OXIDANT STRESS REGULATES BASAL ENDOTHELIN-1 PRODUCTION BY CULTURED RAT PULMONARY ENDOTHELIAL CELLS

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Divisions of 1Respiratory, Critical Care, and Occupational Pulmonary Medicine and 2Nephrology and Hypertension, Department of Medicine, Veterans Affairs Medical Center, University of Utah School of Medicine, Salt Lake City, Utah 84132; and 3Section of Pulmonary and Critical Care Medicine, Department of Medicine, Overton Brooks Veterans Affairs Medical Center, Louisiana State University School of Medicine, Shreveport, Louisiana 71130

Michael, John R., Boaz A. Markewitz, and Donald E. Kohan. Oxidant stress regulates basal endothelin-1 production by cultured rat pulmonary endothelial cells. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L768–L774, 1997.—Endothelin-1 (ET-1) is a pluripotent mediator that modulates vascular tone and influences the inflammatory response. Patients with inflammatory lung disorders frequently have elevated circulating ET-1 levels. Because these pathophysiological conditions generate reactive oxygen species that can regulate gene expression, we investigated whether the level of oxidant stress influences ET-1 production in cultured rat pulmonary arterial endothelial cells (RPAEC). Treatment with the antioxidant 1,3-dimethyl-2-thiourea (10 mM) or the iron chelator deferoxamine (1.8 µM) doubles basal ET-1 release. Conversely, exposing cells to H2O2 generated by glucose and glucose oxidase (0.1–10 µM) for 4 h causes a concentration-dependent decrease in ET-1 release. This effect occurs at concentrations of glucose oxidase that do not affect [3H]leucine incorporation or specific [3H]GTP release from RPAEC. Catalase prevents the decrease in ET-1 synthesis caused by glucose and glucose oxidase. Glucose and glucose oxidase decrease not only ET-1 generation but also ET-1 mRNA as assessed by semiquantitative polymerase chain reaction. Our results indicate that changes in oxidative stress can either up- or downregulate basal ET-1 generation by cultured pulmonary endothelial cells.

E N D O T H E L I N - 1 (ET-1) is a cytokine that can potently modulate pulmonary vascular tone, lung smooth muscle growth, and inflammatory processes. Proinflammatory effects of ET-1 include priming neutrophils, activating mast cells, stimulating oxygen radical production by macrophages, releasing growth factors from smooth muscle cells, and increasing adhesion molecule expression on endothelial cells (19). ET-1 also stimulates monocytes to produce interleukin-6, interleukin-8, and prostaglandin E2, all important in modulating immune responses (17). Besides the proinflammatory effects of ET-1, its ability to regulate vascular and airway tone, augment airway and vascular smooth muscle cell growth, and stimulate fibroblast growth, migration, and collagen synthesis indicate its potential to participate in the tissue reaction to ongoing inflammation (19).

Patients with a variety of inflammatory lung diseases, including the acute respiratory distress syndrome, have evidence for increased ET-1 production and reactive oxygen species generation in their lungs (7, 13, 21, 32). Because reactive oxygen species can regulate gene expression, we investigated the effect of oxidant stress on ET-1 production by cultured pulmonary endothelial cells.

MATERIALS AND METHODS

Reagents

We purchased phenol red-free (PRF) Dulbecco’s modified Eagle’s medium (DMEM), medium 199, liquid 10x Hanks’ balanced salt solution without phenol red (HBSS), Moloney murine leukemia virus reverse transcriptase (RT), 5x first strand buffer, dithiothreitol, Taq DNA polymerase, and random primers from Gibco Laboratories Life Technologies (Grand Island, NY); fetal bovine and bovine calf sera were from HyClone Laboratories (Logan, UT); tissue culture plastic ware was from Costar (Cambridge, MA); [3H]sodium chromate (32Cr), [3H]leucine, and the guanosine 3',5'-cyclic monophosphate (cGMP) 125I assay system were from American Life Science (Arlington Heights, IL); deferoxamine mesylate was from Ciba Pharmaceutical (Summit, NJ); 1,3-dimethyl-2-thiourea (DMTU) was from Aldrich Chemical (Milwaukee, WI); bovine liver thymol-free catalase and glucose oxidase type II-S derived from Aspergillus niger were from Sigma Chemical (St. Louis, MO); ET-1 radioimmunoassay (RIA) kits were from Peninsula Laboratories (Belmont, CA); bicinchoninic acid (BCA) protein assay reagents were from Pierce Chemical (Rockford, IL); dNTP set solution was from Pharmacia LKB Biotechnology (Alameda, CA); formamide and random pd(N)6 were from Boehringer Mannheim (Indianapolis, IN); deoxynucleotide triphosphates, Wizard polymerase chain reaction (PCR) primer sets, and PCR purification system, and RNASin ribonuclease (RNase) inhibitor were from Promega (Madison, WI); [α-32P]GTP was from ICN Biomedicals (Costa Mesa, CA); 2-mercaptoethanol was from Bio-Rad Laboratories (Richmond, CA); phenol saturated solution was from Amresco (Solon, OH); chloroform and calcium chloride were from Mallinckrodt (Paris, KY); ethyl alcohol was from Quantum Chemical (Tuscola, IL); and RNase-free presiliconized microcentrifuge tubes were from Intermountain Scientific (Bountiful, UT). All other reagents and chemicals were purchased from Sigma Chemical unless otherwise stated.

Cell Culture

Rat pulmonary artery endothelial cells (RPAEC) were initially isolated with microcarrier beads as previously described and were generously provided by Dr. Una Ryan (28). The isolated cells have a cobblestone morphology by light and electron microscopy and have been identified as endothelial cells by the presence of factor VIII antigen, by the expression of angiotensin-converting enzyme activity, and by the uptake of acetylated low-density lipoproteins. The cells were main-
tained in monolayer culture at 37°C and 5% CO₂, using Ryan's red medium (medium 199, 6.7% bovine calf serum, 3.3% fetal calf serum, 10⁻² M thymidine, 1.3 mM L-glutamine, 60 U/ml penicillin, 60 μg/ml streptomycin, and 20 μg/ml gentamicin). The cells were passed without the use of enzymes and were plated onto 6- or 24-well plates. All studies were performed at confluence.

Protocols for Measurement of ET-1

RPAEC were exposed to various treatments for 4 h in serum-free PRF-DMEM. To test the effect of antioxidants or an iron chelator on basal ET-1 release, cells were incubated with DMTU (10 mM), ascorbic acid (1 mM), deferoxamine, (1.8 μM), and urea (10 mM) for 4 h. To study the effect of H₂O₂ on ET-1 production, cells were exposed to varying concentrations of glucose oxidase (0, 0.1, 1, 2.5, and 10 μU/ml) in the presence of 5.6 mM glucose. We also studied the effect of treatment with catalase (2,000 U/ml), DMTU (10 mM), or urea (10 mM) in the presence of glucose and glucose oxidase (10 μU/ml) for 4 h.

ET-1 Assay

After incubation, the supernatants were removed, and ET-1 was measured using a commercially available RIA kit as previously described (15). The lower limit of sensitivity for ET-1 detection was 2 pg. Intra-assay variation was <9% and interassay variation was <15%. After extraction, the protein level in each well was measured from an aliquot of the solubilized cells using the BCA protein assay reagents. ET-1 measurements were expressed as picograms ET-1 per milligram total cell protein.

Protocol for Measuring Intracellular cGMP

We tested the effect of glucose and glucose oxidase exposure for 15 min and 4 h on intracellular cGMP levels. In the experiments with 15 min of exposure to glucose and glucose oxidase, confluent monolayers of pulmonary endothelial cells were equilibrated with Krebs buffer containing 0.1 mM 3-isobutyl-1-methylxanthine (IBMX) at 37°C for 30 min before adding the glucose oxidase. The Krebs buffer contained (in mM) 145 NaCl, 10 N-2-hydroxyethylpiperaazine-N'-2-ethanesulfonic acid, 5 glucose, 5 KCl, 1 Na₂HPO₄, 2.5 CaCl₂, and 1.8 MgSO₄·7H₂O at pH 7.3. The cells were then incubated in Krebs buffer with or without 10 μM glucose oxidase for 15 min at 37°C. Additional studies were performed in which the cells were incubated in media without IBMX and were exposed to varying concentrations of glucose oxidase (0, 2.5, 5, and 10 μU/ml) for 4 h. At the end of the experiments, the buffer was removed, and 1 ml of 100% ethanol-HCl solution (50 ml of 100% ethanol and 2 drops of concentrated HCl) was left on the cells overnight at 4°C.

cGMP Levels

cGMP levels were measured using a commercially available RIA kit. The cell homogenate was dried in a Speed-Vac (Savant, Farmingdale, NY). The pellet was resuspended in 500 μl of RIA assay buffer, and a 50 μl aliquot was incubated with 50 μl of 125I-labeled cGMP and 50 μl of anti-cGMP antiserum overnight at 4°C. Amerlex-M donkey anti-rabbit serum (200 μl) was then added for 10 min at room temperature. The samples were centrifuged at 3,000 revolutions/min for 10 min at 4°C, and the counts per minute in the pellet were determined. Total protein measurements were performed as outlined above, and the results were expressed as cGMP in femtomoles per milligram cell protein.

Measurement of ET-1 and β-Actin mRNA by RT-PCR Amplification of RNA

Isolation of RNA. Confluent cultures of RPAEC were incubated in growth media for 24 h, the supernatant was removed, and the cells were washed with medium 199 without serum. Cells were exposed for 4 h to serum-free PRF-DMEM alone or with PRF-DMEM containing glucose oxidase (10 μU/ml). The supernatant was removed, and the cells were overlaid with 4 M guanidinium isothiocyanate, 1% 2-mercaptoethanol, and 1% sarcosyl (pH 7.0). The cells were homogenized by several passages through a 25-gauge needle, and 1/10th volume of 2 M sodium acetate was added. The RNA was phenol/chloroform extracted, precipitated in isopropanol, washed in 70% ethanol, and suspended in Tris(hydroxymethyl)aminomethane (Tris)-HCl-EDTA. Each sample was quantified spectrophotometrically.

RT-PCR Amplification of RNA

RT-PCR amplification of RNA was performed as previously described by this laboratory (16). Five micrograms of total RNA from each sample were reverse transcribed by incubation with 250 pmol random hexamers, 400 units Moloney murine leukemia virus RT, 80 units RNasin, 2 mM deoxynucleotide triphosphates, 0.5 mM dithiothreitol, 75 mM KCl, 3 mM MgCl₂, and 50 mM Tris-HCl (pH 8.3, final volume 50 μl) for 3 h at 37°C. The RT was inactivated by heating for 10 min at 94°C. The cDNA was stored at 4°C.

The cDNA was amplified by PCR. Each sample was measured for ET-1 and β-actin in separate tubes using specific primers. The upstream and downstream primers for ET-1 were 5'-GCCAACAGCAGACAAAGACTCCCGAG-3' and 5'-GCTCTGTAGCTAATGTCGTT-3', respectively. These give a 247-bp fragment that is complementary to position 371–618 in rat ET-1 cDNA (29). PCR of rat genomic DNA yields a single 1,300-bp product, indicating that these primers span an intron. The upstream and downstream primers for β-actin were 5'-TGGAGAAGACGTATGAGCTGCTGT-3' and 5'-GTGCCACCAAGACACCTGTTGTGTT-3', respectively, which produces a single band corresponding to a 201-bp cDNA fragment. PCR of rat genomic DNA with the β-actin primers yields a 289-bp product that is complementary to position 2499–2788 in the β-actin gene, confirming that this primer set spans an intron. Finally, the ET-1 and β-actin product sequences were verified by Margaret Robinson in Dr. Ray White's laboratory at the University of Utah, using fluorescein primers and cycle sequencing.

PCR was performed by incubating 5 μl of sample cDNA with 50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl₂, 0.01% gelatin, 200 μM total dNTP, 2 units Taq DNA polymerase, 2.5% formamide, 0.15 μCi [32P]dCTP, and 100 pmol ET-1 or β-actin primers in 50 μl final volume (final pH 8.3 at room temperature). PCR using β-actin primers was carried out for 25 cycles (15 s at 94°C, 15 s at 65°C, 30 s at 72°C) after 1 min of early DNA denaturation at 94°C using a Perkin-Elmer Cetus 9600 GeneAMP PCR System. PCR using ET-1 was carried out for 30 cycles under identical conditions. Different primers were never combined in the same tube. Twenty microliters of the final PCR reaction were electrophoresed on a 7% nondenaturing polyacrylamide gel. Gels were stained with ethidium bromide, and the bands corresponding to the cDNA product were excised, mixed with scintillation cocktail, and the counts per minute were determined on a Beckman beta counter.

ET-1 and β-actin cDNA obtained from PCR of reverse transcribed RNA were used to generate standard curves. The cDNA was amplified by PCR, and the resultant amplified product was divided into small fractions that were, in turn,
reamplified. After removal of primers using Magic PCR Prep (Promega), the purity of the final product was confirmed by electrophoresis. At the end of the purification, the amount of standard cDNA was quantified spectrophotometrically. Standard curves for β-actin or ET-1 were made by simultaneously amplifying sample cDNA and, in separate tubes, standard cDNA (10⁻¹⁵ to 10⁻⁸ ng/tube). Every PCR amplification included a standard curve. All PCR consisted of simultaneous amplification (in separate tubes) of cDNA for ET-1 and β-actin. All results are expressed as femtograms ET-1 cDNA per picogram β-actin cDNA to control for the amount of RNA initially reverse transcribed. The accuracy of this semiquantitative PCR technique has been described previously in detail (8, 12).

**51Cr Release**

When monolayers of RPAEC were 70–80% confluent in six-well culture plates, they were incubated in 1.5 ml/well Ryan’s medium containing 1 µCi/ml 51Cr for 16–18 h. After labeling, the monolayers were washed three times with 1.5 ml/well PRF-DMEM, followed by incubation in 2 ml/well PRF-DMEM in the absence or presence of varying concentrations of glucose oxidase (0, 1, 2.5, 5, and 10 µU/ml). All of the experiments were performed at 37°C in an environment containing 5% CO₂ for 4 h unless otherwise indicated. The supernate was then removed and centrifuged, and the cell-free supernate was used. The cells were then dissolved in 2 ml/well of 0.1 N NaOH. The samples were counted for 1 min in a Packard 5530 gamma counter (Packard Instrument, Downers Grove, IL). The percent 51Cr release was calculated as follows: (S – BK)/(S – BK) + (C – BK)) × 100, where S represents the counts per minute in the cell-free supernatant, C represents the counts per minute remaining within the cells, and BK represents the background counts per minute. Specific 51Cr release represents the measured release minus baseline release.

**Leucine Incorporation Assay**

Protein synthetic rate was estimated by [3H]leucine incorporation into trichloroacetic acid (TCA)-precipitable protein. Confluent cultures of RPAEC in 24-well plates were exposed to PRF-DMEM alone or containing 1, 2.5, 5, or 10 µM glucose oxidase for 4 h at 37°C in 5% CO₂. [3H]leucine (1 µCi/ml) in PRF-DMEM was added for 10 min at 37°C, and the cells were rinsed two times with phosphate-buffered saline and were solubilized with 0.1% sodium dodecyl sulfate. Proteins were precipitated in 10% TCA at 4°C for 2 h in the presence of 2 mg bovine serum albumin. The precipitate was centrifuged and rinsed two times in 10% TCA, and the counts per minute were determined in a Beckman LS6000 β-counter (Beckman Instruments, Fullerton, CA).

**Measurement of H₂O₂ Produced by Glucose and Glucose Oxidase**

The amount of H₂O₂ produced by glucose and glucose oxidase in PRF-HBSS was determined. Experiments were performed in six-well plates. A 0.2-ml aliquot of the sample was mixed with 0.8 ml of 100 mM potassium phosphate buffer (pH 7.0). The final concentrations of the reagents in the 1 ml reaction mixture were 20 U/ml horseradish peroxidase (type II, 200 purpurogallic U/mg), 1.5 mM 4-aminoantipyrine, and 0.11 M phenol. Absorbance was measured at 510 nm with a Hitachi U-3210 spectrophotometer (Hitachi, Tokyo, Japan). The concentration of H₂O₂ was calculated using a molar extinction coefficient of 6.58 mM⁻¹·cm⁻¹.

**Statistical Analysis**

Data were analyzed by the Mann-Whitney test, unpaired Student’s t-test, or analysis of variance. Statistical significance was taken as P < 0.05. Values are presented as means ± SE.

**RESULTS**

**Effect of Antioxidants on Basal ET-1 Release**

Treatment of RPAEC with the antioxidant DMTU or the iron chelator deferoxamine doubles ET-1 production (Fig. 1). In contrast, ascorbate does not affect ET-1 release (Fig. 1). Because DMTU is a urea derivative, we used urea as a control, and it did not alter ET-1 production (Fig. 1).

**Effect of Oxidant Stress on Basal ET-1 Release**

Glucose and glucose oxidase selectively generate H₂O₂ in a dose-dependent fashion. Glucose oxidase (10 mM) and glucose (5.6 mM) produce ~195 µM H₂O₂ over 4 h. Exposure of cells to glucose oxidase and glucose causes a concentration-dependent decrease in ET-1 production (Fig. 2). The decrease in ET-1 synthesis occurs at doses that do not affect [3H]leucine incorporation into protein or specific 51Cr release (Table 1).

Catalase completely prevents the decrease in ET-1 production caused by glucose oxidase (Fig. 3). Exposing cells to both DMTU and glucose oxidase results in ET-1 levels that are intermediate between the effects of DMTU alone and glucose plus glucose oxidase by themselves [DMTU 209 ± 28% (SE) control, glucose and glucose oxidase 58 ± 8% control, and DMTU + glucose oxidase 121 ± 6% control]. Urea does not prevent the inhibitory effect of glucose oxidase on ET-1 release (Fig. 3).

![Fig. 1. Effect of an iron chelator and an antioxidant on basal release of endothelin-1 (ET-1) from rat pulmonary arterial endothelial cells. Cells were exposed to phenol red-free Dulbecco's modified Eagle's medium (DMEM) with or without deferroxamine (1.8 µM), 1,3-dimethyl-2-thiourea (DMTU, 10 mM), urea (10 mM), or ascorbate (1 mM) for 4 h. Values are expressed as %control (mean ± SE). Control ET-1 release was 48 ± 5 pg/mg cell protein; n = 12 for control, defereroxamine, and ascorbate; n = 8 for DMTU and urea. *P < 0.001 vs. control and †P < 0.01 vs. control.](http://ajplung.physiology.org/Download.html)
Oxidant exposure not only decreases ET-1 release (Fig. 2) but also ET-1 mRNA as assessed by semiquantitative PCR (Fig. 4). We tested the possibility that the H$_2$O$_2$ generated by glucose and glucose oxidase might increase intracellular cGMP levels and thereby reduce ET-1 release. Exposure of RPAEC to glucose and glucose oxidase for 15 min or 4 h, however, does not affect intracellular cGMP levels (Fig. 5).

**DISCUSSION**

The current study demonstrates that changes in oxidant stress regulate ET-1 generation. Treatment with the antioxidant DMTU or the iron chelator deferoxamine substantially increases baseline endothelial ET-1 release (Fig. 1), implying that basal oxidant stress tonically reduces ET-1 production. In contrast, increasing oxidant stress with glucose oxidase reduces in a dose-dependent manner ET-1 production by rat pulmonary arterial endothelial cells. H$_2$O$_2$ mediates this effect of glucose and glucose oxidase since catalase, which converts H$_2$O$_2$ to water and oxygen, completely abrogates this inhibitory effect on pulmonary endothelial ET-1 release. Previous work indicates that the superoxide anion or hydroxyl radical may reduce the level of immunoreactive ET-1 by inducing structural changes in the molecule (9, 34). We and others, however, have demonstrated that H$_2$O$_2$ by itself does not alter ET-1 immunoreactivity (9, 34). Although H$_2$O$_2$ may cause cytotoxicity, we found no evidence of generalized cell damage or dysfunction (as assessed by $[^3]$H)leucine incorporation into protein and $^{51}$Cr release at

### Table 1. Concentration effect of glucose oxidase on $[^3]$H)leucine incorporation and $^{51}$Cr release from rat pulmonary arterial endothelial cells

<table>
<thead>
<tr>
<th>Glucose Oxidase Concentration, mU/ml</th>
<th>$[^3]$H)leucine incorporation, %control</th>
<th>Specific $^{51}$Cr release</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 ± 5</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>1</td>
<td>95 ± 11</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>2.5</td>
<td>90 ± 9</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>91 ± 16</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>10</td>
<td>77 ± 7*</td>
<td>4 ± 1*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–12 for each point. Cells were exposed to glucose oxidase and glucose in phenol red-free Dulbecco's modified Eagle's medium for 4 h. *P < 0.01 vs. control value without glucose oxidase.
concentrations of glucose oxidase that substantially reduce ET-1 production. Additionally, exposure to glucose and glucose oxidase causes a concomitant decrease in ET-1 mRNA as assessed by semiquantitative PCR. Our finding that both endogenous and exogenous oxidants reduce ET-1 synthesis without frank cell damage provides strong evidence that short-term oxidative stress can inhibit pulmonary endothelial ET-1 production.

Catalase, which eliminates H$_2$O$_2$, completely prevents the effect of glucose and glucose oxidase (Fig. 3). Unlike catalase, DMTU does not alter H$_2$O$_2$ levels but likely scavenges subsequent radicals, such as the hydroxyl radical. Combining DMTU, glucose, and glucose oxidase results in ET-1 levels that are intermediate between the effects of DMTU alone versus glucose plus glucose oxidase by themselves. The intermediate ET-1 level seen with DMTU and glucose oxidase may reflect their net effect on intracellular oxidant stress. The ability of catalase, but not DMTU, to completely block the glucose oxidase-mediated decrease in ET-1 suggests that H$_2$O$_2$ by itself can lead to a decrease in ET-1 synthesis.

Relatively little data exist on oxidant regulation of ET-1 production. H$_2$O$_2$ (0.1–20 mM) has been reported to reduce ET-1 release by human umbilical vein endothelial cells (22). This study, however, did not assess cytotoxicity. In contrast, another group detected no effect of exogenous superoxide anion or H$_2$O$_2$ on ET-1 release from bovine pulmonary artery endothelial cells (24). These investigators found, however, that exogenous hydroxyl radical production augmented ET-1 release, but at concentrations that elicited cell damage (24). This report did not measure ET-1 mRNA levels. Other studies suggest that H$_2$O$_2$ may increase ET-1 mRNA levels in bovine aortic endothelial cells (ET-1 release was not measured; see Ref. 25) and ET-1 release by, and mRNA levels in, human renal mesangial cells (9). The reasons for these disparate results are unknown but may relate to differences in cell type, duration, or concentration of oxidant exposure, technique (most studies have failed to closely assess cytotoxicity), or other factors. As an example of the potential for cell-specific differences, one mechanism by which H$_2$O$_2$ may regulate gene expression is by activating transcription factors, such as nuclear factor (NF)-κB (18, 30). The ability of H$_2$O$_2$ to activate NF-κB, however, depends on the cell type studied (18, 30). Additionally, the capacity of H$_2$O$_2$ to induce NF-κB in endothelial cells varies with the vascular bed studied (1, 2). H$_2$O$_2$, for example, activates NF-κB in porcine aortic endothelial cells (1) but not in human umbilical vein endothelial cells (2).

The observation that oxidants can modulate vasoactive factor production has precedence. Reactive oxygen species, for example, can activate phospholipase A$_2$ and enhance arachidonic acid release and mediator production from pulmonary endothelium (5), stimulate thromboxane A$_2$ production in alveolar macrophages (31), differentially alter the synthesis of prostacyclin and 15-hydroxyeicosatetraenoic acid in coronary artery endothelium (4), increase platelet-activating factor synthesis in pulmonary endothelial cells (14), and augment cytokine-induced nitric oxide synthesis (20). Additionally, superoxide anion can combine with nitric oxide, thereby inactivating nitric oxide.

The mechanisms by which oxidants regulate ET-1 production remain speculative. Relatively few factors decrease ET-1 synthesis, and, frequently, they appear to act by enhancing cGMP accumulation (11). This mechanism does not explain the findings in the current study since glucose oxidase had no effect on RPAEC cGMP levels. Reactive oxygen species can affect a multitude of signaling pathways involved in regulating gene expression, including factors interacting with...
Oxidant stress regulates ET-1 production

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REFERENCES


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