Elevated Mitochondrial Cytochrome P450 2E1 and Glutathione S-Transferase A4-4 in Streptozotocin-Induced Diabetic Rats

Tissue-Specific Variations and Roles in Oxidative Stress

Haider Raza, Subbuswamy K. Prabu, Mari-Anne Robin, and Narayan G. Avadhani

Oxidative stress is an important factor in the etiology and pathogenesis of diabetes. We investigated changes in mitochondrial production of reactive oxygen species (ROS) and mitochondrial antioxidant defense systems in different tissues of streptozotocin (STZ)-induced diabetic rats. Our results show that increased ROS production and oxidative stress differentially affect mitochondrial and cytosolic glutathione (GSH) metabolism. Of the four tissues investigated, the pancreas, kidney, and brain appear to be affected more severely than the liver. We show a five-to eightfold increase of cytochrome P450 2E1 (CYP2E1) and glutathione S-transferase (GST) A4-4 levels in mitochondria from STZ-treated rat tissues compared with those in nondiabetic rat tissues, suggesting possible roles in the disease process. Transient transfection of COS cells with CYP2E1 cDNA caused a similar accumulation of CYP2E1 and GST A4-4 in mitochondria and increased production of mitochondrial ROS. Our results also show an increase in steady-state levels of Hsp70 in the mitochondrial and cytosolic fractions of different tissues of diabetic rats. These results indicate, for the first time, a marked increase in mitochondrial oxidative stress in target tissues of STZ-treated rats and implicate a direct role for mitochondrial CYP2E1 in the generation of intramitochondrial ROS. Diabetes 53:185–194, 2004

Increased cellular oxidative stress and altered antioxidant pool have been implicated in both clinical and experimental type 1 diabetes (1,2). Hyperglycemia, auto-oxidation of glycated proteins, increased production of reactive oxygen species (ROS), decreased antioxidant defense, increased lipid peroxidation, and associated membrane degeneration are implicated as main causes of cellular apoptosis or necrosis, which are common in diabetes (3–5). Mitochondrial dysfunction, apoptosis, and reduced ATP biosynthesis have all been implicated in type 1 diabetes (6,7). Accumulation of lipid peroxides, 4-hydroxynonenal (HNE), and malondialdehyde (MDA) in mitochondria has also been reported in diabetic patients, possibly causing further damage to mitochondrial genetic and metabolic systems (8,9).

Streptozotocin (STZ), an analog of N-acetylglucosamine, has been used to generate animal models of type 1 diabetes. Release of nitric oxide, increased glycation of pancreatic proteins, and an increased production of ROS have been proposed as possible causes of STZ-induced pancreatic β-cell damage (10–12). Additionally, STZ has been shown to deplete the antioxidant pool of target cells, thereby making them more susceptible to oxidative damage (13). In STZ-induced diabetic rats, hyperglycemia has been shown to induce typical apoptotic changes in the pancreas and other target tissues (14).

Elevated oxidative stress and ROS production in diabetic rats often parallels an increased expression of cytochrome P450 2E1 (CYP2E1) (14). CYP2E1 is known to metabolize endogenous compounds such as fatty acids, lipid hydroperoxides, and ketone bodies into aldehyde and many xenobiotics and carcinogens into nucleophilic reactive species (14,15). In vitro–reconstituted CYP2E1 and that expressed in intact cells by transient transfection has been shown to produce superoxide (O2•−) and H2O2 (14,15). Several studies have shown increased expression of CYP2E1 in STZ-induced experimental diabetes, human diabetic subjects, obese individuals, and alcohol abusers (16–22). The precise intracellular site(s) of CYP2E1 accumulation and ROS production under these pathological conditions, however, remain unclear. The aim of this study was to examine the extent of ROS production, steady-state levels of CYP2E1, and alterations in the antioxidant defense system in the mitochondrial compartment of STZ-
induced diabetic rat tissues. Mitochondria are an important source of ROS production and the primary targets of ROS-induced toxicity and apoptosis. Our results show a marked increase in mitochondrial CYP2E1 and glutathione S-transferase (GST) A4-4 and lowered mitochondrial GSH pool in different tissues of STZ-induced diabetic rats. Additionally, transient transfection studies show that mitochondrial CYP2E1 plays a direct role in ROS production. Our results suggest that altered mitochondrial biosynthetic and metabolic activities may be contributing factors in type 1 diabetes.

**RESEARCH DESIGN AND METHODS**

Most of the biochemicals, enzymes, substrates, and antibodies were purchased from Sigma Chemical (St. Louis, MO). 4-HNE was purchased from Oxis Research (Portland, OR). Polyclonal antibody against CYP2E1 was obtained from Amersham (Piscataway, NJ). Antibody for cytosolic GST A4-4 was a generous gift from Prof. Bengt Mannervik (Upsalla University, Upsalla, Sweden). Antibody against mitochondrial innermembrane translocator protein 44 (TIM44) was a gift from Dr. D. Pain (University of Medicine and Dentistry of New Jersey, Newark, NJ). Cell culture reagents were purchased from Invitrogen (Grand Island, NY). Reagents for electrophoresis and Western blotting and other chemicals were purchased from Bio-Rad (Richmond, CA).

**Animals and induction of diabetes.** Male Sprague-Dawley rats (150–200 g) were purchased from Harlan. Rats were maintained at 20°C under a light/12-h dark cycle, with access to water and food ad libitum. Diabetes was induced by a single intraperitoneal injection of buffered solution (0.1 mol/l streptozotocin (STZ), pH 4.5) of STZ at a dosage of 60 mg/kg body wt. The animals were considered diabetic when their blood glucose values exceeded 300 mg/dl (range 300–430) 5 weeks after STZ treatment. The animals were divided into three groups of two to three rats, each as control and as many groups of two to three each of diabetic rats (STZ treated) in each group. Control rats received vehicle alone. Animals were maintained for 4 additional weeks after STZ treatment, as described before (19).

**Isolation of tissues and subcellular fractionations.** Animals from control and STZ-treated diabetic groups were sacrificed according to National Institutes of Health-approved protocols. Tissues (liver, kidney, pancreas, and brain) from the control and treated animals from individual group were pooled and used for isolating mitochondria, cytosol, and microsome by differential centrifugation method, essentially as previously described (23). Mitochondria were further purified by treatment with digitonin (75 µg/mg protein for 2 min), and the purity of membrane fractions was ascertained by assaying for organelle-specific marker enzymes, as previously described (24).

Mitochondrial preparations containing <1% cross-contaminations were used in these experiments. Both mitochondrial and microsomal pellets were suspended in 50 mmol/l potassium phosphate buffer (pH 7.5) containing 1% methylsulfonylglycerol, 0.1 mmol/l EDTA, 0.1 mmol/l dithiothreitol, and 0.1 mmol/l phenylmethylsulfonylfluoride and frozen quickly in dry ice. The cytosolic fractions were also frozen at −70°C until they were used for analysis. Aliquots of subcellular fractions from each experimental and control groups were assayed for various parameters reported in this study.

**Enzyme assay and measurement of oxidative stress.** GSH levels were determined as protein-free sulfhydryl content using Ellman’s reagent (25) or by the method of Tietze (26). Lipid peroxidation was estimated by measuring thiobarbituric acid-reactive substances (TBARS), using MDA as a standard (27). Although thiobarbituric acid is known to react with lipoporphic compounds and lipid breakdown products at a lower affinity, this is the most widely used method to measure the lipid peroxidation (LPO) products. The cytosolic and mitochondrial GST activities were measured using 1-chloro-2,4-dinitrobenzene (CDNB) or 4-HNE as substrates according to the method of Habig, Pabst, and Jakoby (28) or Alin, Danielson, and Mannervik (29), respectively. CYP2E1 activities in mitochondria and microsomes were determined using dimethylsulfoximine (DMN) as a substrate (30). The rate of ROS production was measured as NAD(P)H oxidase–dependent lucigenin-enhanced chemiluminescence (31) using freshly isolated mitochondria and microsomes. Mitochondrial cytochrome c oxidase (COX) and NADH dehydrogenase activities were measured as described before (32). The protein concentration was estimated by the method of Bradford (33), using BSA as a standard. Statistical analysis of the data was carried out by Student’s t test using average and SE means from four to six animals assayed in triplicates.

**Transfection of COS cells with CYP2E1 cDNA.** Rat CYP2E1 cDNA cloned in pcMV vector was used to transfect COS cells grown in Dulbecco’s modified Eagle’s medium to 50–70% confluency (30). Forty-eight hours after transfection, cells were harvested by trypsinization and subcellular fractions (mitochondria, microsomes, and cytosol) were isolated as described earlier (30) and used for assaying enzyme activities and oxidative stress as described above.

**Western blot analysis.** The protein fractions (50–100 µg) were subjected to SDS-PAGE on 12% gels (34) and transferred to nitrocellulose membrane for immunoblot analysis (35). Cross-reactivity to antibodies against GSTs, Hsp70, and CYP2E1 was determined by incubating the membranes with antibodies followed by incubation with horseradish peroxidase–conjugated secondary antibodies. The immunoblots were developed using the Super signal west femto kit from Pierce Biotechnology (Rockford, IL), and the blots were imaged and quantitated using a BioRad Fluor-S-Imager as described (34).

**RESULTS**

Mitochondrial oxidative stress in STZ-induced diabetes. Blood glucose levels were measured using a glucose oxidase kit from Sigma Chemical. Throughout this study, control rats with a blood glucose level of 85–105 mg/dl and diabetic rats (STZ treated) with a level of 300–430 mg/dl were used.

Figure 1 shows the extent of ROS production and lipid peroxidation in isolated mitochondrial and microsomal membrane fractions from the pancreas, liver, kidney, and brain of control and diabetic rats. Figure 1A (left panel) shows that ROS production in the isolated mitochondrial fractions from different tissues of control rats (180–600 light units · min⁻¹ · mg protein⁻¹) was generally higher than with the corresponding microsomal fractions (right panel, 50–160 light units · min⁻¹ · mg protein⁻¹). Furthermore, a dramatic 8- to 10-fold increase in ROS production was observed in mitochondria from the pancreas and brain of diabetic rats. A modest but significant increase (P < 0.05) of ROS production (25–30%) was observed in mitochondria from the kidney and liver of diabetic rats.

With the microsomal fractions of diabetic rats, however, a twofold increase was observed in the pancreas and a 25% increase in the kidney. There was a significant increase in the brain, whereas there was a significant decrease in the liver (Fig. 1A, right panel). Figure 1B shows that total LPO (TBARS) was significantly higher in the mitochondrial fractions of pancreas and liver (6–8 nmol/mg protein) of control rats than in the microsomal fractions of these tissues (5 nmol/mg protein). The microsomal fractions of kidney and brain, however, showed a two- to threefold higher LPO (10–20 nmol/mg protein) than the corresponding mitochondrial fractions (4–6 nmol/mg protein). Mitochondria from the pancreas, kidney, and brain of diabetic rats showed a 20–30% increase in lipid peroxidation, while mitochondria from liver showed 20% reduced activity. The microsomal fractions of the pancreas, kidney, and liver from diabetic rats showed a 10–20% increase in TBARS, while microsomes from brain showed a 20% decrease. The reasons for the decreased ROS production in the liver microsome (Fig. 1A, right panel) and decreased TBARS production in liver mitochondria and brain microsomes of diabetic rats, or the physiological significance of these variations, currently remain unclear. These results, however, show that mitochondria from different tissues are important and preferential targets of STZ-induced diabetes.

**Mitochondrial and microsomal CYP2E1 levels in STZ-treated rat tissues.** A number of studies suggest a possible role for CYP2E1 in ROS production and oxygen-mediated tissue injury (14). We therefore investigated the levels of CYP2E1 in the mitochondrial and microsomal...
compartments of tissues from diabetic rats. The immunoblot in Fig. 2A (top panel) shows that the steady-state level of the microsomal CYP2E1 in the diabetic pancreas, kidney, and liver was increased by about two to threefold, although the basal activities varied markedly between different tissues. There was no significant increase in the brain microsomes. The levels of CYP2E1 in the mitochondrial fractions of pancreas, kidney, liver, and brain were induced five- to sixfold in diabetic animals. In fact, the steady-state levels of CYP2E1, based on mitochondrial fractions per milligram protein, were higher than in the corresponding microsomal fractions. The levels of calreticulin (Fig. 2, top panel) and TIM44 (bottom panel), used as loading controls, did not vary significantly under the experimental conditions. Furthermore, the microsomal fraction contained very low levels of TIM44, and the mitochondrial fractions contained very low levels of calreticulin, indicating the purity of the respective membrane preparations.

Figure 2B shows the DMNA N-demethylase activity, a marker for CYP2E1 activity, in the mitochondrial (left panel) and microsomal (right panel) fractions of tissues from control and diabetic rats. It is seen that the DMNA activity is increased significantly (25–80%) in mitochondria from pancreas, kidney, and liver of diabetic rats. The brain mitochondria from diabetic rats showed only a marginal increase of 10%. The microsomal fractions of pancreas, kidney, and liver also showed a 25–50% increase, whereas the brain microsomes showed no increase. Despite a general trend of increased activity, the DMNA activity profile did not directly reflect the CYP2E1 protein level (Fig. 2A), which may indicate altered catalytic activity of the enzyme in diabetic tissues due to either post-translational modification or other unknown factors. The latter is known to affect the interaction of CYP2E1 with the mitochondrial-specific electron transfer protein, adrenodoxin (Adx) (36).

Mitochondrial and cytosolic GST A4-4 levels in STZ-treated rat tissues. The immunoblot in Fig. 3A shows four- to fivefold increased GST A4-4 in the mitochondrial fraction of pancreas and kidney and about a threefold increased level in mitochondria from diabetic rat livers. Brain mitochondria, on the other hand, contained very low levels of GST A4-4. The microsomal GST A4-4 levels were increased two- to threefold in the pancreas, kidney, and liver from diabetic rats, and there was no detectable protein in both control and diabetic brain. Hsp70, another stress-related protein, was also increased by 20% in the mitochondrial fraction of kidney to about twofold in other tissues. The increase in the cytosol of diabetic rat was variable in that pancreas and kidney showed a four- to sixfold induction, while liver and brain showed no significant induction. The results show that the extent of GSA4-4 induction in STZ-induced diabetes was generally more in the mitochondrial fraction than in the cytosol. In the case of Hsp70, mitochondrial content was increased in all tissues, whereas the induction in the cytosolic compartment was mixed.

4-HNE is a preferred substrate for GST A4-4, and CDNB is a general substrate for many different isozymes of GST (28,29). Total GST activity measured with CDNB as the substrate was increased twofold (P ≤ 0.05) in the brain mitochondria, while there was a 10–15% increase (P > 0.05) in the liver and kidney mitochondria of diabetic rats. There was a reduction in the mitochondrial GST activity in the pancreas (Fig. 4A). CDNB metabolic activity in the cytosol of all tissues, with the exception of the liver, was marginally decreased. Using 4-HNE as a specific substrate for GST A4-4, however, there was a 20–50% increase of activity in both the mitochondrial and cytosolic fractions.
of all four tissues (Fig. 4B). The overall level of 4-HNE metabolism was about two- to threefold higher in the cytosolic than in the mitochondrial fraction. Consistent with the increase of GST A4-4 protein levels, these results show that the mitochondrial 4-HNE metabolism was increased in all four tissues of the diabetic rats studied.

Figure 5A shows that the mitochondrial GSH level in the pancreas, kidney, and liver was moderately increased (20–35%) in STZ-induced diabetic animals, whereas brain mitochondria showed an ~18% decrease. GSH in the cytosolic fractions was increased only in the liver (~10%), reduced by ~20% in the kidney, and did not vary in the pancreas and brain (Fig. 5A, right panel). These results suggest that either the mitochondrial GSH biosynthesis in the four tissues increases or the cytosolic GSH is transported to mitochondria during STZ-induced diabetes. A similar stress-induced shift in the intracellular GSH pools was shown in a recent study (24).

Figure 5B shows that mitochondrial respiratory chain activity was also affected in STZ-induced diabetes. We observed a 10–20% decrease of COX activity in all tissues after STZ administration, and the NADH dehydrogenase activity was increased by 10–20%. In all cases, the changes were statistically significant (P < 0.05).

Oxidative stress in COS cells transfected with CYP2E1 cDNA. To assess the physiological significance of increased mitochondrial CYP2E1 in oxidative stress and its role in ROS production in STZ-mediated diabetes, we
used COS cells transfected with CYP2E1 cDNA as a model system. The immunoblot in Fig. 6A shows that mitochondria and microsomes from cells transfected with CYP2E1 cDNA contain three- to fourfold more antibody-reactive proteins than mock-transfected cells. The results also show that the mitochondrial fraction contained negligible calreticulin and the microsome fraction contained no detectable TIM44, indicating the purity of the subcellular fractions used. Quantitation of the gel pattern in Fig. 6A shows that mitochondrial CYP2E1 represents ~25–35% of the level in the microsomes. Figure 6B shows that the CYP2E1-transfected cells also exhibit 50% to twofold increased steady-state levels of Hsp70 and GST A4-4 in both cytosolic and mitochondrial compartments, indicating an increase in oxidative stress (Fig. 6B). The immunoblots also show that mitochondrial fraction did not contain significant β-actin, a cytosol-specific protein. The cytosolic fraction, on the other hand, did not contain significant TIM44, a mitochondrial inner membrane–specific protein.

Increased oxidative stress in COS cells transfected with CYP2E1 was also ascertained by measuring ROS production and LPO and GSH pools in the mitochondrial and microsomal fractions of transfected cells. As shown in Table 1, the mitochondrial ROS in transfected cells was increased by 45%, LPO by 30%, and CYP2E1 activity, as measured by DMNA N-demethylation, increased by 133% compared with mock-transfected cells. The mitochondrial GST activity with CDNB substrate was increased by 44%.
whereas that with 4-HNE was increased by 82%. The mitochondrial GSH concentration, however, was not significantly affected by overexpression of CYP2E1. COX activity in the mitochondrial fraction was decreased by ~30% in cells transfected with CYP2E1 cDNA. In the microsomal fraction, however, despite a fourfold increase in CYP2E1, the ROS production and LPO level was only marginally ($P > 0.05$) altered (Table 1). An increase in cytosolic GST activity (55–60%) was also observed after CYP2E1 transfection. On the other hand, cytosolic GSH level was not altered significantly ($P > 0.05$).

We sought direct evidence for the contribution of mito-
chondrial CYP2E1 in the production of ROS using COS cells transfected with CYP2E1 cDNA. We reasoned that in this system, prevention of flow of electrons from Adx to CYP2E1 should also reduce ROS production. Previous studies from our laboratory (36,37) showed that a peptide corresponding to sequence 265–279 of CYP1A1 (36,37), a portion of the helical region that is involved in binding to Adx, can effectively abrogate enzyme activity of both P450MT2 (mitochondrial counterpart of CYP1A1) and P450MT5 (mitochondrial counterpart of CYP2E1). Mitochondria from transfected cells were incubated with wild-type or mutant peptides for 10 min at room temperature and used for assaying ROS production. Table 2 shows that increasing concentrations of wild-type MT2 peptide yielded increasing inhibition (34–60%) of ROS production. Mutated MT2 peptide, carrying substitutions at the Adx binding sites, on the other hand, did not inhibit mitochondrial ROS production. Since the MT2 peptide specifically interferes with the interaction of Adx with mitochondrial CYPs, these results conclusively show the role of CYP2E1 in mitochondrial ROS production.

DISCUSSION

Mitochondria are susceptible to the damaging effects of ROS leading to both accidental cell death (necrosis) and programmed cell death (apoptosis) (38) that are invariably associated with many degenerative disorders (39). In STZ-treated diabetic rats, hyperglycemia induces apoptotic changes and associated oxidative stress (40). Increased oxidative stress and changes in antioxidant pools have been observed in both clinical and experimental diabetes. Mitochondrial ROS generation is thought to be a continuous and normal physiological process, and consequently these organelles contain efficient antioxidant enzyme systems, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione transferase, GSH, NADPH, and, finally, the mitochondrial respiration itself (41,42).

Various groups, including ours, have reported alterations in the GSH pool and GSH-metabolizing enzymes in addition to increased CYP2E1 expression in STZ-induced diabetic rat tissues (19–22). However, most of the studies thus far have focused on the cytosolic and microsomal compartments of target tissues or cells. The present study demonstrates that mitochondria from pancreas, liver, and kidney of STZ-induced diabetic rats exhibit high oxidative stress, increased tissue degeneration. Indeed, reduced mitochondrial FADH2-driven ATP production and its sequential effects on ATP-dependent K+ channel and voltage-gated Ca2+ channel activities have been implicated as possible causes of reduced insulin secretion in type 1 diabetes.

FIG. 6. Levels of mitochondrial CYP2E1, Hsp70, and GST A4-4 in COS cells transfected with CYP2E1 cDNA. COS cells (~1.5 × 10^6) were transfected with rat CYP2E1 cDNA cloned in pCMV vector, as previously described (38). Cells were harvested after 48 h of transfection, and cell fractions (mitochondria, microsomes, and cytosol) were prepared by differential centrifugation. Proteins (50 μg) were separated by 14% SDS-PAGE and probed by Western blot analysis. A: Blots were probed with antibody to CYP2E1. Antibodies to calreticulin and TIM44 were used to evaluate protein loading and to assess cross-contamination. B: Two companion blots were probed with antibodies to Hsp70 and GST A4-4. One blot was also probed with antibodies to β-actin and TIM44 to evaluate protein loading and assess cross-contamination. Mean ± SE values were calculated from blots run with three different cell culture experiments. In each case, the intensity with the mitochondrial fraction for calculating the relative intensities of other samples was regarded as 1.
TABLE 1
Effect of overexpression of CYP2E1 on oxidative stress parameters in COS cells

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mitochondria</th>
<th>Microsomes</th>
<th>Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mock cDNA</td>
<td>CYP2E1 cDNA</td>
<td>Mock cDNA</td>
</tr>
<tr>
<td>ROS (light units ⋅ min⁻¹ ⋅ mg protein⁻¹)</td>
<td>220.1 ± 19</td>
<td>319.2 ± 40*</td>
<td>6.3 ± 0.04</td>
</tr>
<tr>
<td>LPO (nmol ⋅ min⁻¹ ⋅ mg⁻¹)</td>
<td>0.52 ± 0.04</td>
<td>0.68 ± 0.08*</td>
<td>0.06 ± 0.05</td>
</tr>
<tr>
<td>GSH (nmol/mg)</td>
<td>9.0 ± 0.08</td>
<td>10.5 ± 1.3</td>
<td>ND</td>
</tr>
<tr>
<td>GST (CDNB) (nmol ⋅ min⁻¹ ⋅ mg⁻¹)</td>
<td>90.5 ± 7.5</td>
<td>131 ± 14*</td>
<td>ND</td>
</tr>
<tr>
<td>GST (HNE) (nmol ⋅ min⁻¹ ⋅ mg⁻¹)</td>
<td>22.2 ± 2.0</td>
<td>40.5 ± 4.2*</td>
<td>ND</td>
</tr>
<tr>
<td>CYP2E1 (DMNA) (nmol ⋅ min⁻¹ ⋅ mg⁻¹)</td>
<td>0.30 ± 0.02</td>
<td>0.71 ± 1.0*</td>
<td>0.5 ± 0.05</td>
</tr>
<tr>
<td>COX (μmol ⋅ min⁻¹ ⋅ mg⁻¹)</td>
<td>60 ± 5.3</td>
<td>43 ± 3.9</td>
<td>ND</td>
</tr>
</tbody>
</table>

Mitochondria cell fractions and additions
.Cells transfected with empty vector (mock) and CYP2E1 cDNA–cloned vector, as described in Fig. 6, were assayed for ROS, LPO, GSH, GST, and COX activities, as described in RESEARCH DESIGN AND METHODS. Mean ± SE values were calculated based on three different experiments, as described in Fig. 6. *P ≤ 0.05 vs. control values. ND, not determined.

TABLE 2
Effects of adrenodoxin-binding peptide on ROS production in mitochondrial fraction of CYP2E1 overexpressing cells

<table>
<thead>
<tr>
<th>Cell fractions and additions</th>
<th>ROS (light units ⋅ min⁻¹ ⋅ mg protein⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondria alone</td>
<td>255.0 ± 18.0</td>
</tr>
<tr>
<td>Plus wild-type MT2 peptide (1 nmol)</td>
<td>170.0 ± 15.0*</td>
</tr>
<tr>
<td>Plus wild-type MT2 peptide (2 nmol)</td>
<td>155.0 ± 18.0*</td>
</tr>
<tr>
<td>Plus mutant MT2 peptide (2.5 nmol)</td>
<td>260.0 ± 25.0</td>
</tr>
</tbody>
</table>

Mitochondria from COS cells transfected with CYP2E1 cDNA, as described in Fig. 6 were used for assaying ROS production. Mitochondria were preincubated with indicated amounts of adrenodoxin peptides (wild type or mutant) for 10 min on ice before initiating the reaction. Values represent the mean of three separate cell culture experiments. *P ≤ 0.05 vs. control values. Wild-type MT2: (265) MFKKLKEHYRTFEK (279); Mutant MT2: (265) MFPKNLHYRTFEK (279).
reduced level of ROS production in mitochondrial isolates from CYP2E1 transfected cells by incubation with wild-type MT2 peptide, which selectively interferes with CYP interaction with Adx. These results suggest that disruption of electron flow from Adx to CYP2E1 causes a vastly reduced ROS production. Our results therefore provide a direct role for mitochondrial CYP2E1 in ROS production. Since increased mitochondrial accumulation of CYP2E1 in transfected cells also caused a steady and continuous inhibition of COX activity, we hypothesize that mitochondrial CYP2E1 is probably the important initial contributor of mitochondrially generated ROS that subsequently causes altered electron transfer chain activity.

In summary, we provide evidence for increased mitochondrial oxidative stress in STZ-treated diabetic rat tissues. The extent of increase in stress, steady-state levels of CYP2E1, and activation of GSH-dependent oxidant metabolism are differentially affected in different tissues. These results provide insight for controlling or preventing oxidative injury in different target tissues during diabetes.

ACKNOWLEDGMENTS

This work was supported by National Institute of Health Grant GM-34883-18 (to N.G.A.). Support from the research committee (Faculty of Medicine and Health Sciences, United Arab Emirates University; to H.R.) is also acknowledged.

We thank members of the Avadhani lab for valuable suggestions and criticisms.

REFERENCES