Oxidative stress is intimately linked to the progression of many diseases such as preeclampsia (6), diabetes (39), and atherosclerosis (22). These conditions share widespread vascular abnormalities, which is likely the result of endothelial cell dysfunction. The mechanism(s) by which oxidative stress mediates endothelial cell function, and ultimately vascular reactivity, is not fully understood. However, one manifestation of oxidative stress, the formation of peroxynitrite, is hypothesized to be involved in the progression of a variety of diseases (3).

Under conditions of oxidative stress, the oxygen free radical, superoxide anion, will preferentially react with available nitric oxide rather than its endogenous neutralizer superoxide dismutase, thus increasing the formation of peroxynitrite in tissues (3). Peroxynitrite formation has been observed in the maternal vasculature of women with preeclampsia (29), in the placental vasculature of women with preeclampsia and women with diabetes (23), and in platelets from patients with diabetes (38) as well as in atherosclerotic plaques (2). The localization of peroxynitrite to these tissues suggests that it may be involved in altering vascular function. However, the direct effect(s) of peroxynitrite on endothelial cells is largely unknown.

The endothelium has an important function in maintaining vascular tone, which is mediated in part by the enzymes nitric oxide synthase (NOS) and prostaglandin H synthase (PGHS). NOS catalyzes the reaction of L-arginine to nitric oxide, whereas PGHS uses arachidonic acid as a substrate, forming prostaglandin H2 (PGH2). PGH2 is converted to vasoactive molecules, such as prostacyclin and thromboxane, via specific synthases (prostacyclin synthase and thromboxane synthase, respectively). Both NOS and PGHS have inducible isoforms (iNOS and PGHS-2), which are oxidant sensitive through the activation of nuclear factor-κB (NF-κB) (11, 30).

The involvement of NOS and PGHS in altering vascular function has been implicated in conditions characterized by oxidative stress (3, 36). Enhanced NOS activity in an environment of oxidative stress would result in scavenging of NO by superoxide anion, forming the potent pro-oxidant peroxynitrite, thus reducing nitric oxide bioavailability as a vasodilator (3). PGHS-2 also may mediate vascular dysfunction in conditions characterized by oxidative stress. For example, in carotid arteries and macrophages from patients with atherosclerosis, PGHS-2 expression is elevated (2, 36). Furthermore, the enzymatic activity of prostacyclin synthase is inhibited by the pro-oxidant peroxynitrite, which could result in reduced prostacyclin-mediated vasodilation (44). Thus endothelial cells maintain a balance of vasodilators and vasoconstrictors, in part through NOS- and PGHS-dependent mechanisms, which may be disrupted by oxidative stress. However,
the involvement of peroxynitrite in regulating these pathways has not been well elucidated.

The aim of this study was to determine the effect of peroxynitrite on endothelial cell function, focusing on pathways that modulate vessel reactivity. We hypothesized that peroxynitrite would increase the levels of iNOS and PGHS-2, through the activation of NF-κB, and decrease protein levels of prostacyclin synthase. The results from this study will help determine the mechanisms by which peroxynitrite may alter endothelial cell function, leading to the vascular abnormalities that are characteristic of patients with diabetes, atherosclerosis, and preeclampsia.

MATERIALS AND METHODS

Reagents. α-Minimum essential medium (α-MEM) with or without phenol red, horse serum, l-glutamine, trypsin, phosphate-buffered saline (PBS), and penicillin/streptomycin were purchased from GIBCO (Gaithersburg, MD). Nystatin, 3-morpholinosydnonimine N-ethylcarbamate (SIN-1), pyrrolidine dithiocarbamate (PDTC), gentamycin, kanamycin, and bovine serum albumin (BSA) were purchased from Sigma (Oakville, ON, Canada).

Endothelial cell culture. Bovine microvascular endothelial cells were selected for our study because they originate from a resistance-sized vascular bed and therefore intrinsically function to regulate systemic vascular resistance. When cultured, these cells show typical endothelial cell characteristics: growth in a monolayer, cobblestone morphology, and positive detection of von Willebrand factor. Cells were grown at 37°C in an atmosphere of 5% CO₂/95% air. Growth media (α-MEM) were supplemented with 1% l-glutamine, 10% horse serum, 5 μg/ml gentamycin, 20 μg/ml kanamycin, penicillin-streptomycin, and nystatin. At confluence, cells were plated into six-well plates (10⁶ cells/well) in a volume of 1 ml. After 24 h, cells were quiesced with phenol red-free media overnight. Before stimulation, plates were replaced with fresh media. After experimentation, the total protein content of the cells was determined using the Bradford Method (4) with BSA used as a standard.

Experimental protocol. Our experiments involved the use of SIN-1, a peroxynitrite donor that breaks down at physiological pH to form nitric oxide and superoxide anion simultaneously (12). SIN-1 is commonly used as an effective pharmacological agent for administering peroxynitrite at the level of the cell in a relatively stable form, as opposed to using authentic peroxynitrite, which is highly volatile and may decompose into inactive metabolites before reaching the cell. We determined the formation of peroxynitrite in our cells by detection of nitrotyrosine, using both Western immunoblots and immunocytochemistry (Fig. 1). Furthermore, to confirm that the effects of SIN-1 were mediated by peroxynitrite, we studied the effect of exogenous peroxynitrite (Cayman Chemicals, Ann Arbor, MI) as well as the effect of the nitric oxide donor sodium nitroprusside (Sigma) on endothelial cell function.

SIN-1 was prepared immediately before use and was tested initially in a range of doses (0.1–1.0 mM) and for various lengths of stimulation (2, 6, and 18 h) based on the literature (24, 25, 42). From these experiments, 0.5 mM and 6 h were selected as the most effective dose and length of stimulation and thus used for all subsequent experiments. Higher doses and longer incubation times resulted in an increased incidence of cell damage, as assessed by the lactate dehydrogenase (LDH) assay. After 24 h of quiescence, cells were stimulated for 6 h with 0.5 mM SIN-1 in the presence or absence of 0.5 mM PDTC, an NF-κB inhibitor (21). Treatments were performed in triplicate, and at least six separate experiments were conducted. At the end of the stimulation period, the supernatant was collected and stored at −80°C. Cells were rinsed with PBS, scraped, and collected into 200 μl of homogenizing buffer (25 mM Tris-Cl with 0.1% Triton X-100). Cells were then sonicated for ~5 s and stored at −80°C.

Nuclear protein preparation. At confluence, cells were quiesced for 24 h in a T-75 flask. Afterward, cells were stimulated with 0.5 mM SIN-1 for 4 h. This time frame was chosen on the basis of previous accounts of NF-κB activation (8). Nuclear protein was extracted from the cells by following the method described by Schreiber et al. (31). Briefly, cells were washed with 10 ml of Tris-buffered saline (TBS) and pelleted by centrifugation at 1,500 g for 5 min. The pellet was resuspended in 1 ml of TBS and pelleted again by spinning in a microfuge for 12,000 g for 15 s. The pellet was then resuspended in cold buffer A (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1.0 mM dithiorthiotol (DTT), and 0.5 mM phenylmethylsulfonyl fluoride (PMSF)). The cells were allowed to swell on ice for 15 min, and then 25 μl of 10% Nonident P-40 (Boehringer Mannheim, Mannheim, Germany) were added. The tube was vortexed for 10 s and centrifuged for 30 s at 12,000 g. The nuclear pellet was resuspended in 50 μl of ice-cold buffer B (20 mM HEPES, 0.4 M NaCl, 1.0 mM EDTA, 1.0 mM EGTA, 1.0 mM DTT, and 1.0 mM PMSF). The tube was agitated for 15 min at 4°C and then centrifuged for 5 min, and the supernatant containing the nuclear protein was stored at −80°C for future use.

Western immunoblotting. Western immunoblots were performed to measure levels of endothelial NOS (eNOS), inducible NOS (iNOS), PGHS-1, PGHS-2, prostacyclin synthase, and nitrotyrosine in endothelial cells and NF-κB in endothelial cell nuclei. Samples were diluted 1:4 with sample buffer (1.0 M Tris-Cl, glycerine, 2% SDS, 2% bromphenol blue, and β-mercaptoethanol) and boiled for 3 min. Protein from each sample was loaded (8 μg/well), and 10 μl of Kaleidoscope molecular weight marker (Bio-Rad, Hercules, CA) were loaded in a separate well to allow for accurate determination of molecular weight. eNOS and iNOS proteins were run on an 8% acrylamide gel, whereas PGHS-1, PGHS-2, prostacyclin synthase, NF-κB, and nitrotyrosine were run on a 10% gel. Protein was separated by electrophoresis at 120 V for 1.5 h in a mini-gel apparatus after the procedure of Laemmli (19).

After separation, protein was transferred onto a nitrocellulose membrane (Osmonics, Westborough, MA). Membranes
were incubated for 2 h at room temperature with primary antibody (rabbit polyclonal anti-PGHS-2, mouse monoclonal anti-PGHS-1, or anti-prostacyclin synthase (Cayman Chemicals, Ann Arbor, MI) or mouse monoclonal anti-iNOS, anti-nitrotyrosine (Transduction Laboratories, San Diego, CA) or overnight at 4°C [rabbit polyclonal anti-NF-κB p65 (Santa Cruz Biotechnology, Santa Cruz, CA)]. Secondary antibody was incubated for 1 h at room temperature [polyclonal anti-rabbit or anti-mouse horseradish peroxidase conjugated (Jackson ImmunoResearch, West Grove, PA)]. Afterward, protein bands were visualized using the enhanced chemiluminescence detection system (ECL; Amersham Life Sciences, Amersham, UK) and quantified using the Fluor-S MultiImager (Bio-Rad).

Immunocytochemistry. Cells were plated onto 22 × 22-mm glass coverslips and treated with 1.0 mM SIN-1 for 6 h. Afterward, the coverslips were fixed with 10% formalin-phosphate and stored overnight at 4°C. SIN-1-treated cells were incubated with 1:100 rabbit polyclonal anti-NF-κB (Santa Cruz) or 1:100 monoclonal anti-nitrotyrosine (Transduction Laboratories, San Diego, CA) or overnight at 4°C. A standard immunostaining protocol was followed by using the Vectastain ABC kit from Vector Laboratories (Burlingame, CA).

Total RNA isolation. After stimulation, cells were lysed directly in a six-well plate by adding 1 ml of Trizol reagent (GIBCO, Burlington, ON, Canada), passing the cell lysate through a pipette several times, and allowing the cells to sit for 5 min at room temperature. The lysate was transferred into Eppendorf tubes, 0.2 ml of chloroform was added, and each tube was vigorously shaken. Tubes were centrifuged for 3 min at 12,000 g at 4°C. The aqueous phase was transferred into a fresh tube, 0.5 ml of isopropanol was added, and tubes were incubated at room temperature for 10 min. Afterward, the tubes were centrifuged at 4°C at 12,000 g for 10 min. The RNA pellet was washed with 75% ethanol and resuspended in 50 μl of TE buffer (10 mM Tris-Cl, pH 7.5, and 1 mM EDTA, pH 8.0). The RNA concentration was determined by measuring the absorbency at 260 nm and calculating the optical density.

Real-time PCR. First-strand cDNAs were synthesized by incubating 1.0 μg of total RNA from endothelial cells with 1.0 μM random primers (Stratagene, La Jolla, CA), in a 20-μl reaction volume containing cDNA buffer (50 mM Tris-Cl, 75 mM KCl, 3 mM MgCl2, and 5 mM DTT), 2.5 mM deoxynucleotide triphosphates (dNTPs), and 1 unit of reverse transcriptase (Superscript II; GIBCO). The mixtures were incubated for 50 min at 48°C, followed by 5 min at 85°C. The PCR reaction contained 25 μl of the SYBR green Master Mix Kit (containing DNA polymerase, dNTPs, and MgCl2; Applied Biosystems) 100 nM sense and antisense primers, and 2 μl of the reverse transcriptase reaction. The temperature profile was 3 min at 95°C followed by 38 cycles of 30 s at 95°C and 30 s at 60°C (or 30 s at 53°C for cyclophilin, a housekeeping gene). The following primers were used for bovine prostacyclin synthase: forward, 5′-AGGATGAGAAGGAGAAGCTGG-3′, and reverse, 5′-GGGCTCCTCAGATGTTCTCCA-3′. The following primers were used for cyclophilin: forward, 5′-CACCGGTGTCTTCTGAGATCAC-3′, and reverse, 5′-CCA-GTGCTCAGAGCTCGAGAAG-3′.

Data analysis. Western immunoblot density was quantified using the Fluor-S Max Quantity One software (Bio-Rad). Protein values are percent control expressed as means ± SE. RNA content was measured and quantified using the I-Cycler software (Bio-Rad). The starting quantity of RNA was determined on the basis of the number of cycles required to amplify the cDNA above a set threshold. Starting quantity values (for both cyclophilin and prostacyclin synthase) were obtained from a standard curve, created from control RNA (ranging from 1.0 to 1,000 ng RNA), and then normalized to cyclophilin RNA for each experimental group. Data are standardized amount of RNA per amount of cyclophilin RNA, expressed as means ± SE. Either Student’s t-test (for comparison between 2 groups) or one-way ANOVA followed by a Fisher least significant difference post hoc test was used to determine statistical significance between groups (P < 0.05).

RESULTS

Effect of a peroxynitrite donor on iNOS protein levels and NF-κB activation in endothelial cells. We investigated the effect of peroxynitrite on endothelial cell enzymes that are important for vascular function and known to be oxidant sensitive. In this study SIN-1 was used as an endogenous peroxynitrite donor (24, 25, 42). Cell damage was assessed using a LDH assay and was negligible at all doses.

Untreated (control) endothelial cells showed low levels of constitutive iNOS expression, which also has been shown in a variety of other cell types (18, 26). SIN-1 treatment significantly increased iNOS protein levels compared with untreated endothelial cells (167 ± 24.2%, P < 0.05; Fig. 2A), whereas eNOS protein mass was not altered by SIN-1 treatment (Fig. 2B). Because we observed a SIN-1-induced increase in iNOS protein mass, we examined whether the NF-κB inhibitor PDTC could block this effect. Indeed, coinoculation of endothelial cells with SIN-1 in combination with PDTC prevented the increase in iNOS protein levels (78 ± 18.3%, P < 0.05).

To further substantiate the hypothesis that peroxynitrite is activating NF-κB, we investigated the effect of SIN-1 on endothelial cell nuclei. Because NF-κB activation requires protein translocation into the nucleus, we assessed nuclear NF-κB levels in the presence or absence of SIN-1 treatment. Untreated cells showed low levels of NF-κB in the nucleus, which is consistent with the constitutive expression of iNOS reported above. However, we found that treatment of cells with 0.5 mM SIN-1 significantly increased NF-κB protein mass in the nucleus (135 ± 10.0%, P < 0.05; Fig. 3). Furthermore, using immunocytochemistry as a qualitative tool, we found that NF-κB immunostaining was diffuse and localized mainly to the cytosol in untreated cells (Fig. 4A), whereas in SIN-1-treated cells, NF-κB immunostaining was prominently localized to the nucleus (Fig. 4B).

Effect of a peroxynitrite donor on PGHS-2 and prostacyclin synthase expression. We also studied the effect of peroxynitrite on the enzymes PGHS-1, PGHS-2, and prostacyclin synthase. Contrary to our hypothesis, treating endothelial cells with a peroxynitrite donor did not increase PGHS-2 protein levels (104 ± 8.7%, P < 0.05; Fig. 5A). The constitutively expressed PGHS-1 also was not altered by SIN-1 treatment (Fig. 5B). On the other hand, prostacyclin synthase, the enzyme downstream of PGHS that forms the vasodilator prostacyclin, was significantly inhibited by SIN-1.
treatment of the endothelial cells (78 ± 8.9%, P < 0.05; Fig. 6).

We further investigated whether this downregulation of prostacyclin synthase was at the level of transcription by using PCR to measure changes in mRNA expression in endothelial cells after SIN-1 treatment. The results (expressed as a ratio of standardized prostacyclin synthase to cyclophilin RNA) indicate that there was no difference between the amount of prostacyclin synthase RNA in control vs. SIN-1-treated endothelial cells (0.66 ± 0.2 vs. 0.59 ± 0.1, Fig. 7). Therefore, peroxynitrite does not seem to be altering prostacyclin synthase at the level of gene expression.

**Effect of authentic peroxynitrite on iNOS and prostacyclin synthase.** To confirm the cellular effects of peroxynitrite formed by SIN-1, we treated endothelial cells with 100 μM authentic peroxynitrite for 6 h. Our data show that the same changes in protein levels occur with peroxynitrite treatment as with SIN-1. For example, iNOS protein levels increased (133 ± 9%, P = 0.07; Fig. 8A), whereas prostacyclin synthase was significantly reduced (73 ± 5.5%, P < 0.05, Fig. 8B). Therefore, these observations support the results obtained with the peroxynitrite donor SIN-1 and strongly suggest that peroxynitrite is capable of mediating changes in protein levels in the endothelium.

**Fig. 2.** Western immunoblot analysis of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) protein mass. Equal amounts of protein extracts (8 μg) were separated on a 8% SDS-PAGE gel. A: representative immunoblot of iNOS: lanes 1 and 2, unstimulated cells; lanes 3 and 4, endothelial cells treated with 0.5 mM SIN-1 for 6 h. B: representative immunoblot of eNOS: lanes 1 and 2, unstimulated cells; lanes 3 and 4, cells treated with 0.5 mM SIN-1 for 6 h. Summary data are percent control expressed as means ± SE of 6 separate experiments. *P < 0.05 vs. control.

**Fig. 3.** Western immunoblot analysis of nuclear factor (NF)-κB protein mass in endothelial cell nuclei. Nuclear protein was extracted as previously described (17). Equal amounts of protein (10 μg) were separated on a 10% SDS-PAGE gel. Representative immunoblot of 1 experiment: lane 1, unstimulated endothelial cell nuclei; lane 2, endothelial cell nuclei treated with 0.5 mM SIN-1 for 4 h. Summary data are percent control expressed as means ± SE of 4 separate experiments. *P < 0.05 vs. control.

**Fig. 4.** Immunocytochemistry of endothelial cells stained for NF-κB. Cells were plated onto glass coverslips and treated with 0.5 mM SIN-1 for 4 h. Cells were then incubated overnight at 4°C with 1:100 anti-NF-cob p65 polyclonal antibody. A: representative slide of unstimulated endothelial cells. B: representative slide of endothelial cells stimulated with 0.5 mM SIN-1. C: immunostaining for nonspecific IgG, negative control.
DISCUSSION

This study shows that peroxynitrite alters endothelial cell pathways, which are important for vascular function. We have shown, for the first time, that the peroxynitrite donor SIN-1 significantly upregulates iNOS protein mass in endothelial cells. This novel finding has important implications for the development of vascular dysfunction, especially in diseases characterized by oxidative stress. By increasing iNOS levels in the vasculature, peroxynitrite is capable of stimulating large quantities of nitric oxide, which, in an environment of oxidative stress, will rapidly be scavenged, forming more peroxynitrite and culminating in a vicious positive feedback cycle (Fig. 9). Thus the detrimental effect(s) of peroxynitrite may be exacerbated by this feed-forward mechanism, in addition to reducing the bioavailability of nitric oxide as a potent vasodilator.

A role for iNOS in vascular pathophysiology is supported by data in the spontaneously hypertensive rat, showing a reduction in the development in hypertension by a specific iNOS inhibitor (13). Furthermore, patients with atherosclerosis show elevated iNOS in coronary plaques, which colocalizes with nitrotyrosine, a marker for peroxynitrite (7). Our data using endothelial cells suggest that peroxynitrite induces iNOS protein. However, it also has been reported that peroxynitrite inhibits iNOS activity in lung epithelial cells (27). This is one potential mechanism by which peroxynitrite may counterbalance the positive feedback cycle, as discussed above. Moreover, the increased expression of iNOS by peroxynitrite also may help explain paradoxical observations in women with preeclampsia; while nitric oxide-mediated vasorelaxation is reduced in maternal blood vessels (1), there is reportedly either no change (5) or elevated (32) levels of nitric oxide metabolites. Thus the impaired endothelium-dependent relaxation, with no comitant decrease in nitric oxide per se, suggests that there is increased nitric oxide scavenging by oxygen free radicals. Indeed, we have reported evidence of peroxynitrite formation in the vasculature of women with preeclampsia (29).

Currently, little is known about the effect(s) of peroxynitrite on cellular signaling pathways; therefore, we studied the mechanism by which peroxynitrite is altering the expression of iNOS. Because NF-κB is an oxidant-sensitive transcription factor, we investigated whether the pro-oxidant peroxynitrite could potentially activate NF-κB. Using Western blot analysis of cell nuclei, a technique used by others (34), we showed that SIN-1 treatment was capable of increasing NF-κB protein mass. Equal amounts of protein extracts (8 μg) were separated on a 10% SDS-PAGE gel. Representative immunoblot of 1 experiment: lane 1, unstimulated endothelial cells; lanes 2 and 3: endothelial cells treated with 0.5 mM SIN-1 for 6 h. Summary data are percent control expressed as means ± SE of 6 separate experiments.

Fig. 6. Western immunoblot analysis of prostacyclin synthase protein mass. Equal amounts of protein extracts (8 μg) were separated on a 10% SDS-PAGE gel. Representative immunoblot of 1 experiment: lane 1, unstimulated endothelial cells; lanes 2 and 3: endothelial cells treated with 0.5 mM SIN-1 for 6 h. Summary data are percent control expressed as means ± SE of 6 separate experiments. *P < 0.05 vs. control.
levels in the endothelial cell nuclei. These data provide strong correlative evidence that peroxynitrite can induce nuclear translocation of NF-κB. Our results are further supported by immunocytochemical data, illustrating diffuse cytosolic immunostaining in control cells but intense nuclear staining in SIN-1-treated cells. Furthermore, the peroxynitrite-induced increase in iNOS protein levels was diminished in the presence of a pharmacological NF-κB inhibitor, PDTC. Together, our data support the hypothesis that peroxynitrite is a potential activator of NF-κB in endothelial cells.

The activation of NF-κB by peroxynitrite has been implicated in other cell types. In lipopolysaccharide-stimulated human whole blood, peroxynitrite can induce interleukin-8 gene expression, which is blocked by the NF-κB inhibitor PDTC (9). Additionally, during hepatocyte isolation, NF-κB activation can be inhibited by both NOS blockade (nitro-L-arginine methyl ester) or through administration of the antioxidant/peroxynitrite scavenger Trolox (28). In rat lung epithelial cells, SIN-1 activated a NF-κB-dependent luciferase reporter vector after 8 h of stimulation (14). Therefore, the pro-oxidant peroxynitrite is likely able to activate NF-κB.
κB, and our data extend this mechanism to include endothelial cells.

Peroxynitrite previously has been shown to activate PGHS (10). In our study, we investigated whether peroxynitrite could increase protein expression. We were unable to detect a statistically significant increase in PGHS-2 levels in endothelial cells treated with SIN-1. Because our data suggest that peroxynitrite activated NF-κB in the endothelial cells, it is possible that other nuclear factors are necessary for full activation of PGHS-2 expression, including high-mobility group protein I (Y), which is required for complete upregulation of PGHS-2 under hypoxic conditions (15). Therefore, the formation of peroxynitrite in vivo may act in concert with other physiological events that were not present in our experiments to increase PGHS-2 production in endothelial cells.

Prostacyclin synthase, an enzyme downstream of PGHS, was significantly reduced in endothelial cells treated with SIN-1. However, this effect of peroxynitrite on prostacyclin synthase does not seem to be at the level of transcription, because we did not observe any effect of SIN-1 on prostacyclin synthase mRNA expression. One possible mechanism by which peroxynitrite may decrease prostacyclin synthase protein mass is through increasing proteolytic degradation of the enzyme. Zou et al. (45) have illustrated that peroxynitrite inhibits the activity of prostacyclin synthase through a tyrosine nitration-dependent mechanism. Furthermore, recent findings show that nitration of tyrosine residues in certain proteins can increase proteolytic degradation by enhanced targeting to the proteasome (33). Thus prostacyclin synthase is a potential candidate for enhanced proteolytic degradation by peroxynitrite-induced tyrosine nitration.

The effects of peroxynitrite on the PGHS pathway illustrate another important mechanism by which peroxynitrite can alter endothelial cell function. We have shown, for the first time, that peroxynitrite is capable of inhibiting prostacyclin synthase at the level of protein, in addition to the well-documented effects of peroxynitrite on prostacyclin synthase activity (Fig. 9) (45). Furthermore, peroxynitrite has been shown to increase PGHS activity (10). Therefore, in endothelial cells, peroxynitrite formation shifts the balance away from the vasodilator prostacyclin and toward the vasoconstrictors PGH2 and thromboxane. Again, this theory correlates well with data from women with preeclampsia, who show reduced levels of prostacyclin and elevated levels of thromboxane metabolites (41). Furthermore, in atherosclerosis, PGHS-2 is highly expressed and colocalizes with iNOS and nitrotyrosine (2). Finally, PGHS-2-mediated vasoconstriction is more pronounced in aging (37), which also is a state of oxidative stress in which peroxynitrite levels have been shown to be elevated (40).

Although it has been proposed that peroxynitrite may have physiological effects similar to those of nitric oxide in certain biological systems (20), our results are likely specific to peroxynitrite. There are reports illustrating nitric oxide-dependent modulation of NF-κB; however, most of these show an inhibitory effect of nitric oxide on NF-κB activity (16, 35). Furthermore, for comparative purposes, we observed that a nitric oxide donor did not increase iNOS in our endothelial cells (data not shown), whereas authentic peroxynitrite induced changes in protein levels similar to those of SIN-1. Therefore, in an endothelial cell culture model, we find that SIN-1 is an effective, endogenous peroxynitrite donor that can alter intracellular enzyme expression.

In conclusion, it is well documented that peroxynitrite is elevated in the vasculature of many conditions characterized by oxidative stress. Peroxynitrite is increased in the maternal vasculature of women with preeclampsia (29) and in patients with diabetes as well as patients with atherosclerosis (2, 38). In addition, in the placental blood vessels of women with diabetes and women with preeclampsia, peroxynitrite levels are increased (23), which correlates with the vascular dysfunction and reduced placental perfusion (17). Furthermore, in ischemia-reperfusion injury, peroxynitrite mediates coronary vasospasm in bovine coronary arteries (43). Although peroxynitrite is hypothesized to be involved in the vascular pathophysiology of these conditions, few studies have focused on the effects of peroxynitrite on endothelial cell pathways that regulate vessel function. This study shows that peroxynitrite is a novel mediator of endothelial cell function. By activating NF-κB, thus increasing the expression of iNOS and inhibiting prostacyclin synthase, peroxynitrite can contribute to the altered vascular reactivity in a variety of conditions in which the clinical manifestations are mediated by oxidative stress.

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