Columbia/Yukon Heart and Stroke Foundation for their on-going support of our work and to the Canadian Diabetes Foundation for a predoctoral studentship award to one of the authors (P.M.T.). We also wish to thank Dr. Joanne Weinberg for assistance in design of the stress protocol.

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