Vanadyl Sulfate Inhibits NO Production via Threonine Phosphorylation of eNOS

Zhuowei Li,[1] Jacqueline D. Carter,[2] Lisa A. Dailey,[2] and Yuh-Chin T. Huang[2]

[1]Center for Environmental Medicine, Asthma and Lung Biology, University of North Carolina, Chapel Hill, North Carolina, USA; [2]National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

Exposure to excessive vanadium occurs in some occupations and with consumption of some dietary regimens for weight reduction and body building. Because vanadium is vasoactive, individuals exposed to excessive vanadium may develop adverse vascular effects. We have previously shown that vanadyl sulfate causes acute pulmonary vasoconstriction, which could be attributed in part to inhibition of nitric oxide production. In the present study we investigated whether NO inhibition was related to phosphorylation of endothelial nitric oxide synthase (eNOS). $VOSO_4$ produced dose-dependent constriction of pulmonary arteries in isolated perfused lungs and pulmonary arterial rings and a right shift of the acetylcholine-dependent vasorelaxation curve. $VOSO_4$ inhibited constitutive as well as A23187-stimulated NO production. Constitutive NO inhibition was accompanied by increased Thr^{495} (threonine at codon 495) phosphorylation of eNOS, which would inhibit eNOS activity. Thr^{495} phosphorylation of eNOS and inhibition of NO were partially reversed by pretreatment with calphostin C, a protein kinase C (PKC) inhibitor. There were no changes in Ser^{1177} (serine at codon 1177) or tyrosine phosphorylation of eNOS. These results indicate that $VOSO_4$ induced acute pulmonary vasoconstriction that was mediated in part by the inhibition of endothelial NO production via PKC-dependent phosphorylation of Thr^{495} of eNOS. Exposure to excessive vanadium may contribute to pulmonary vascular diseases. *Key words:* boilermakers, protein kinase C, pulmonary hypertension, vanadium. *Environ Health Perspect* 112:201–206 (2004). doi:10.1289/ehp.6477 available via *http://dx.doi.org/* [Online 22 October 2003]

Vanadium was first discovered in 1971 as a trace element that is essential for normal growth. Since then, vanadium has been found to regulate the activity of various enzymes that induce pronounced changes in metabolic functions. Some of these enzymes include Na^+,K^+ ATPase, acid phosphatase, alkaline phosphatase, and protein tyrosine phosphatases. Its vanadyl (IV) derivative, vanadyl sulfate ($VOSO_4$), has a potent effect on transportation and metabolism of glucose.

Humans may be exposed to excessive vanadium in several situations, for example, over-consumption of vanadium-rich foods (e.g., some wines and seafood) (Bu-Olayan and al-Yakoob 1998; Teissedre et al. 1998), ingestion of certain dietary regimens, and inhalation of vanadium-rich environmental pollutants in certain occupations (Levy et al. 1984; Woodin et al. 1999, 2000). These occupations include boilermakers and power plant workers, who are often exposed to high levels of vanadium-rich compounds at work. Vanadium exposures have been associated with acute upper and lower respiratory symptoms in these workers (Levy et al. 1984; Woodin et al. 1999, 2000).

Exposure to excessive vanadium may also produce adverse effects on the cardiovascular system. Rats fed vanadium-containing drinking water for 2 months developed pulmonary hypertension (Susic and Kentera 1986). $VOSO_4$ and vanadium-rich pollutant dust (residual oil fly ash) produced an acute increase in pulmonary artery pressure in buffer-perfused rabbit lungs (Huang et al. 2002) and vasoconstriction in isolated rat aortic rings (Cadene et al. 1997). In boilermakers, the vanadium concentration in particulate air was associated with increases in the heart rate variability index, indicating alterations in cardiac autonomic functions (Magari et al. 2002). The mechanisms by which $VOSO_4$ induced cardiovascular adverse effects, however, are unclear.

We have shown previously that $VOSO_4$ induced acute pulmonary vasoconstriction in perfused rabbit lungs and that this effect was associated with inhibition of nitric oxide production (Huang et al. 2002). The inhibition occurred within 10 min after the administration of $VOSO_4$, indicating the mechanisms for the inhibition may be at the levels of nitric oxide synthase (NOS) activity. Because phosphorylation of endothelial NOS (eNOS) is an important posttranslational regulatory mechanism for its activity, in this study we investigated the effects of $VOSO_4$ on the phosphoryation of threonine (Thr) residues of eNOS, which would inhibit its enzymatic activity. The hypothesis was tested in isolated perfused lungs, isolated pulmonary artery rings, and pulmonary artery endothelial cells.

Materials and Methods

Reagents and chemicals. Vanadyl sulfate ($VOSO_4$), zinc sulfate, copper, zinc superoxide dismutase (Cu,ZnSOD), PAPANONOate, calphostin C, and A23187 (a calcium ionophore) were obtained from Sigma Chemical Co. (St. Louis, MO). Human pulmonary artery endothelial cells and endothelial cell growth medium were purchased from Cell Applications, Inc. (San Diego, CA), and the monoclonal antibodies to phosphothreonine and phosphoserine were purchased from Alexis Corp. (San Diego, CA). Rabbit anti-phosphothreonin(495) (phospho-Thr^{495}) eNOS, mouse anti-phosphotyrosine (PY99), rabbit anti-eNOS (NOS3, N-20), rabbit anti-p-Akt1(serine at codon 473), goat anti-human Akt, protein A agarose, and secondary antibodies (both horseradish peroxidase-conjugated and fluorescein-conjugated) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Molecular mass standards, polyacrylamide, and buffers were purchased from Bio-Rad (Richmond, CA). We obtained ECL Western blotting detection reagents from Amersham Pharmacia Biotechnology (Piscataway, NJ).

Isolated perfused rabbit lung. The isolated buffer-perfused lung (IPL) model using male New Zealand white rabbits (Robinson's Farm, Clemens, NC) weighing 2.5–3.0 kg has been described previously (Huang et al. 2002). Briefly, the pulmonary artery and the left atrium were cannulated for monitoring the mean pulmonary artery pressure and the left atrial pressure, respectively. A reservoir was connected with the perfusion circuit downstream from the left atrium and was suspended freely from a force displacement transducer (Model FT100; Grass Instrument

Company, Quincy, MA) for monitoring the weight gain of the lung. The reservoir was placed at the lowest portion of the lung to maintain a left atrial pressure of zero. The perfusion circuit also consisted of a roller pump, a bubble trap, and a heat exchanger connected with Tygon tubing. The volume of the system was approximately 250 mL. The trachea was also cannulated for monitoring tracheal pressure. The lung was ventilated with 21% O_2 + 5% CO_2 through the tracheostomy using an animal respirator delivering 30 breaths/min at 2–3 cm H_2O positive end-expiratory pressure. The tidal volume was adjusted to achieve a peak tracheal pressure of 7–10 mmHg (~20 mL). All pressures and weight gain were transmitted to a 4-channel analog-to-digital converter and amplifier connected to a personal computer equipped with data acquisition software (DATAQ; DATAQ Instruments, Inc. Akron, OH). After the pulmonary circulation was washed free of blood with Krebs-Henseleit (KH)-3% albumin buffer (82.8 mM sodium chloride, 4.7 mM potassium chloride, 2.4 mM monobasic potassium phosphate, 25 mM sodium bicarbonate, 1.2 mM magnesium sulfate, 2.7 mM calcium chloride, 11.1 mM dextrose, and 3% w/v bovine serum albumin, fraction V at a pH of 7.3–7.4), a recirculated perfused lung system with a flow of 100 mL/min was established. The lung was then allowed to stabilize for 10–15 min. Lungs with visible leaks and/or high pulmonary artery pressure (> 20 mmHg) during this period were excluded.

Measurements of microvascular pressure and pulmonary vascular resistance. At the end of the experiments, we measured the microvascular pressure (P_{mv}) using the double occlusion technique (Linehan et al. 1982). Pulmonary vascular resistance was partitioned into upstream (arterial; R_a) and downstream (venous; R_v) resistance using the following equations:

$$R_a = \frac{\left(P_{pa} - P_{mv}\right)}{Flow}$$

$$R_v = \frac{\left(P_{mv} - P_{la}\right)}{Flow}$$

where P_{la} is left atrial pressure. In our study, the flow was a constant at 100 mL/min, and P_{la} was set at zero.

Isolated rat pulmonary artery ring. Segments of the right and left main pulmonary arteries of Sprague-Dawley rats (250–350 g) approximately 2–3 mm in length were removed carefully and placed in KH buffer. The artery segments were then suspended in the Radnoti 4-unit organ bath system (Glass Technology Inc., Monrovia, CA). Each reservoir held 20 mL of KH buffer

and was bubbled constantly with a 21% O_2 + 5% CO_2 gas mixture. After a 10–15 min stabilization period, the baseline tension of the rings was adjusted to 1 g before all experiments. In acetylcholine experiments, all rings were preconstricted with 1 µM phenylephrine. The degree of relaxation and constriction was expressed as a percentage of constriction induced by 1 µM phenylephrine.

Cultured human pulmonary artery endothelial cells (HPAEC). HPAEC (Cell Applications, Inc. San Diego, CA) were cultured in the endothelial cell growth medium in 6-well plates and were used during passages 3–5. HPAEC were isolated from normal human pulmonary arteries and have been shown to respond to a variety of vasoactive substances (Ryan et al. 1976a; 1976b).

Measurements of nitrite/nitrate. To determine the production of NO, we measured nitrite/nitrate concentrations in serial perfusate samples and in the culture medium of HPAEC by the Griess reaction using a colorimetric nitrite/nitrate assay kit (Nitric Oxic Assay Kit; R &D Systems, Minneapolis, MN).

Immunoprecipitation and Western blot. Confluent HPAEC cells were washed once with ice-cold phosphate-buffered saline (PBS) and then lysed with RIPA buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS in PBS, pH 7.4) containing 0.1 mM $VOSO_4$ and protease inhibitors

(0.5 mg/mL aprotinin, 0.5 mg/mL E-64, 0.5 mg/mL pepstatin, 0.5 mg/mL bestatin, 10 mg/mL chymostatin, 0.1 ng/mL leupeptin). Cells were scraped up and passed through a 21-gauge needle 3–5 times. The cell samples were then centrifuged at 3,000 × g for 10 min at 4°C and the supernatants were collected. For immunoprecipitation, the supernatant samples were pre-cleared with protein A-agarose for 30 min at 4°C and centrifuged at 10,000 × g for 1 min. A rabbit anti-human eNOS antibody (1 µg) was added to the supernatant (250 µg) and incubated for 2 hr at 4°C, followed by another 2-hr incubation with 20 µL protein A-agarose at 4°C with end-to-end rotation. After centrifugation at 10,000 × g for 1 min, the pellets were collected, washed twice with 500 µL ice-cold RIPA buffer and once with ice-cold PBS, resuspended in electrophoresis sample buffer, and boiled for 5 min. Supernatant samples were then separated by 7.5% SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. The blots were blocked with 3% gelatin in TBS for 2 hr at room temperature and probed with antibodies against phosphothreonine, phosphoserine, or phosphotyrosine (PY99) overnight at 4°C. This was followed by incubation with appropriate HRP-conjugated secondary antibodies. To determine the amount of eNOS, the blot was stripped and reprobed with a rabbit
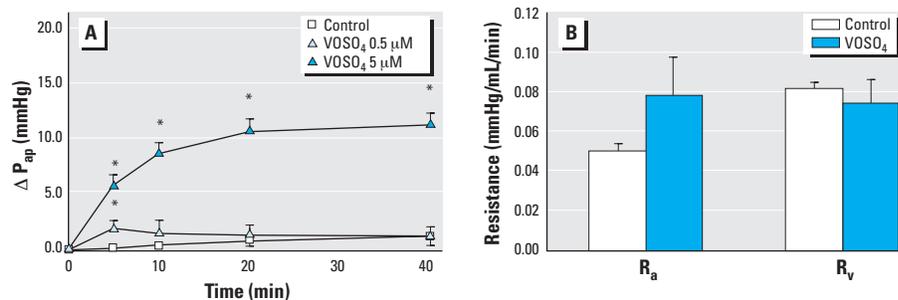

Figure 1. Effects of $VOSO_4$ on IPL. (*A*) Changes in mean P_{pa} in IPL treated with $VOSO_4$, which was injected into the pulmonary artery and monitored for 40 min. (*B*) Pulmonary vascular resistance [upstream (R_a) and downstream (R_v)] in IPL treated with 5 µM $VOSO_4$.
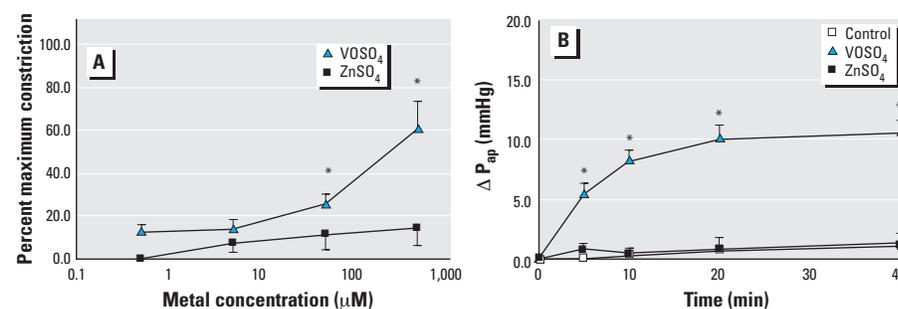*$p < 0.05$ versus control; $n = 7$–9.

Figure 2. $VOSO_4$-induced constriction in (*A*) rat pulmonary artery rings (expressed as percentage of vasoconstriction produced by 1 µM phenylephrine, with $ZnSO_4$ used as a negative control) and (*B*) isolated perfused lung (expressed as the change in mean P_{pa}).
*$p < 0.05$ versus $ZnSO_4$.

anti-human eNOS antibody and HRP-conjugated goat anti-rabbit IgG. Bands were detected using chemiluminescence reagents and film.

Immunocytochemical staining of phosphorylated eNOS. HPAEC grown on slides were treated with $VOSO_4$ (50 μM) for 10 min. The cells were fixed with 4% paraformaldehyde and blocked with 3% donkey serum. The fixed cells were probed with an antibody against phospho-Thr[495] eNOS overnight at 4°C followed by incubation with a fluorescein-conjugated secondary antibody for 30 min. Images were examined under a fluoresence microscope equipped with a fluorescein isothiocyanate filter. Photographs were taken using a digital camera system (Nikon Microphot-SA; Southern MicroInstruments, Marietta, GA) and imaging software (ACT-1, version 2.10, Nikon).

Statistical analysis. We present data as mean ± SE. Time-series data were analyzed using repeated measures analysis of variance (ANOVA). We evaluated differences among groups at selective time points using one-way ANOVA followed by the Fisher protected least-significant-difference post-hoc test. The statistical analysis was performed using Statview software (SAS Institute Inc., Cary,

NC). We report actual *p*-values where statistical analyses were performed. A *p*-value of < 0.05 was considered statistically significant.

Results

$VOSO_4$-induced pulmonary artery constriction. The baseline mean pulmonary artery pressure of the IPL was 13 ± 1 mmHg. Vanadyl sulfate at 0.5 μM injected into the pulmonary artery produced a transient increase in pulmonary artery pressure, which peaked at 5 min and returned to the baseline by 40 min (Figure 1A). At 5 μM, $VOSO_4$ produced an acute and persistent increase in pulmonary artery pressure, which plateaued at approximately 24 mmHg after 20 min. At 50 μM, $VOSO_4$ produced such severe pulmonary hypertension and lung edema that the IPL could not be sustained for 40 min. All subsequent experiments in IPL were thus performed with 5 μM of $VOSO_4$.

The increase in pulmonary vascular resistance induced by $VOSO_4$ was primarily due to constriction of upstream pulmonary vessels (Figure 1B). $VOSO_4$ produced vasoconstriction in pulmonary artery rings (Figure 2A) and IPL, whereas zinc sulfate had little vasoactive effect (Figure 2B).

$VOSO_4$ shifted the acetylcholine-induced vasorelaxation curve to the right (Figure 3A) in

pulmonary artery rings, which is consistent with a loss of vasodilatory activity of NO. Inhibition of NO production by an NOS inhibitor, L-NAME, increased pulmonary artery pressure in our IPL system (Huang et al. 1997). $VOSO_4$-induced pulmonary hypertension was attenuated by an NO donor, PAPANONOate (1 mM) and Cu,ZnSOD (Figure 3B and 3C).

$VOSO_4$ inhibited NO production. In control IPL, NO production measured as nitrite/nitrate accumulation in the perfusate increased gradually over time; the total increase was 3.4 ± 0.8 μM during 40 min of perfusion (Figure 4A). $VOSO_4$ (5 μM) inhibited nitrite/nitrate accumulation, which occurred as early as 10 min after the treatment. At 40 min, $VOSO_4$ inhibited nitrite/nitrate accumulation by approximately 80%. The average change in nitrite/nitrate concentration after 40 min of perfusion was decreased to 0.7 ± 0.6 μM ($p = 0.003$ vs. control) (Figure 4A). In HPAEC, $VOSO_4$ inhibited constitutive and A23187-stimulated NO production (Figure 4B and 4C).

Effect of $VOSO_4$ on eNOS phosphorylation. In HPAEC, $VOSO_4$ induced an approximately 2-fold increase in Thr phosphorylation of eNOS (Figure 5A and 5B). Immunostaining
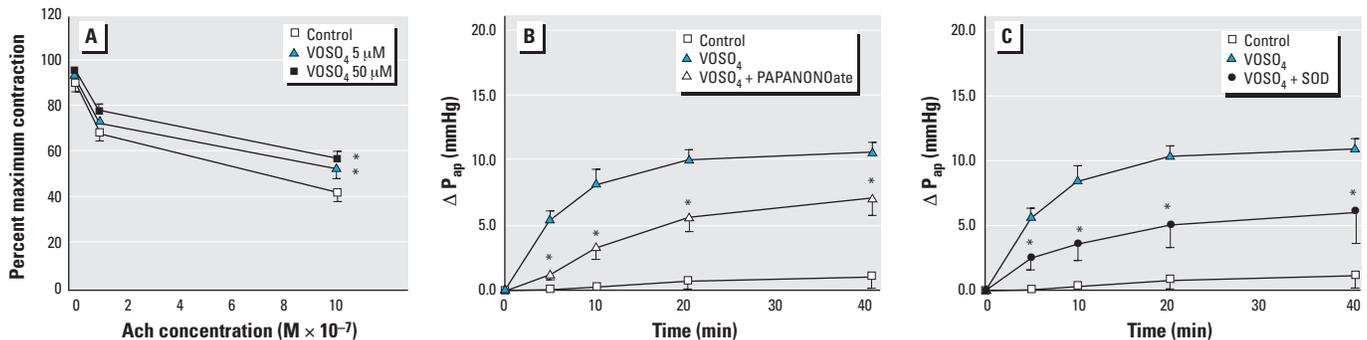

Figure 3. Effects of $VOSO_4$ on artery rings. (*A*) Inhibition of acetylcholine (Ach)-induced vasorelaxation by $VOSO_4$ ($n = 10–15$ separate experiments). (*B*) Attenuation of $VOSO_4$-induced pulmonary hypertension by 1 mM PAPANONOate, an NO donor ($n = 5–9$). (*C*) Attenuation of $VOSO_4$-induced pulmonary hypertension by 100 U/mL Cu,ZnSOD ($n = 5–9$). See "Materials and Methods" for details of experiments.
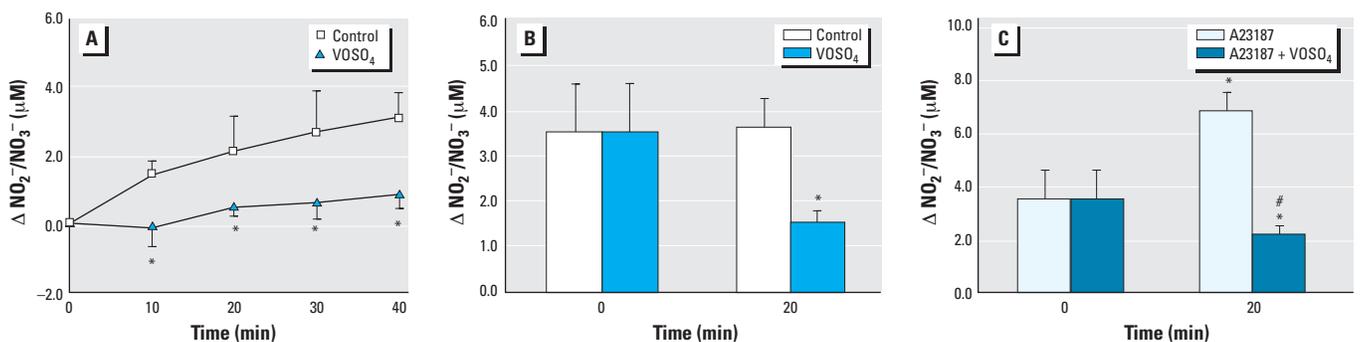*$p < 0.05$ versus control in (*A*) and $p < 0.05$ versus $VOSO_4$ for (*B*) and (*C*).

Figure 4. Inhibition of NO production measured as the accumulation of nitrite/nitrate concentration. (*A*) Inhibition of NO production in the perfusate of IPL after treatment with 5 mM $VOSO_4$ ($n = 7–9$). (*B*) Inhibition of constitutive NO production by 50 μM $VOSO_4$ in HPAEC ($n = 6$). (*C*) Inhibition of A23187-stimulated NO production by $VOSO_4$ in HPAEC pretreated simultaneously with $VOSO_4$ and 1 μM A23187 ($n = 6$). The baseline nitrite/nitrate concentration for HPAEC in (*B*) and (*C*) was pooled from all 12 experiments.
*$p < 0.05$ versus control in (*A*) and (*B*) and $p < 0.05$ versus time 0 in (*C*). #$p < 0.05$ vs. A23187 alone.

with a phospho-Thr[495] eNOS antibody was also increased in $VOSO_4$-treated HPAEC (Figure 5C). Thr[495] was located in the calcium–calmodulin binding domain of eNOS, and phosphorylation of this Thr residue has been shown to inhibit eNOS activity (Fulton et al. 2001; Govers and Rabelink 2001). These findings are consistent with the $VOSO_4$-induced inhibition of NO production stimulated by A23187, which enhances calcium binding to calmodulin. $VOSO_4$ did not alter phosphorylation on Ser[1177] tyrosine residues of eNOS or Akt1 phosphorylation (data not shown).

Effects of protein kinase C inhibitor on $VOSO_4$ effects. The $VOSO_4$-induced pulmonary artery hypertension in perfused rabbit lungs was partially reversed by pretreatment with calphostin C (0.1 μM), a protein kinase C (PKC) inhibitor (Figure 6A). The attenuation of pulmonary hypertension was associated with a reversal of $VOSO_4$-induced inhibition of NO production (Figure 6B). In HPAEC, 0.1 μM calphostin C also attenuated $VOSO_4$-induced inhibition of NO production (Figure 7A). This was associated with decreased Thr[495] phosphorylation of eNOS (Figure 7B).
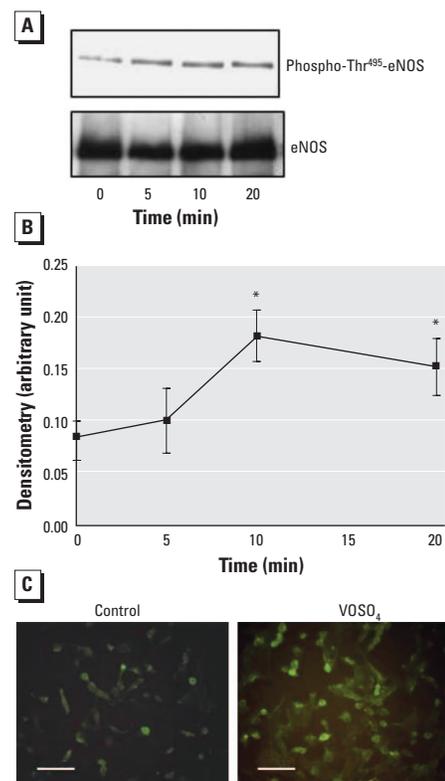
Figure 5. Increases in Thr[495] phosphorylation of eNOS in HPAEC by 50 μM $VOSO_4$ (see "Materials and Methods" for details). (*A*) Representative Western blot of four independent experiments. (*B*) Densitometry analysis of (*A*). (*C*) Immuno-fluorescent staining of phospho-Thr[495] eNOS. Bar = 100 μm.

Discussion

Vanadium compounds have been known to cause vasoconstriction in systemic arteries. Orthovanadate (V^{+5}) constricts renal arteries (Benabe et al. 1984; Kumar and Corder 1980; Larsen and Thomsen 1980a, 1980b), splanchnic arteries (Larsen and Thomsen 1980a), and the aorta (Borchard et al. 1981), and $VOSO_4$ (V^{+4}) constricts mesenteric arteries and the aorta (Cadene et al. 1997). In the present study, we showed in intact lungs and isolated pulmonary artery rings that $VOSO_4$ produced pulmonary artery constriction, which could be attributed in part to the loss of vasodilator activity provided by endothelial NO. This was supported by the inhibition of nitrite/nitrate accumulation in the perfusate of IPL and HPAEC and by the rightward shift of the acetylcholine-induced vasorelaxation curve induced by $VOSO_4$.

$VOSO_4$-induced NO inhibition in our study was associated with increased Thr[495] phosphorylation of eNOS. Thr[495] phosphorylation is one of the few negative posttranslational mechanisms that regulate eNOS activity (Fulton et al. 2001; Govers and Rabelink 2001). Many agonist stimuli, such as vascular endothelial growth factor (VEGF) (Michell et al. 2001), bradykinin (Fleming et al. 2001; Harris et al. 2001), and ischemic stress (Chen ZP et al. 1999), inhibit phosphorylation

at the Thr[495] residue. Phosphorylation of Thr[495] by antagonists such as $VOSO_4$, as shown in our study, would thus interfere with the binding of calcium–calmodulin and inhibit eNOS activity. Several previous studies in systemic arteries have shown that the vanadium-induced contraction was related to entry of extracellular calcium (Benabe et al. 1984; Cadene et al. 1997). Extracellular calcium may activate protein phosphatases (e.g., PP1, PP2A) and dephosphorylate Thr[495] (Michell et al. 2001).

The mechanism for Thr[495] phosphorylation was partially mediated by activation of PKC because the PKC inhibitor, calphostin C, attenuated $VOSO_4$-induced Thr phosphorylation and restored NO production in HPAEC. These findings agree with those in bovine and porcine aortic endothelial cells, in which PKC activation phosphorylates Thr[495] of eNOS (Fleming et al. 2001; Michell et al. 2001).

There are other phosphorylative mechanisms that regulate eNOS activity posttranslationally. Phosphorylation of Ser[1177] enhances eNOS activity, a mechanism shared by many NO agonists such as insulin (Montagnani et al. 2001; Schnyder et al. 2002), insulin-like growth factor-1 (Michell et al. 1999), VEGF (Brouet et al. 2001; Dimmeler et al. 2000; Fulton et al. 1999; Michell et al. 2001), estrogen (Hisamoto
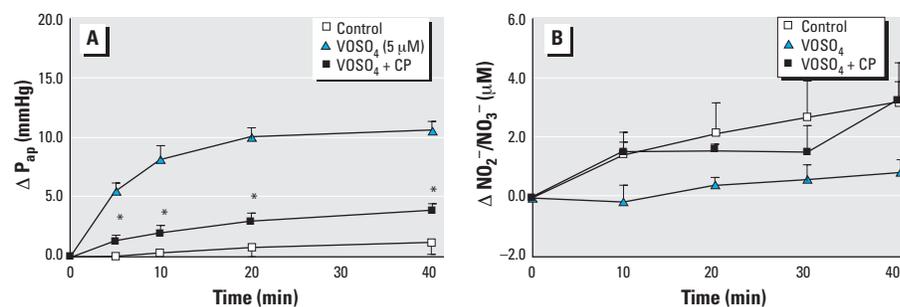
Figure 6. Effects of PKC on (*A*) $VOSO_4$-induced pulmonary hypertension (expressed as the change in mean P_{pa}) and (*B*) NO inhibition in perfused rabbit lungs. $VOSO_4$-induced pulmonary hypertension and NO inhibition were partially reversed by pretreatment of calphostin C (CP; 0.1 μM), 20 min before the addition of 5 μM $VOSO_4$; $n = 5–9$.
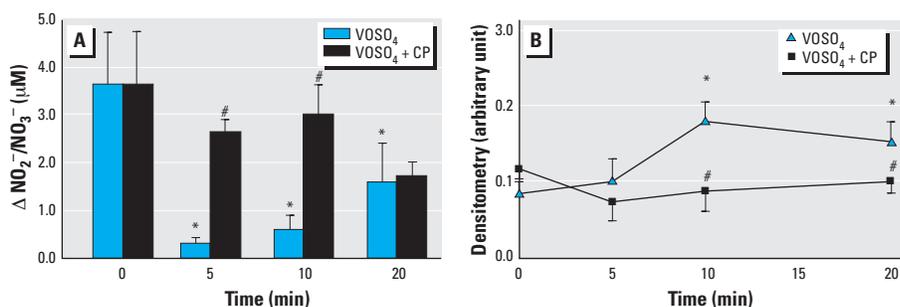*$p < 0.05$ vs. $VOSO_4$.

Figure 7. Effects of PKC on $VOSO_4$-induced NO inhibition in HPAEC. (*A*) Time-dependent inhibition of NO production in HPAEC by 50 μM $VOSO_4$, which was partially reversed by pretreatment with 0.1 μM calphostin C (CP). (*B*) Time course of Thr[495] phosphorylation of eNOS by 50 μM $VOSO_4$ with or without CP; $n = 4$ independent experiments.
*$p < 0.05$ vs. time 0. #$p < 0.05$ vs. $VOSO_4$ at respective time points.

et al. 2001), and stress (Boo et al. 2002). In our study, VOSO$_4$ did not affect Ser phosphorylation of eNOS or Akt1. eNOS may also be phosphorylated at tyrosine residues, although the functional consequence of the enzyme is less clear. Tyrosine phosphorylation of eNOS produced by hydrogen peroxide and orthovanadate decreased enzyme activity (Garcia-Cardena et al. 1996). In contrast, VEGF-, estrogen- or stress-induced eNOS activation could be inhibited by tyrosine kinase inhibitors (Ayajiki et al. 1996; Chen Z et al. 1999; Corson et al. 1996; Papapetropoulos et al. 1997). We did not see tyrosine phosphorylation of eNOS in HPAEC exposed to 50 μM of VOSO$_4$, but we could not exclude tyrosine residues of other proteins functionally linked to NOS activation.

Our findings that VOSO$_4$ produces pulmonary vasoconstriction by inhibiting NOS activity have several clinical implications. First, certain occupations, such as boilermakers, are exposed to fuel oil ash that contains a high concentration of vanadium (up to 30 μg/m^3) for many hours a day during a boiler overhaul (Hauser et al. 1995, 1998; Woodin et al. 1999, 2000). Inhalation of vanadium in this situation has been associated with not only airway inflammation and constriction (Hauser et al. 1995, 2001, 2002; Hessel et al. 1998; Woodin et al. 1998, 2000) but also cardiovascular effects measured as increased heart rate variability (Magari et al. 2002). Vanadium contained in vanadium-rich residual oil fly ash given intratracheally can gain access into the pulmonary circulation (Huang et al. 2002).

Second, certain weight-reduction and muscle-building regimens contain significant amounts of VOSO$_4$. For example, BioLean Free contains 400 μg VOSO$_4$ per serving (four tablets), Mass Appeal contains 5 mg VOSO$_4$ per serving, and Satiete contains 660 μg of VOSO$_4$ per serving (all from Wellness International Network, Ltd., Plano, TX). With the recommended doses of several servings a day on the label, regular users can consume a significant amount of VOSO$_4$. The pulmonary vasoconstrictor property of VOSO$_4$ demonstrated in our study raised the possibility of increased risk for pulmonary hypertension in these individuals, especially in view of the long elimination half-life of vanadium (12 days) after ingestion (Ramanadham et al. 1991). Indeed, rats fed with vanadium for 2 months developed pulmonary hypertension and right ventricular hypertrophy (Susic and Kentera 1986).

Vanadium is a unique metal that is known to avidly permeate cell membranes. Once inside the cells, vanadium chelates to many intracellular ligands, such as creatine phosphate, proteins, glutathione, and ascorbic acids (Nechay et al. 1986), and presumably is detoxified. When vanadium loads exceed the capacity of the chelators, however, free vanadyl ions (V^{+4}) and the more potent vanadate (V^{+5}) may be released at high local concentration and may attack important biomolecules, resulting in cellular dysfunction (Hirao 2000; Liochev and Fridovich 1987; Nechay 1984). Our results indicate that eNOS in endothelial cells is a potential target. Given the long elimination half-life of vanadium, the endothelial dysfunction with loss of NO-mediated vasodilator activity raised the possibility that individuals exposed to excessive vanadium in the environment may be at a greater risk for developing pulmonary hypertension and perhaps other vascular diseases. Our results indicate the need for further studies on the prevalence of cardiovascular diseases in at-risk workers and individuals consuming vanadium-rich dietary supplements on a regular basis.

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