Lead-Induced Downregulation of Soluble Guanylate Cyclase in Isolated Rat Aortic Segments Mediated by Reactive Oxygen Species and Cyclooxygenase-2

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Abstract. Lead exposure is a known cause of hypertension. Although most studies have focused on lead-induced endothelial dysfunction and on the involvement of reactive oxygen species (ROS), it has been recently demonstrated that the vascular wall of lead-exposed rats has both an altered the endothelium-independent relaxing response and a reduced expression of soluble guanylate cyclase (sGC). The aim of the present study was to determine in *in vitro* incubated rat isolated aortic segments if lead downregulates sGC expression, analyzing the involvement of ROS and cyclooxygenase-2 (COX-2). The experiments were performed in isolated aortic segments from Wistar rats that were incubated with lead for 24 h. Lead significantly reduced sGC-β_1 subunit expression in a concentration-dependent manner. The maximal reduction in sGC-β_1 subunit expression was achieved with 1 ppm lead. Vitamin C (30 μmol/L) partially restored sGC-β1 subunit expression in lead (1 ppm)-exposed aortic segments. A similar protection of sGC-β_1 subunit expression was obtained with both a protein kinase A inhibitor, H89 (1 μmol/L) and with rofecoxib (1 μmol/L), an inhibitor of COX-2 activity. Moreover, lead exposure increased COX-2 expression in the arterial wall. While vitamin C reduced both COX-2 expression and superoxide anion production related to lead exposure, rofecoxib failed to modify superoxide anion generation in lead-incubated aortic segments. In conclusion, the present results suggest the involvement of ROS and COX-2 in the downexpression of sGC-β_1 subunit induced by lead in the rat vascular wall.

Lead exposure is a well-known cause of hypertension in humans and experimental animals (1,2). Although different considerations have been raised to explain the pathogenesis of lead-induced hypertension, several studies have suggested the primary involvement of the increased production of reactive oxygen species (ROS) observed in lead-exposed animals (3,4). Elevated levels of ROS reduce the bioavailability of nitric oxide (NO), thus inducing an impaired endothelium-dependent vasorelaxation (5).

NO is generated in the endothelium and induces vasodilatation by stimulating soluble guanylate cyclase (sGC) in the adjacent smooth muscle cells (6). Exogenous nitrovasodilators also cause vasodilatation of the vascular wall, independently of the endothelium, by activating the formation of cyclic GMP (cGMP) in the adjacent smooth muscle cells (7).

sGC is a heterodimer composed of a large (α_1) and a small ($\beta_{1)}$ subunit (8). Recent cloning and expression experiments have revealed that, although both the α_1 and β_1 subunits seem to contain a catalytic domain, the sGC enzymatic activity requires the presence of both subunits (9). In this regard, we recently demonstrated an impaired endothelium-independent vasodilating response associated with downexpression of the sGC β_1-subunit (10). Moreover, although we are aware of no data in the literature about a possible role for ROS in the regulation of sGC expression, our previously mentioned study demonstrated that administration of vitamin C, an antioxidant, prevented the downexpression of sGC observed in the vascular wall of lead-exposed rats (10).

Although the intracellular signal regulators of sGC expression are still not completely established, different studies have demonstrated that in addition to nerve growth factor, endotoxin and interleukin-1β, cyclic adenosin monophosphate (cAMP)-stimulating agents, *i.e.*, prostaglandins, downregulate sGC expression (11–13). Therefore, the inhibition of the cAMP-coupled signaling pathway, such as protein kinase A activation, could protect the expression of sGC. Interestingly, it has been also demonstrated that ROS upregulate cyclooxygenase-2 (COX-2) expression, a COX isoform primarily responsible for the synthesis of prostaglandins involved in pathologic processes including hypertension (14,15). Therefore, on the basis of the above-mentioned findings, the aim of the present study was to determine in *in vitro* incubated rat isolated aortic segments if lead downregulates sGC expression in the vascular

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wall of rats, analyzing the involvement of ROS generation and COX-2 expression.

Materials and Methods

Incubation of Isolated Aortic Segments

Studies were approved by the Institutional Ethics Committee for animal studies and were performed in accordance with the international convention on animal experimentation. The experiments were carried out on male 300-g Wistar Kyoto rats. The animals were anesthetized with pentobarbital (30 mg/kg intramuscularly), and the descending thoracic aorta was removed and cut into 5-mm-long segments and individually suspended into culture solution (RPMI medium containing 5% fetal calf serum) at 37°C. Increasing concentrations of lead acetate (0 to 1 ppm) were added to the culture solution, and the aortic segments were incubated for 24 h in the presence and in the absence of vitamin C (30 μmol/L), rofecoxib (1 μmol/L), a COX-2 inhibitor (16), and an inhibitor of protein kinase A activity, H-89 (1 μmol/L) (17). The aortic segments were then immediately frozen in liquid nitrogen for molecular biology determinations. The lead concentrations in the present study were chosen on the basis of previous *in vitro* studies from Vaziri *et al.* (18,19), who elegantly demonstrated that 0.1 ppm to 1 ppm lead promoted lipid peroxidation and 1 ppm lead upregulated endothelial nitric oxide synthase expression in rat and human cultured endothelial cells, respectively.

Determination of sGC-β_1 Subunit and COX-2 Expression by Western Blot Analysis

The expression of sGC-β_1 subunit and COX-2 proteins was analyzed by Western blot as described elsewhere (20). In brief, the aortic rings were pulverized and solubilized in Laemmli buffer containing 2-mercaptoethanol (21). The proteins obtained were separated in denaturing SDS/10% polyacrylamide gels. Equal amounts of proteins (20 μg per lane) estimated by bicinchonic acid reagent (Pierce, Rockford, IL) were loaded. To verify that equal amounts of proteins were loaded in the gel, a parallel gel was run and stained with Coomasie, and the intensities of the protein bands were examined. The proteins were then blotted into nitrocellulose (Immobilion-P; Millipore Ibérica, S.A.) The blots were blocked overnight at 4°C with 5% nonfat dry milk in TBS-T (20 mmol/L Tris-HCl, 137 mmol/L NaCl, 0.1% Tween 20). Western blot analyses were performed with monoclonal antibodies against sGC-β_1 subunit and COX-2, respectively. The blots were incubated with the first antibody (1:2500) for 1 h at room temperature and, after extensive washing, with the second antibody (horseradish peroxidase-conjugated anti-rabbit Ig antibody) at a dilution of 1: 1500 for another hour. Specific sGC-β_1 subunit and COX-2 proteins were detected by enhanced chemoluminiscence (ECL, Amersham Corp) and evaluated by densitometry (Molecular Dynamics). Prestained protein markers (Sigma) were used for molecular mass determinations. To compare the sGC-β_1 subunit and COX-2 isoform expression with the expression of another protein, we analyzed the expression of β-actin by Western blot, using a β-actin monoclonal antibody (Sigma Aldrich). For this purpose, a parallel gel with identical samples was run; after blotting onto nitrocellulose, Western blot analysis was then performed with the β-actin monoclonal antibody (1:2000).

Measurement of cGMP

cGMP levels in the vascular wall were determined as described previously (10). In brief, aortic segments were incubated for 24 h in the presence and in the absence of 1 ppm lead in the culture solution (RPMI medium containing 5% fetal calf serum at 37°C). The vessels were then washed and incubated in the same medium that contained the phosphodiesterase inhibitor 3-isobutyl-1-methylxantine (2 mmol/L) to prevent breakdown of cGMP for 20 min at 37°C. Similar experiments were performed incubating the different aortic segments with the nitric oxide donor, sodium nitroprusside (SNP, 10^{-4} mol/L). The vessels were then immediately stored in liquid nitrogen. The frozen segments were extracted at 4°C with a 49:1 (vol/vol) mixture of 0.1 mol/L ethanol/HCl. The extracts were evaporated using a speed vac evaporator (Model VR-1/120/240; Heto Lab-Equipment A/S, Denmark). cGMP concentrations were measured in acetylated samples by means of a RIA kit (Amersham International). The sensitivity of the assay was 0.5 fmol. The intraassay and interassay variations were $<$8.9% and $<$16%, respectively.

Superoxide Anion Generation in the Aortic Segments

The amount of superoxide anion generated by the vascular wall was determined by measuring the SOD-inhibitable reduction of ferricytochrome C, as described (22). In brief, aortic segments were incubated in culture RPMI-1640 medium containing 1% FCS and 0.1 mmol/L ferricytochrome C at 37°C for 30 min, as reported. The supernatants were collected, and the generation of superoxide anion was calculated as the difference in absorbance between aortic segments incubated with and without SOD 100 μg/mL. The difference was then divided by the molar extinction coefficient change between ferricytochrome C and ferrocytochrome C to determine the amount of superoxide radicals produced over 30 min. All observations were made in triplicate and the data averaged. The absorbance was measured in a spectrophotometer at 550 nm.

In addition to this quantitative method, we detected the generation of superoxide anion by confocal microscopy. The oxidative fluorescent dye dihydroethidium (DHE, 5 μmol/L) was used to evaluate *in situ* production of superoxide. DHE is freely permeable to cells and in the presence of superoxide anion is oxidized to ethidium, and trapped by intercalating with the DNA (23). Ethidium is excited at 488 nm with an emission spectrum of 610 nm. For confocal experiments, aortic segments incubated in the presence and in the absence of 1 ppm lead were cut into 30-μm-thick sections and placed on a glass. DHE (2 μmol/L) was added to each tissue section. The slices were incubated in a light-protected humidified chamber at 37°C for 30 min. Images were obtained with a Leica Confocal Microscope (Leica TCS SP2 Microsystems, Heidelberg, Germany).

Statistical Analyses

Results are expressed as mean $\pm$ SEM. Each of the above-mentioned studies was performed in at least a minimum of five different aortic segments. Comparisons were performed by ANOVA or paired and unpaired *t* test when appropriate. Bonferroni correction for multiple comparisons was used to determine the level of signifcance of *P*. $P < 0.05$ was considered significant.

Results

Effect of Lead Exposure on sGC Expression

The Western blot experiments showed that the *in vitro* exposure of rat vascular wall to lead induced a dose-dependent decrease in sGC-β_1 subunit protein expression (Figure 1A). The maximal reduction in sGC-β_1 subunit expression was achieved with 1 ppm lead (Figure 1A). This lead concentration was used in most of the following experiments. No changes in β-actin expression were observed in control and lead-incubated aortic segments (Figure 1A).

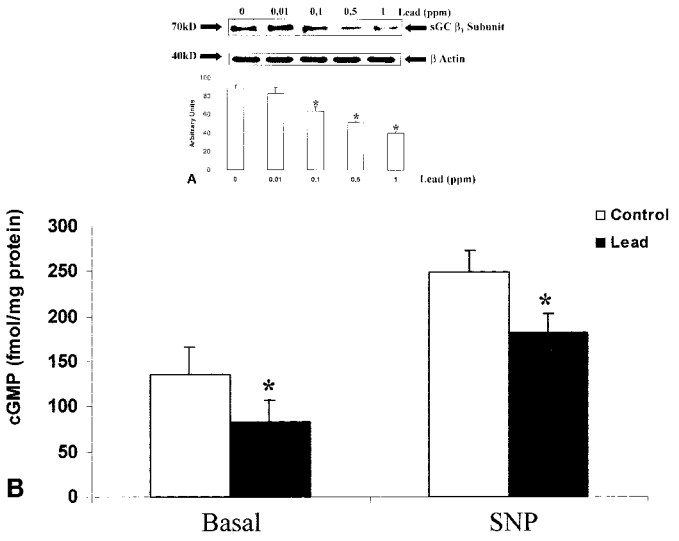

Figure 1. (A) Representative Western blot showing soluble guanylate cyclase (sGC) β_1-subunit expression in isolated rat aortic segments incubated in the absence and in the presence of increasing concentrations of lead. The expression of the constitutive protein β-actin is also shown. The bottom shows the densitometric analysis of the Western blot. Results are represented as mean ± SEM of five different aortic segments. * $P < 0.05$ with respect to control. (B) Bar graph showing cyclic GMP (cGMP) accumulation in the vascular wall of control and 1 ppm lead-incubated rat aortic segments before and after the addition of the nitric oxide donor, sodium nitropruside (SNP, 10^{-4} mol/L). Results are represented as mean ± SEM of four different aortic segments. * $P < 0.05$ with respect to each corresponding control aortic segment.

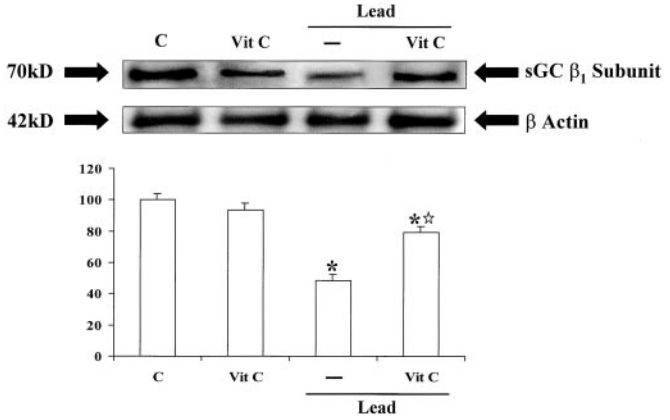

Figure 2. Representative Western blot showing sGC β_1-subunit expression in isolated rat aortic segments exposed to 1 ppm lead for 24 h in the presence and in the absence of vitamin C (30 μmol/L). Similar experiments were performed in isolated non–lead-exposed aortic segments. The bottom shows the densitometric analysis of the Western blot. Results are represented as mean ± SEM of six different experiments. * $P < 0.05$ with respect to control. * $P < 0.05$ with respect to lead exposure alone.

The decreased expression of sGC-β_1 subunit observed in the vascular wall after lead exposure was associated with a reduced content of cGMP both under basal conditions and after stimulation with an exogenous nitric oxide donor, 10^{-4} mol/L SNP (Figure 1B).

sGC expression, Vitamin C, and Superoxide Anion

In a previous work, we demonstrated *in vivo* that antioxidant therapy with vitamin C inhibited the downexpression of sGC-β subunit protein elicited by lead administration and restored NO-dependent vascular relaxation (10). We thus analyzed *in vitro* whether vitamin C modified sGC expression in lead-incubated aortic segments.

Vitamin C (30 μmol/L) partially although significantly protected sGC-β_1 subunit expression in 1 ppm lead-exposed aortic segments; however, the expression of sGC-β_1 subunit remained reduced with respect to that detected in control aortic segments (Figure 2). Vitamin C concentrations greater than 30 μmol/L failed to preserve sGC-β_1 subunit expression to a higher magnitude than that observed with 30 μmol/L vitamin C (data not shown). In the absence of lead, sGC-β_1 subunit expression was not significantly modified by the addition of 30 μmol/L vitamin C (Figure 2).

Incubation of the rat aortic segments with lead stimulated the release of superoxide anion (control, 3.15 ± 0.84 μg O_2^-/mg protein; lead, 16.87 ± 1.53 μg O_2^-/mg protein; $P < 0.5$). Vitamin C (30 μmol/L) reduced the amount of superoxide anion released from lead-exposed rat aortic segments (8.03 ± 1.12 μg O_2^-/mg protein; $P < 0.05$ with respect to lead-exposed aortic segments).

Confocal microscopy experiments confirmed these findings. A greater superoxide anion content was observed in the vascular wall of the segments incubated in the presence of 1 ppm lead when compared with control rings (Figure 3). Moreover, the addition of vitamin C reduced the superoxide anion content in the vascular segments exposed to 1 ppm lead (Figure 3).

The expression of gp^{91}phox-containing NADPH oxidase, a NADPH oxidase subtype considered as one of the major vascular superoxide anion sources, was not different between control and lead-exposed aortic segments (Figure 4). Moreover, vitamin C failed to modify gp^{91}phox-containing NADPH expression in lead-incubated aortic segments (Figure 4).

Relationship among sGC, COX-2 Expression, and Superoxide Anion Production

A protein kinase A inhibitor, H-89 (1 μmol/L), partially restored the downexpression of sGC-β_1 subunit induced by lead exposure of the aortic segments (Figure 5). Moreover, lead-exposed aortic segments showed a marked upexpression of COX-2 protein, an effect that was prevented by 30 μmol/L vitamin C (Figure 6). Rofecoxib (1 μmol/L), an inhibitor of COX-2 activity, partially protected sGC-β_1 subunit expression in lead-incubated aortic segments (Figure 5). However, rofecoxib did not significantly modify the increased release of superoxide anion induced by lead exposure of the aortic segments (15.84 ± 2.21 μg O_2^-/mg protein; P = NS with respect to lead-exposed aortic segments). This was further confirmed in confocal microscopic sections of lead + rofecoxib-incubated aortic segments labeled with the oxidative dye DHE in

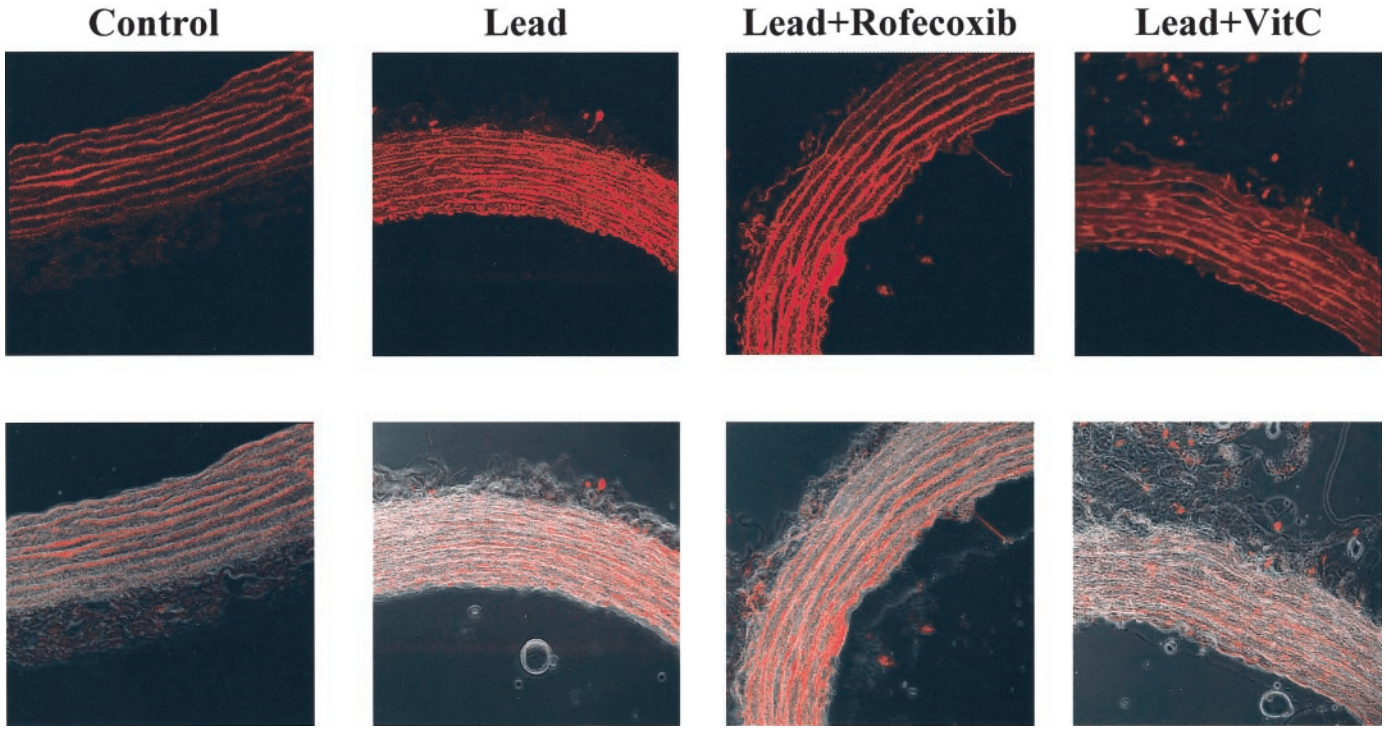

Figure 3. In situ detection of superoxide anion in rat aorta. Fluorescent photomicrographs of confocal microscopic sections of the aorta labeled with the oxidative dye DHE (red fluorescence when oxidized to ethidium by superoxide anion). Isolated aortic segments were incubated in the absence and in the presence of 1 ppm lead for 24 h. Additional experiments were performed in lead-exposed aortic segments in the presence of vitamin C (30 μmol/L) and rofecoxib (1 μmol/L). In the upper panels are shown the immunofluorescence picture, whereas the bottom panels show the combination of the immunofluorescence pictures with the optical microscopy photomicrograhs.

which we did not observe any significant differences in the content of superoxide anion with respect to aortic segments exposed to lead alone (Figure 3).

Discussion

In this study, we have shown that (*1*) lead exposure decreased sGC β_1-subunit protein expression in *in vitro* incubated rat aortic segments; (*2*) this effect was associated with an increased content of superoxide anion in the vascular wall after lead exposure, and it was prevented by the use of an antioxidant agent such as vitamin C; (*3*) lead-exposure upregulated COX-2 expression which was prevented by vitamin C; and (*4*) COX-2 activity was involved in the downregulation of sGC-β_1 subunit elicited by lead exposure.

Lead-induced hypertension is the element of chronic lead intoxication that has received more attention in the literature. Different considerations have been raised to explain the pathogenesis of lead-induced hypertension. In this regard, several studies have focused on the ability of the endothelium to generate NO in this model of hypertension. These studies demonstrated the existence of an altered NO synthesis, probably because of a diminished eNOS activity and/or increased NO catabolism by oxygen free radicals (3,5). However, we recently demonstrated that, independently of the NO-generating system, lead administration downregulated sGC, the main receptor of NO, in the vascular wall, an effect that was prevented by the co-administration of the antioxidant vitamin C, suggesting the involvement of reactive oxygen species (10). In the present study, we have confirmed *in vitro* that lead induces a downexpression of sGC β_1-subunit in the vascular wall. In this regard, it is well established that the presence of both α_1 and β_1 subunits is required for sGC activity and the reduction in one of these subunits is enough to decrease the ability of sGC to generate cGMP in response to NO (9). In the same line of evidence, lead-exposed aortic segments showed a reduced content of cGMP under basal conditions and after stimulation with the exogenous nitric oxide donor, sodium nitroprusside.

The fact that vitamin C protected sGC β_1-subunit expression in lead-exposed aortic segments suggested the involvement of ROS in the modulation of sGC expression. In our experiments, lead exposure stimulated the ability of the vascular wall to release superoxide anion, which is in accordance with previous works from Vaziri *et al.* (3) and Ding *et al.* (18,24), which demonstrated an increased lipid peroxidation and enhanced hydroxyl radical generation in rats and cultured endothelial cells after lead exposure. In this regard, ROS stimulation by lead has been recently associated with inactivation of nitric oxide, which was correlated with a compensatory up-expression of endothelial nitric oxide synthase in rat aortic segments and cultured human coronary endothelial cells (19,25).

It has been recently postulated that gp^{91}phox-containing NADH oxidase is one of the main sources of superoxide anion in the arterial wall (26). However, the increased ability of the arterial wall to release superoxide anion after lead exposure

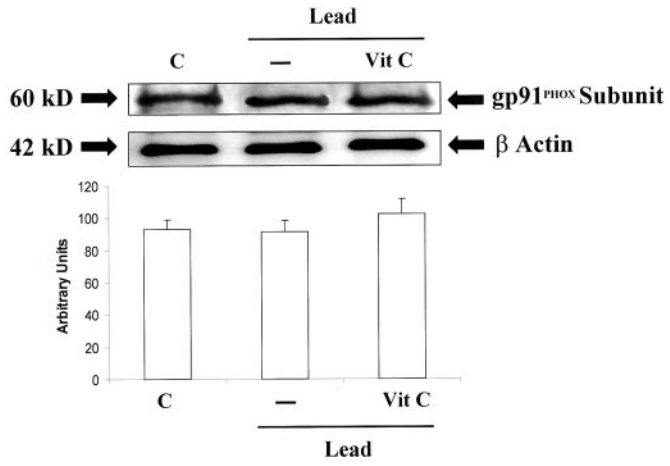

Figure 4. Representative Western blot showing the expression of gp^{91}phox-containing NADPH in control, 1 ppm lead-exposed, and 30 μmol/L vitamin C + 1 ppm lead-exposed isolated aortic segments. The bottom shows the densitometric analysis of the Western blot. The Western blot was performed by using a goat polyclonal antibody against the carboxy terminal of gp^{91}phox of human origin (1:500; Santa Cruz Biotecnology Inc.). The immunoblot shows a typical band for gp^{91}phox at $\approx$ 60 kDa. The gp^{91}phox refers to the molecular weight in human tissues where the subunit is heavily glycosylated. Results are represented as mean $\pm$ SEM of five different experiments.

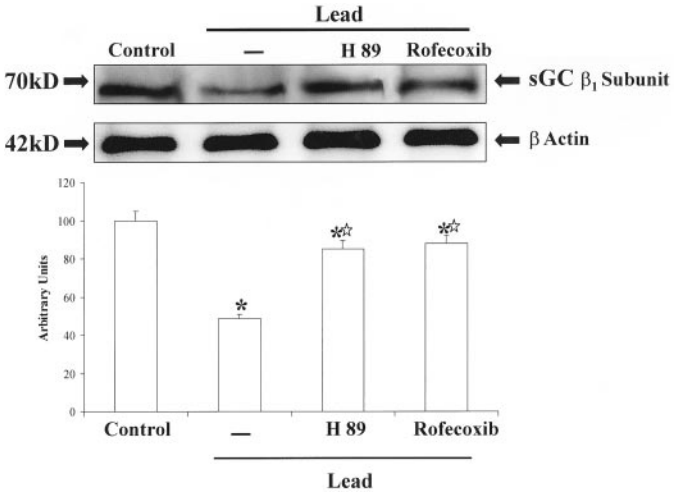

Figure 5. Representative Western blot showing the expression of the sGC β_1-subunit in control and 24-h lead (1 ppm)-exposed aortic segments. Additional experiments were performed in 1 ppm lead-exposed aortic segments in the presence of the protein kinase A inhibitor, H89 (1 μmol/L), and an inhibitor of COX-2 activity, rofecoxib (1 μmol/L). Results are represented as mean $\pm$ SEM of five different experiments. * $P < 0.05$ with respect to control; ☆ $P < 0.05$ with respect to lead exposure alone.

was not associated with an increased expression of gp^{91}phox-containing NADH oxidase. The present experimental design did not allow us to determine whether lead stimulated the activity of gp^{91}phox-containing NADH and/or modified the

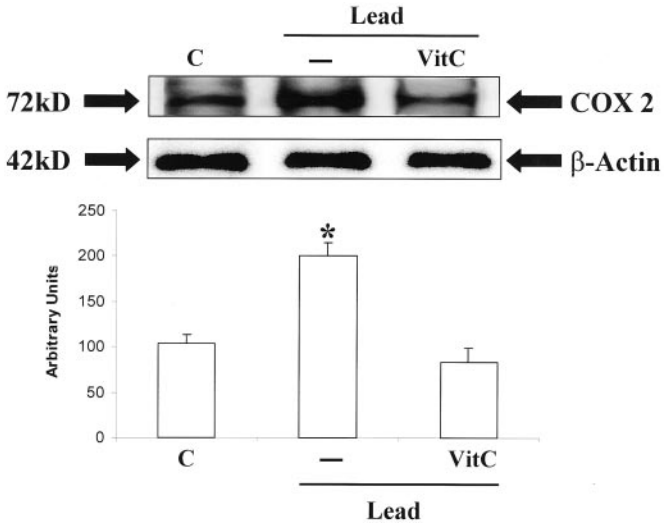

Figure 6. Representative Western blot showing the expression of the COX-2 isoform in control, lead (1 ppm)-exposed and 1 ppm lead + 30 μmol/L vitamin C-exposed isolated aortic segments. Results are represented as mean $\pm$ SEM of five different experiments. * $P < 0.05$ with respect to control.

expression of other enzymes related with superoxide anion generation in the arterial wall such as nox-1 (27). Further investigations are needed to elucidate these issues.

Cyclic AMP is a second messenger of the products of COX-2 pathway (28). Interestingly, attenuation of sGC expression has been recently demonstrated to be induced by cyclic AMP-elevating mediators (13,29). In this regard, we observed that lead exposure not only reduced the expression of sGC-β1 subunit but also increased the expression of the COX-2 isoform. Moreover, lead-induced up-expression of COX-2 protein was associated with the downregulation of sGC-β_1 subunit expression because rofecoxib, an inhibitor of COX-2 activity, attenuated the reduction of sGC-β_1 subunit expression. This was further supported by the fact that an inhibitor of protein kinase A activity, H-89 (17), also attenuated the down-expression of sGC-β_1 subunit to a similar level as rofecoxib.

The question then raised was if COX-2 enhanced superoxide anion production by lead or the increased superoxide anion generation induced by lead stimulated the expression of COX-2 protein. In our experiments, while the addition of vitamin C prevented lead-induced COX-2 expression, rofecoxib failed to modify the enhanced superoxide anion production induced by lead exposure of the vascular wall, suggesting that superoxide anion was involved in the upregulation of COX-2 expression elicited by lead. In this regard, increased ROS activity has been demonstrated to exhert an upregulation of COX-2 expression in luteal cells (30). Moreover, Fang *et al.* (31) have demonstrated that transfection of catalase-encoding cDNA induces the transcriptional activation of the COX-2 gene and increases mRNA stability, thus supporting that peroxides upregulate COX-2 expression.

It is noteworthy that both vitamin C and rofecoxib only partially restored the downexpression of the sGC β_1 subunit by

lead, suggesting that other signaling pathways stimulated by lead and/or lead itself may be involved in the downregulation of the sGC-β_1 subunit expression in the vascular wall. In this regard, it has been demonstrated that heavy metals, including lead, may modulate the expression of several other proteins (32,33). Further experiments are warranted to test these hypotheses.

In conclusion, our data have shown for the first time the involvement of ROS via COX-2 expression in the downregulation of sGC-β1 subunit expression induced by lead in the rat vascular wall. These results suggest a role for ROS and COX-2 in the pathogenesis of lead-induced hypertension.

Acknowledgments

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References

1. Sharp DS, Osterloh J, Becker CE, Bernard B, Smith AH, Fisher JM, Syme SL, Holman BL, Johnson T: Blood pressure and blood lead concentration in bus drivers. *Environ Health Perspect* 78: 131–137, 1988
2. Sharp DS, Becker CE, Smith AH: Chronic low-level lead exposure. Its role in the pathogenesis of hypertension. *Med Toxicol* 2: 210–232, 1987
3. Vaziri ND, Ding Y, Ni Z, Gonick HC: Altered nitric oxide metabolism and increased oxygen free radical activity in lead-induced hypertension: Effect of lazaroid therapy. *Kidney Int* 52: 1042–1046, 1997
4. Gonick HC, Ding Y, Bondy SC, Ni Z, Vaziri ND: Lead-induced hypertension: interplay of nitric oxide and reactive oxygen species. *Hypertension* 30: 1487–1492, 1997
5. Vaziri ND, Liang K, Ding Y: Increased NO inactivation and sequestration in lead-induced hypertension. Effect of vitamin E. *Kidney Int* 56: 1492–1498, 1999
6. Busse R, Mulsch A, Fleming I, Hecker M: Mechanisms of nitric oxide release from the vascular endothelium. *Circulation* 87: 18–25, 1993
7. Katsuki S, Arnold W, Mittal C, Murad F: Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerine and nitric oxide in various tissue preparations and comparison to the effects of sodium azyde and hychoxylamine. *J Cyclic Nucleotide Res* 3: 23–35, 1977
8. Gerzer R, Bohme E, Hoffman R, Shultz G: Soluble guanylate cyclase purified from bovine lung contains heme and copper. *FEBS Lett* 132: 71–74, 1981
9. Buechler WA, Nakane M, Murad F: Expression of soluble guanylate cyclase activity requires both enzyme subunits. *Biochem Biophys Res Commun* 174: 351–357, 1991
10. Marques M, Millas I, Jimenez A, García-Collis E, Antonio Rodriguez-Feo J, Velasco S, Barrientos A, Casado S, López-Farré A: Alterations of the soluble guanylate cyclase system in the vascular wall of lead-induced hypertensive rats. *J Am Soc Nephrol* 12: 2594–2600, 2001
11. Liu H, Force T, Bloch KD: Nerve growth factor decreases soluble guanylate cyclase in rat PC12 cells. *J Biol Chem* 272: 6038–6043, 1997
12. Arriero MM, Cedrán A, Jimenez P, García A, de la Pinta JC, Manzalbertia F, Muñoz L, Reyero A, Escribano M, Casado S, López Farré A: Aspirin protected the nitric oxide-cyclic GMP generating system in human peritoneum. *Peritoneal Dyal Int* 21: S48–S53, 2001
13. Parapetropoulos A, Marczin N, Mora G, Milici A, Murad F, Catravas JD: Regulation of vascular smooth muscle soluble guanylate cyclase activity, mRNA and protein levels by cAMP-elevating agents. *Hypertension* 26: 696–704, 1995
14. Nakamura T, Sakamoto K: Reactive oxygen species up-regulates cyclooxygenase-2, p53 and Bax mRNA expression in bovine luteral cells. *Biochem Biophys Res Commun* 284: 203–210, 2001
15. Cheng HF, Wang CJ, Moeckel GN, Zhang MZ, McKanna JA, Harris RC: Cycloxygenase-2 inhibitor blocks expression of mediators of renal injury in model of diabetes and hypertension. *Kidney Int* 62: 929–939, 2002
16. FitzGerald GA, Patrono C: The coxibs selective inhibitors of cyclooxigenase-2. *N Engl J Med* 345: 433–442, 2001
17. Sánchez de Miguel L, Casado S, Farré J, García-Durán M, Montón M, Romero J, Rico L, Bellver T, Mata P, Esteban A, López-Farré A: Comparison in vitro effects of trifusal and acetylsalicylic acid on nitric oxide synthesis by human neutrophils. *Eur J Pharmacol* 343: 57–65, 1998
18. Ding Y, Gonick HC, Vaziri ND: Lead promotes hydroxyl radical generation and lipid peroxidation in cultured aortic endothelial cells. *Am J Hypertens* 13: 552–555, 2000
19. Vaziri ND, Ding Y: Effect of lead on nitric oxide synthase expression in coronary endothelial cells: Role of superoxide. *Hypertension* 37: 223–226, 2001
20. López-Farré A, Rodríguez-Feo JA, García Collis E, Gomez J, López-Blaya A, Fortes J, de Andres R, Rico L, Casado S: Reduction of the soluble cyclic GMP vasorelexing system in the vascular wall of stroke prone spontaneously hypertensive rats. Effect of the α 1:MDNM^{-}-receptor blocked doxazosin. *J Hypertens* 20: 463–470, 2002
21. Laemmli NK: Change of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685, 1970
22. Sanchez de Miguel L, Arriero MM, Farré J, Jimenez P, García-Mendez A, de Frutos T, Jimenez A, García R, Cabestrero F, Gomez J, de Andres R, Montón M, Martín E, de la Calle-Lombana LM, Rico L, Romero J, López Farré A: Nitric oxide production by neutrophils obtained from patients during acute coronary syndromes: Expression of the nitric oxide synthase isoforms. *J Am Coll Cardiol* 39: 818–825, 2002
23. Rothe G, Valet G: Flow cytometric analysis of respiratory burst activity in phagocytes which hydroethidine and 2',7'-dichlorofluorescein. *J Leukoc Biol* 55: 253–258, 1995
24. Ding Y, Gonick HC, Vaziri ND, Liang K, Wei L: Lead-induced hypertension III. Increased hydroxyl radical production. *Am J Hypertens* 14: 169–173, 2001
25. Vaziri ND, Ding Y, Ni Z: Compensatory up-regulation of nitric oxide synthase isoforms in lead-induced hypertension; reversal by a superoxide dismutase-mimetic drug. *J Pharmacol Exp Therapeutics* 298: 679–685, 2001
26. Görlach A, Brandes RP, Nguyen K Amidi M, Dehghani F, Busse R: A gp^{91}phox containing NADPH oxidase selectively expressed

in endothelial cells is a major source of oxygen radical generation in the arterial wall. *Circ Res* 87: 26–32, 2000
27. Lasségue B, Sorescu D, Sjöcs K, Yin QQ, Akers M, Zhang Y, Grant SL, Lambeth JD, Griendling KK: Novel gp^{91}phox homologies in vascular smooth muscle cells: nox-1 mediates angiotensin II-induced superoxide formation and redox sensitive signaling pathways. *Circ Res* 88: 888–894, 2001
28. Pelletier S, Dube J, Villeneuve A, Gobeil F, Yang O, Battistini B, Guillemette G, Sirois P: Prostaglandin E(2) increases cyclic AMP and inhibits endothelin-1 production/secretion by guinea-pig tracheal epithelial cells through EP (4) receptors. *Br J Pharmacol* 135: 999–1008, 2001
29. Shimouchi A, Janssens SP, Bloch DB, Zapol NM, Bloch KD: cAMP regulates soluble guanylate cyclase β_1-subunit gene expression in RFL-6 rat fetal lung fibroblastes. *Am J Physiol* 265: L456–L461, 1993
30. Nakamura T, Sakamoto K: Reactive oxygen species up-regulates cycloxygenase-2, p53, and Bax mRNA expression in bovine luteal cells. *Biochem Biophys Res Commun* 284: 203–210, 2001
31. Fang X, Moore AS, Nwankwo J, Weintranb LN, Oberley WL, Snyder DG, Spector A: Induction of cyclooxygenase-2 by overexpression of the human catalase gene in cerebral microvascular endothelial cells. *J Neuro Chem* 75: 614–623, 2000
32. Zuscik MJ, Pateder DB, Puzas JE, Schiwarz EM, Rosier RN, O'Keefe RJ: Lead alters parathyroid hormone-related peptide and transforming growth factor-beta 1 effects and AP-1 and NF-kappa B signaling in chondrocytes. *J Orthop Res* 20: 811–818, 2002
33. She H, Xiong S, Lin M, Zandi E, Giulivi C, Tsukamoto H: Iron activates NF-kappa B in Kupffer cells. *Am J Physiol* 283: G719–G726, 2002