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Iron regulates xanthine oxidase activity in the lung

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Ghio, Andrew J., Thomas P. Kennedy, Jacqueline Stonehuerner, Jacqueline D. Carter, Kelly A. Skinner, Dale A. Parks, and John R. Hoidal. Iron regulates xanthine oxidase activity in the lung. *Am J Physiol Lung Cell Mol Physiol* 283: L563–L572, 2002. First published May 17, 2002; 10.1152/ajplung.00413.2000.—The iron chelator deferoxamine has been reported to inhibit both xanthine oxidase (XO) and xanthine dehydrogenase activity, but the relationship of this effect to the availability of iron in the cellular and tissue environment remains unexplored. XO and total xanthine oxidoreductase activity in cultured V79 cells was increased with exposure to ferric ammonium sulfate and inhibited by deferoxamine. Lung XO and total xanthine oxidoreductase activities were reduced in rats fed an iron-depleted diet and increased in rats supplemented with iron, without change in the ratio of XO to total oxidoreductase. Intratracheal injection of an iron salt or silica-iron, but not aluminum salts or silica-zinc, significantly increased rat lung XO and total xanthine oxidoreductase activities, immunoreactive xanthine oxidoreductase, and the concentration of urate in bronchoalveolar fluid. These results suggest the possibility that the production of uric acid, a major chelator of iron in extracellular fluid, is directly influenced by iron-mediated regulation of the expression and/or activity of its enzymatic source, xanthine oxidase.

xanthine dehydrogenase; deferoxamine; silica; uric acid

AS OUR SPECIES HAS EVOLVED, *Homo sapiens* has lost uricase activity, acquired efficient renal tubular reabsorption of urate, and, as a consequence, developed plasma urate concentrations that are tenfold higher in humans than most other mammals (42). Teleologically, these events have been explained as necessary for humans to utilize urate as an antioxidant and have been cited as a possible basis for increases in both human lifespan and brain size (2). In addition to functioning as a potent scavenger of hydroxyl radicals, singlet oxygen, oxoheme oxidants, hydroperoxyl radicals, and hypochlorous acid (4), urate can also form stable 2:1 coordination complexes with ferric ions, thereby protecting against iron-catalyzed oxidations (8). On the basis of the estimated stability constant of formation of urate-Fe^{3+} complexes (1.1×10^{11}) and the high concentrations of urate in human plasma (500 μM), it has been suggested that this compound may be one of the major soluble-phase iron-binding protective agents formed by humans (8). This coordination complex does not appear to support electron transport, and therefore, urate inhibits iron-catalyzed oxidations (34).

Only one enzyme, xanthine oxidoreductase, is capable of producing uric acid. This enzyme physiologically uses NAD^+ as the electron acceptor but can be converted from this xanthine dehydrogenase (XDH, EC 1.1.1.204) form to xanthine oxidase (XO, EC 1.1.3.22), which transfers reducing equivalents to molecular oxygen (6). As a result of its ability to generate reactive oxygen species, the XO form has received considerable attention as a pathophysiological cause of ischemia-reperfusion injury (18), renal failure (35), lipopolysaccharide-induced endothelial injury (41), viral pneumonia (1), and cutaneous photosensitivity to hematoporphyrins (3). Invoking a novel mechanism to explain the protection of deferoxamine against XO-dependent injury by lipopolysaccharide to bovine pulmonary artery endothelial cells, Rinaldo and Gorry (41) reported that this iron chelator inhibits the activity of both XO and XDH when added to cultured cells but not when incubated with the enzymes ex vivo. We were intrigued by this finding and wondered if the converse might be true: Would increased iron stimulate lung xanthine oxidoreductase activity? Studying whole lungs and a cultured renal cell line, we found that total xanthine oxidoreductase activity and protein levels appear to be modulated by the supply of iron provided as either a soluble salt or environmentally relevant chelate. This suggests a possible mechanism by which iron regulates urate production in direct response to local need for urate to complex transition metals and protect biological systems from free radical injury.

METHODS

Materials. The silica dust (Min-U-Sil 5) employed in these studies was obtained from U. S. Silica (Berkeley Springs,

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WV). V79-4 Chinese hamster kidney cells were obtained through American Tissue Type Culture (Rockville, MD). Joklik's minimum essential medium was obtained from BioWhittaker (Walkersville, MD), and keratinocyte growth medium was from Clonetics (San Diego, CA). Fetal bovine serum was purchased from Hyclone Laboratories (Logan, UT). Rats were obtained from Charles River Laboratories (Raleigh, NC). All other materials were obtained from Sigma (St. Louis, MO) unless specified.

Preparation of silica dusts. SiO_2 has a capacity to coordinate ferric ion and, through metal-catalyzed radical generation, presents an oxidative stress to the lung. Therefore, silica was used in studies as it exemplifies a relevant environmental exposure to iron (12). Fe^{3+} and Zn^{2+} were complexed to silica as previously described (14). Briefly, 1 g of Min-U-Sil 5 was agitated in 50 ml of 1 N HCl for 1 h and centrifuged at 1,200 *g*, and the supernatant was removed. The dust was then agitated in 50 ml of distilled water, centrifuged at 1,200 *g*, and washed with distilled water twice more to provide acid-washed silica (silica-H). Three hundred milligrams of silica-H were added to either 50 ml of 1 mM $ZnCl_2$ or $FeCl_3$ and agitated for 30 min. This suspension was centrifuged at 1,200 *g*, and the sedimented dusts were washed in distilled water three times to provide silica dusts with surface-complexed zinc (silica-Zn) or surface-complexed ferric iron (silica-Fe). We measured surface concentrations of iron and zinc by inductively coupled plasma emission spectroscopy (ICPES) after agitating 10 mg of dust in 5 ml of 1 N HCl for 1 h (14). The concentration of zinc complexed to silica-Zn was 23.4 ± 0.9 μmol/g dust, whereas that of iron associated with silica-Fe was 54.8 ± 4.3 μmol/g dust. There was no measurable metal on silica-H.

Culture of and experiments with V79 cells. Chinese hamster cells (V79-4) are a kidney cell line previously used for studies of xanthine oxidoreductase activity (22). These cells were grown on Joklik's minimum essential medium modified for spinner investigation (S-MEM) and supplemented with 10% fetal bovine serum. Initially, 10^8 cells were placed into 100 ml of medium in an air-sealed glass spinner flask (Bellco Glass, Vineland, NJ). Cells grew in clumps and were split daily by letting clumps settle out, removing single cells, and adding fresh medium. Cells were maintained at a density that required only daily splitting (~10^6 cells per ml). The volume of medium was increased with each split until the desired cell density was reached.

Cells (5×10^7) in 50 ml of S-MEM were incubated at 37°C in an atmosphere of 5% CO_2 either with or without ($n = 5$ per treatment group) 500 μM of the XO inhibitor allopurinol, 500 μM ferric ammonium sulfate (FAS), 100 μM of the iron chelator deferoxamine, 1,000 μg/ml unmodified silica, or 1,000 μg/ml silica in the presence of 100 μM deferoxamine. After 2 h, we determined cell viability by staining with trypan blue and quantifying dye exclusion in 200 cells. Cells were collected by centrifugation for 2 min at 200 *g*. Medium was aspirated and assayed for lactate dehydrogenase (LDH) activity with a commercially available kit (Sigma) modified for an autoanalyzer (Cobas Fara II centrifugal analyzer; Roche Diagnostic, Montclair, NJ). The cell pellet was then treated with 0.1% Triton X-100, and LDH activity in the lysate was measured, permitting expression of supernatant LDH activity as a percentage of total. In identical incubations, cells were collected by centrifugation at 200 *g* and gently resuspended in 0.3 ml of cold lysis buffer [10 mM dithiothreitol (DTT), 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 5×10^{-7} g/ml leupeptin, and 0.1 mM ethylenediaminetetraacetic acid (EDTA) in 50 mM potassium phosphate, pH 7.4]. Cells were sonicated on ice for 10 s using a Microson cell disrupter at maximum power in pulse mode, and cellular XO activity was measured as described in *Measurement of XO and total xanthine oxidoreductase activities*.

Similarly, 50 ml of cell suspension (5×10^7 cells) were incubated with 1.0 ml of S-MEM or S-MEM containing 1,000 μg silica-H, silica-Zn, or silica-Fe. After 2 h, cell viability was determined by trypan blue dye exclusion and measurement of LDH as described above. After identical incubations, cells were pelleted, resuspended in cold lysis buffer, and sonicated. XO and total xanthine oxidoreductase activities were then determined as described in *Measurement of XO and total xanthine oxidoreductase activities*.

Finally, 2 h of exposure of V79 cells (5×10^7 in 50 ml S-MEM) to saline and μM 500 FAS were repeated either with or without ($n = 5$ per treatment group) 4 μM actinomycin or 1 μM cycloheximide. Cells were collected by centrifugation for 2 min at 200 *g*, and cellular XO activity was measured.

Effect of modifying body iron stores on activity of xanthine oxidoreductase. Sixty-day-old male Sprague-Dawley rats (obtained between 7 and 10 days of age) were fed either a diet with only 3–8 parts/million (ppm) iron (TD80396; Tedlad Premier Laboratory Diets, Madison, WI) or a normal diet containing 198 ppm iron (Rat Chow 5001; Ralston Purina, St. Louis, MO). After 8 wk (that time estimated to deplete stores of the metal), rats ($n = 6$/diet) were anesthetized with 5% halothane and exsanguinated. Samples of blood were collected by intracardiac puncture and placed into microhematocrit tubes for determination of hematocrit. Lungs were excised and weighed. One gram of lung was homogenized (Brinkman Polytron, Westbury, NY) for 30 s on ice in 5 ml of cold lysis buffer [50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 10 mM DTT, 0.2 mM PMSF, and 5×10^{-7} g/ml leupeptin] and centrifuged for 30 min at 40,000 *g* at 4°C. XO and total xanthine oxidoreductase activities were then determined on supernatant.

In separate experiments, rats were intraperitoneally injected twice a week with either 1.0 ml of saline or 1.0 ml of ferric-nitrilotriacetic acid (NTA) equivalent to 15 mg Fe/kg body wt. After 4 wk (that time estimated to elevate concentrations in the lung), rats ($n = 6$/group) were anesthetized with halothane and exsanguinated. Lung tissue was assayed for nonheme iron by hydrolysis (1.00 g wet tissue/10.0 ml 3 N HCl and 10% trichloroacetic acid) at 70°C for 24 h. After centrifugation at 1,200 *g* for 10 min, iron in the supernatant was measured by ICPES. Lungs were excised for determination of XO and total xanthine oxidoreductase activities.

Effect of intratracheal iron salts or silica dusts on activity of lung xanthine oxidoreductase. The treatment and care of animals were conducted under the direction of the Institute for Animal Care and Use Committee, National Health and Environmental Effects Research Laboratory, Environmental Protection Agency (Research Triangle Park, NC). Animals were housed in temperature- and humidity-controlled rooms and fed a standard diet (Rat Chow 5001; Ralston Purina). Food and water were available ad libitum. Sixty-day-old (250–300 g), male Sprague-Dawley rats were exposed to saline, aluminum ammonium sulfate, or FAS. After anesthesia with 2–5% halothane (Aldrich Chemicals, Milwaukee, WI), 0.3 ml of normal saline, 500 μM aluminum ammonium sulfate in 0.3 ml of normal saline, or 500 μM FAS in 0.3 ml of normal saline was intratracheally instilled into the lungs. After 2 or 24 h, animals ($n = 6 \cdot \text{exposure}^{-1} \cdot \text{time point}^{-1}$) were again anesthetized with halothane, were euthanized by exsanguination, and had the lungs resected for measurement of XO and total xanthine oxidoreductase activities.

In separate experiments, rats ($n = 4$/exposure) were intratracheally instilled with either 0.3 ml of saline or 1,000 μg of

unmodified silica, silica-H, silica-Zn, or silica-Fe in 0.3 ml of saline. After 2 h, animals were anesthetized with halothane and exsanguinated, and lungs were excised for measurement of XO and total xanthine oxidoreductase activities.

Measurement of XO and total xanthine oxidoreductase activities. XO and total xanthine oxidoreductase activities were measured in cell lysates at 25°C by a sensitive fluorometric assay (5). Fifty microliters of lysed cell suspension were added to 2.0 ml of a buffer containing 0.1 mM EDTA and 50 mM potassium phosphate at pH 7.4. The reaction was initiated by addition of 20 μl of 1 mM pterine, and the change in fluorescence with time was recorded on a Perkin Elmer LS 50B luminescence spectrophotometer with excitation at 345 nm and emission at 390 nm. XO activity was determined by the rate of change in fluorescence with time in the presence of pterine alone; methylene blue (20 μl of 1 mM) was then added, and the change in fluorescence was again recorded to measure total xanthine oxidoreductase activity. The reaction was then stopped by addition of allopurinol to a final concentration of 1 mM. The fluorescence at 390 nm was then measured before and after addition of 20 μl of 10 μM isoxanthopterin to the assay mixture as an internal standard. The number of units of XO and total xanthine oxidoreductase activity were calculated as previously described (5).

In experiments involving rat intratracheal instillation of silica, we measured total lung XO activity by mixing 100 μl of supernatant from lung homogenate with 1 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA and 0.1 mM xanthine. Change in absorbance at 295 nm with respect to time was recorded at 37°C on a Shimadzu UV 170 U spectrophotometer. The number of units of XO activity, where one unit catalyzes the oxidation of 1 μmol xanthine/min, was based on a change in extinction of $9.6 \times 10^3 \cdot cm^{-1} \cdot M^{-1}$ at 295 nm for conversion of xanthine to uric acid (31). In later experiments involving rat intraperitoneal injection of ferric-NTA, intratracheal instillation of aluminum or FAS, and intratracheal instillation of silica-H, silica-Zn, or silica-Fe, XO activity and total xanthine oxidoreductase activity were measured in supernatant from lung homogenates using this same assay.

Effect of intratracheal iron salts or silica dusts on in situ immunohistochemistry for lung xanthine oxidoreductase. Polyclonal antibody to xanthine oxidoreductase was prepared by cloning 1,073 nucleotides from the 3′-coding sequence of human aldehyde oxidase cDNA (originally reported as XDH) into the *Escherichia coli* expression vector pQE32 (Qiagen, Chatsworth, CA) after DNA amplification by polymerase chain reaction. Aldehyde oxidase protein expression was induced by the addition of isopropyl β-D-thiogalactopyranoside to cultures of *E. coli* carrying the aldehyde oxidase expression vector. The expressed aldehyde oxidase protein was purified under denaturing conditions using nickel chelated agarose and injected into rabbits for antibody production. Antibodies generated were found to recognize aldehyde oxidase and xanthine oxidoreductase but do not discriminate between oxidase and dehydrogenase forms of xanthine oxidoreductase because of the high degree of similarity between the two. This specific antibody has been employed previously (23).

To demonstrate the sensitivity of the antibody employed in immunohistochemical staining, we vacuum slot-blotted bovine XO (Calbiochem, La Jolla, CA) onto 0.45-mm nitrocellulose (Schleicher and Schuell, Keene, NH) in a saline buffer containing 100 mM Tris, pH 8.0. The blot was allowed to air dry, blocked with 5% powdered milk for 30 min, and then incubated with a 1:5,000 dilution of the antibody to XO in 5% dry milk for 2 h. The blot was washed in phosphate-buffered saline-Tween 0.05% and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG in 5% dry milk for 1 h. Detection was accomplished on film using enhanced chemiluminescence reagents as per manufacturer's instructions (Amersham, Arlington Heights, IL). Using comparable slot blot methodology, we found no cross-reactivity demonstrated between the antibody used and aldehyde dehydrogenase.

Sixty-day-old male Sprague-Dawley rats were intratracheally instilled with either 0.3 ml of normal saline and 500 μM aluminum ammonium sulfate in 0.3 ml of saline or 500 μM FAS in 0.3 ml of saline. After 2 and 24 h, animals ($n = 2 \cdot exposure^{-1} \cdot time\ point^{-1}$) were anesthetized with 5% halothane and exsanguinated. Lungs were excised, and inflation was fixed with 10% formalin for 24 h. Thin tissue sections were floated on a protein-free water bath, mounted on silane-treated slides, air-dried overnight, and heat-fixed to stain for xanthine oxidoreductase.

In separate experiments, rats ($n = 2$/exposure) were intratracheally instilled with either 0.3 ml of saline or 1,000 μg silica-H, silica-Zn, or silica-Fe in 0.3 ml of saline. After 2 h, animals were anesthetized with halothane and exsanguinated, and lungs were excised for sectioning and staining for xanthine oxidoreductase.

To stain for xanthine oxidoreductase, we treated sections with hydrogen peroxide in methanol (1:16) to block endogenous peroxidase. Nonspecific staining of highly charged protein was blocked with incubation in Cyto Z Background Buster (Innovex Biosciences, Lenexa, KS) for 10 min at 37°C. The excess solution was removed, and the primary antibody was applied at a dilution of 1:75 in phosphate-buffered saline containing 1% bovine serum albumin. After incubation at room temperature for 45 min, slides were washed in Cyto Q HRP Enhancing Wash buffer three times (2 min each wash). Biotinylated linking antibody from Stat-Q Staining Systems was applied and incubated for 10 min at room temperature. Slides were washed again in Cyto Q HRP-Enhancing Wash buffer three times (2 min each wash). Peroxidase enzyme label was applied and incubated for 10 min at room temperature. Slides were washed again three times with Cyto Q HRP-Enhancing Wash buffer (2 min each wash). Sections were developed with 3,3′-diaminobenzidine-tetrahydrochloride for 3 min at room temperature and washed with tap water for 2 min. Slides were then counterstained with hematoxylin (Fisher Scientific, Raleigh, NC). Controls included stains of lung tissue without the polyclonal antibody added.

Slot blots for lung xanthine oxidoreductase. Animals were intratracheally instilled with 0.3 ml of normal saline, 500 μM aluminum ammonium sulfate in 0.3 ml of saline, or 500 μM FAS in 0.3 ml of saline. At 24 h, the animals were euthanized, the lungs were excised, and the tissue was homogenized. Aliquots of homogenate containing 10 μg of protein were vacuum slot-blotted, allowed to air dry, blocked with 5% powdered milk for 30 min, and then incubated with a 1:500 dilution of the antibody to XO in 5% dry milk for 2 h. The blot was washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG for 1 h. Detection was accomplished on film using enhanced chemiluminescence reagents as per manufacturer's instructions (Amersham). The negative was quantitated using a BioImage Densitometer (BioImage, Ann Arbor, MI).

Effect of iron salts or silica dusts on levels of uric acid in bronchoalveolar lavage. Sixty-day-old male Sprague-Dawley rats were intratracheally instilled with 0.3 ml of normal saline, 500 μM aluminum ammonium sulfate in 0.3 ml of saline, or 500 μM FAS in 0.3 ml of saline. After 24 h, animals ($n = 4 \cdot exposure^{-1} \cdot time\ point^{-1}$) were anesthetized with 5% halothane and exsanguinated. The trachea was cannulated, and lungs were lavaged by instillation and withdrawal three

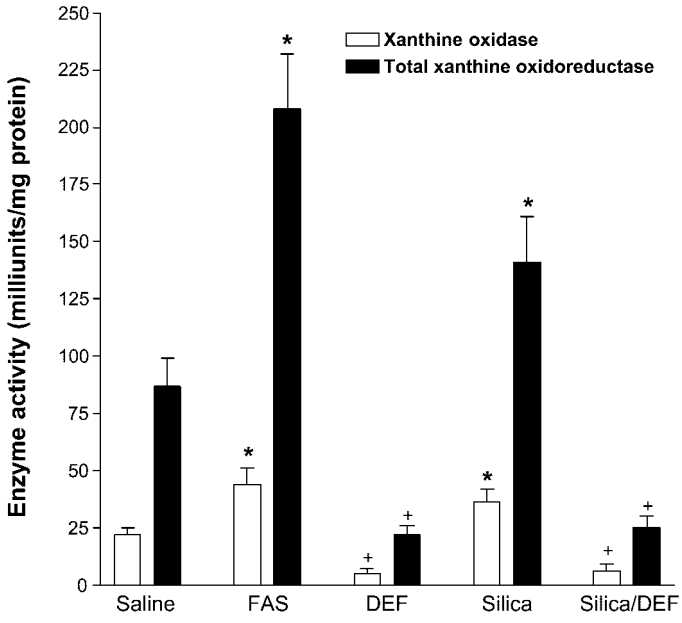

Fig. 1. Effect of deferoxamine and silica on xanthine oxidase (XO) activity in Chinese hamster V79 cells. Cells suspensions were exposed to 500 μM ferric ammonium sulfate (FAS), 100 μM deferoxamine (DEF), 1,000 μg/ml silica, or DEF and silica for 2 h, and then xanthine oxidase (XO) and total xanthine oxidoreductase (XOR) activities were measured by a sensitive fluorometric assay (14). FAS significantly increased both XO and total XOR activities, whereas the iron chelator DEF significantly decreased both. Comparable with the iron compound, silica significantly increased enzyme activities, and the silica-induced increases were attenuated by DEF. $*P < 0.05$ elevated compared with saline; $+P < 0.05$ decreased compared with silica.

times of a single volume (35 ml/kg body wt) of warm (37°C) normal saline. Bronchoalveolar lavage fluid was centrifuged at 400 *g* for 15 min to separate cells. Perchloric acid was then added to the supernatant to a final concentration of 3%, and samples were stored at −80°C until assay for uric acid.

In separate experiments, rats (n = 4/exposure) were intratracheally instilled with either 0.3 ml of saline, or 1,000 μg of silica-Zn or silica-Fe in 0.3 ml of saline. After 2 h, animals were anesthetized with halothane and exsanguinated, and lungs were lavaged as outlined for measurement of uric acid.

Measurement of uric acid in bronchoalveolar lavage. Thawed lung lavage samples were centrifuged at 20,000 *g* for 30 min at 4°C before assay of uric acid by high performance liquid chromatography with electrochemical detection according to the method of Kutnink et al. (28). The system consisted of a Waters 6000A pump (Waters Associates, Milford, MA), a Waters Z module Radial-PAK cartridge column containing μBondapak C18 packing, and a BAS model LC-4B amperometric detector with a glassy carbon electrode (Bioanalytical Systems, West Lafayette, IN). Data were collected and analyzed from peak areas using a Nelson Analytical 3000 series Chromatography Data System (Nelson Analytical, Cupertino, CA). The lowest detectable concentration was determined from the concentration of uric acid standards to be 0.2 nmol/ml (4 pmol injected in 20 μl). Final results were normalized to lavage protein content to correct for increases in uric acid that might have occurred from exudation of plasma into the airway.

Statistics. Data are expressed as means ± SD. Differences between two groups and multiple groups were compared employing *t*-tests of independent means and analysis of variance, respectively, The post hoc test used was Scheffé's. Two-tailed tests of significance were employed. Significance was assumed at $P < 0.05$.

RESULTS

Experiments with V79 cells. V79 cells contained 22 ± 3 milliunits/mg protein XO activity and 87 ± 12 milliunits/mg protein total xanthine oxidoreductase activity. This was decreased to 0 ± 0 for both XO and XDH activities when 500 μM allopurinol was added to culture medium. Inclusion of 500 μM FAS significantly increased XO and total xanthine oxidoreductase activities (Fig. 1). Similar to results previously reported in bovine pulmonary artery endothelial cells (41), deferoxamine also significantly reduced XO activity in V79 cells (Fig. 1). Conversely, silica significantly increased XO activity in V79 cells, and stimulation of XO by silica was blocked by deferoxamine (Fig. 1). Unmodified silica has considerable iron attached to its surface (14), leading us to suspect that complexed iron might be responsible for augmentation of XO activity by silica dust. This is confirmed by results in Fig. 2. Silica-Fe, but not silica-H or silica-Zn, increased activities of both XO and total xanthine oxidoreductase in V79 cells. The ratio between XO and total xanthine oxidoreductase activities was not changed by any of the silica treatments. Allopurinol, FAS, deferoxamine, unmodified silica, silica-Zn, or silica-Fe caused no increase in trypan blue dye exclusion or LDH release into media, but silica-H significantly increased both the percentage of cells staining for trypan blue (16.3 ± 8.8% vs. 6.2 ± 5.3% for media control, $P < 0.05$) and the percentage of LDH released into media (10.9 ± 3.3% vs. 4.2 ± 1.7% for media control, $P < 0.05$). Although cycloheximide significantly diminished elevations in XO activity after 2-h exposures of V79 cells to FAS, actinomycin had no effect (Fig. 3). Measures of total xanthine oxidoreduc-

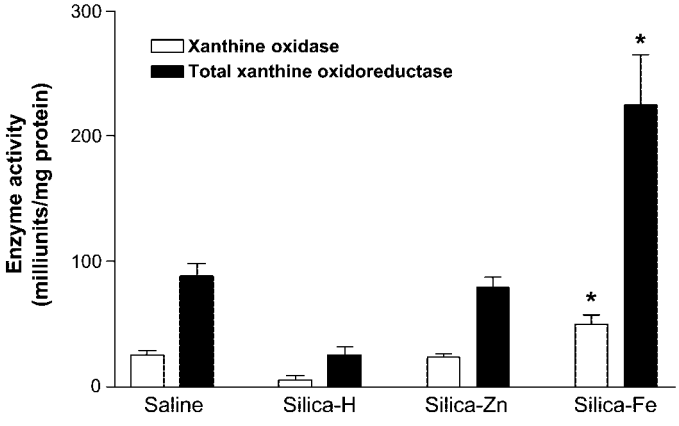

Fig. 2. Effect of metal-free silica or silica complexed to iron or zinc on XO and total XOR activities in Chinese hamster V79 cells. Cells suspensions were exposed to 20 μg/ml acid-washed silica (silica-H), silica complexed with zinc (silica-Zn), or silica complexed with iron (silica-Fe). After 2 h, XO and total XOR activities were measured by a sensitive fluorometric assay (14). Silica-Fe, but not silica-H or silica-Zn, increased activities of both XO and total XOR without changing ratios between the 2 activities. $*P < 0.05$ elevated compared with saline.

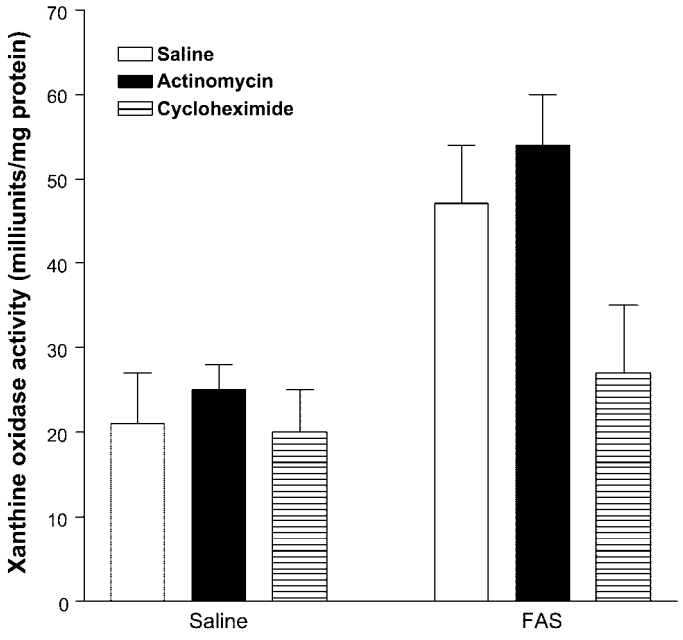

Fig. 3. Effect of actinomycin and cycloheximide on enzyme activity in Chinese hamster V79 cells. Cell suspensions were exposed to either saline or 500 μM FAS after 1 h of pretreatment with either 4 μM actinomycin or 1 μM cycloheximide (neither agent was removed from the 2-h incubation). XO activity was measured by a sensitive fluorometric assay (14). FAS significantly increased XO activity. Treatments with cycloheximide significantly diminished enzyme activities after iron exposure, whereas actinomycin had no effect.

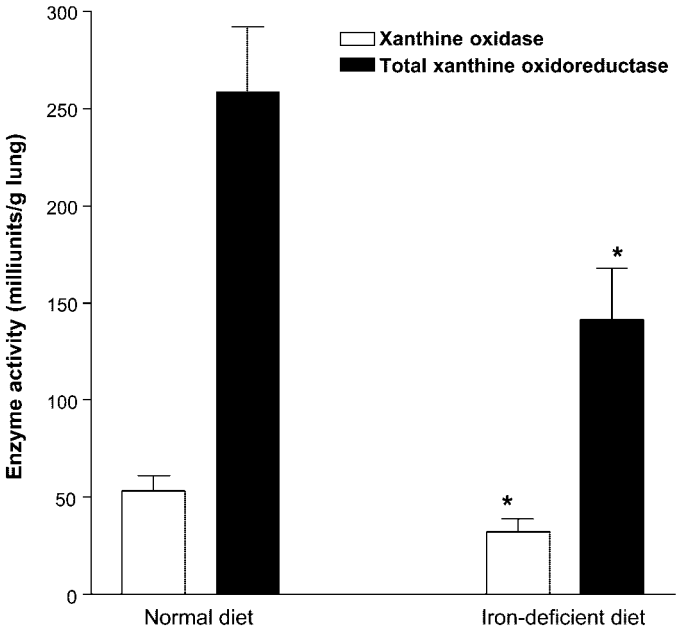

Fig. 4. Iron depletion significantly reduces XO and total XOR activities. Rats were fed a normal or iron-deprived diet, and lung XO and total XOR activities were measured at 8 wk. $*P < 0.05$ compared with respective activity on a normal diet.

tase were similarly decreased after cycloheximide treatment of the cells (data not shown).

Effect of modifying body iron stores on activity of xanthine oxidoreductase. To further test for an association between iron availability and xanthine oxidoreductase activity, we modified body iron stores of rats either with an iron-deficient diet or by parenteral injection of an iron salt. Animals fed an iron-deprived diet had significantly reduced lung XO and total xanthine oxidoreductase activities at 8 wk compared with those on regular chow (Fig. 4). Rats on an iron-deprived diet had slightly lower hematocrits (37.4 ± 1.5 compared with 40.2 ± 4.0% for those on regular chow), but these values were not significantly decreased, suggesting that profound iron deficiency was not induced. In contrast, iron-loading with Fe-NTA significantly increased XO and total xanthine oxidoreductase activities in rat lung at 4 wk (Fig. 5). Rats injected with Fe-NTA had significantly increased concentrations of nonheme iron in the lung (115 ± 17 μM) compared with saline-injected animals (80 ± 16 μM), confirming an elevated burden of the metal in this tissue. In both iron-deprivation and iron-supplementation experiments, the ratio of lung XO to total xanthine oxidoreductase activities did not significantly change.

Effect of intratracheal iron salts or silica dusts on activity of lung xanthine oxidoreductase. Lung XO and total xanthine oxidoreductase activities were also significantly increased 2 h after direct injection of FAS intratracheally (Fig. 6*A*), and they remained elevated at 24 h (Fig. 6*B*). Intratracheal aluminum ammonium sulfate had no effect on lung XO or total xanthine oxidoreductase activities (Fig. 6, *A* and *B*), and iron injection produced no significant change in the ratio of the oxidase and dehydrogenase forms of the enzyme.

As in V79 cells, unmodified silica significantly increased activity of XO in rat lungs 2 h after intratra-

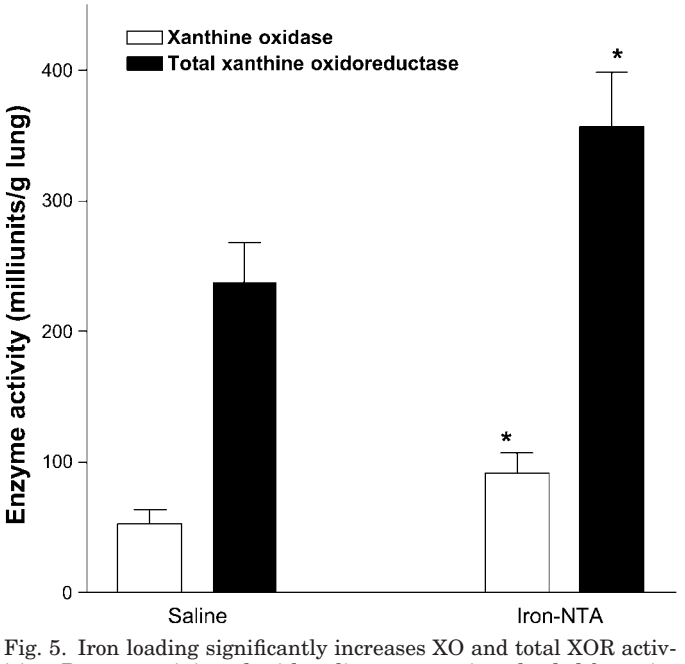

Fig. 5. Iron loading significantly increases XO and total XOR activities. Rats were injected with saline or were iron loaded by twice weekly intraperitoneal injection with ferric-nitrilotriacetic acid (iron-NTA, 15 mg/kg), and lung XO and total XOR activities were measured at 4 wk. $*P < 0.05$ compared with respective activity in saline-injected animals.

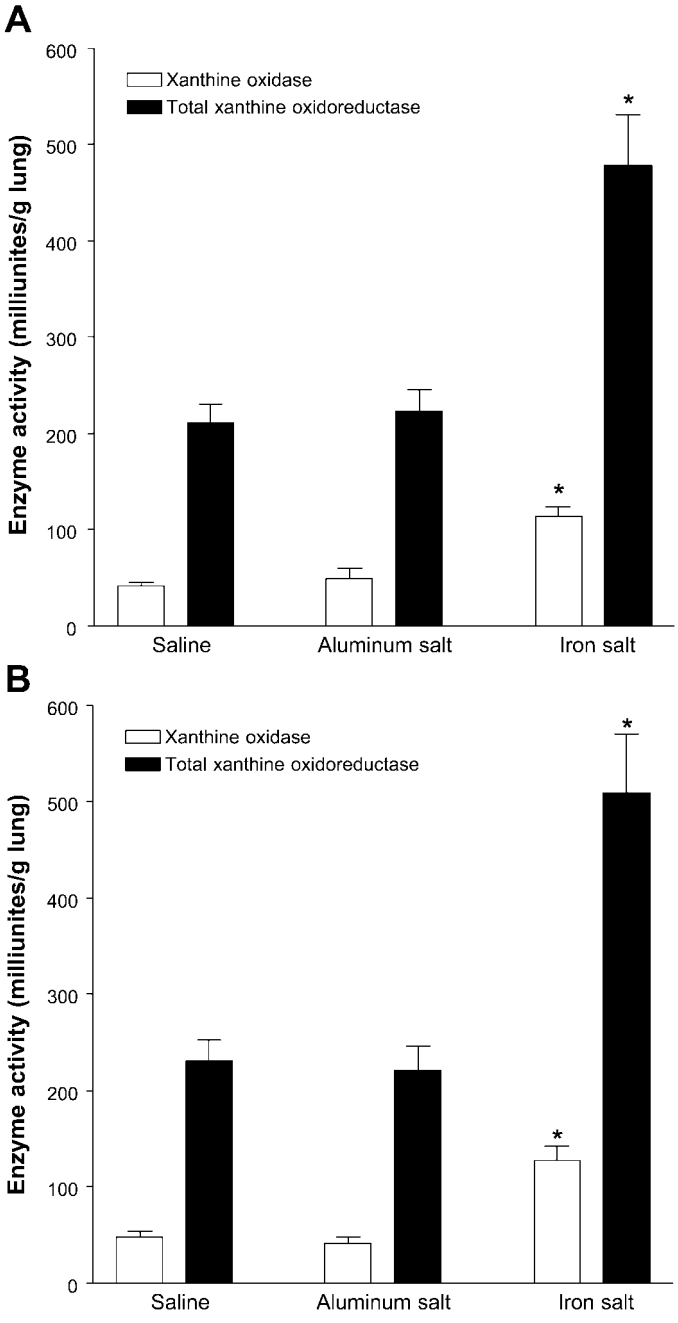

Fig. 6. Intratracheal instillation of iron salts increases lung XO and total XOR activities. Rats were intratracheally injected with 0.3 ml of saline, or 500 μM aluminum ammonium sulfate or FAS in 0.3 ml of saline. Lung XO and total XOR activities were measured 2 (*A*) and 24 (*B*) h later. $*P < 0.05$ compared with respective activity in saline-instilled animals.

cheal injection (45 ± 6 milliunits/g wet lung vs. 20 ± 4 for saline control, $P < 0.05$). Figure 7 shows that XO and total xanthine oxidoreductase activities increased in rat lung at 2 h only after intratracheal injection of silica-Fe, and not silica-H or silica-Zn, confirming that silica enhanced enzyme activity by increasing the locally available lung concentration of iron.

Effect of intratracheal iron salts or silica dusts on in situ immunohistochemistry for lung xanthine oxidoreductase. Slot blots verified a sensitivity of the antibody used for immunohistochemistry toward commercially available XO (Fig. 8). Although not providing quantitative data, in situ immunohistochemistry suggests that the increase in xanthine oxidoreductase in rat lungs in response to iron salts was the result of increased levels of protein rather than simply enhanced enzymatic activity. Compared with lungs instilled with either saline (Fig. 9*A*) or aluminum ammonium sulfate (Fig. 9*B*), lungs demonstrated significantly increased immunoreactivity for xanthine oxidoreductase 2 (Fig. 9*C*) and 24 h (Fig. 9*D*) after

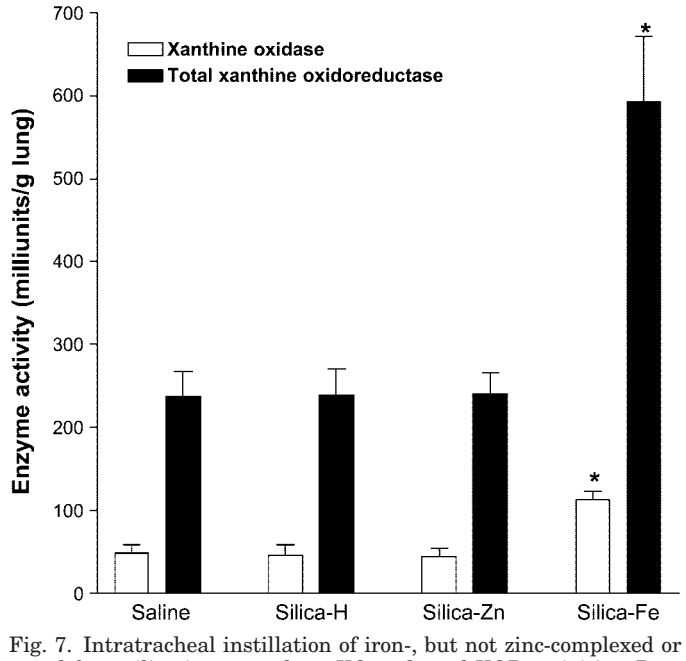

Fig. 7. Intratracheal instillation of iron-, but not zinc-complexed or metal-free, silica increases lung XO and total XOR activities. Rats were intratracheally injected with 0.3 ml of saline or 1,000 μg of silica-Fe, silica-Zn, or silica-H in 0.3 ml of saline. Lung XO and total XOR activities were measured 2 h later. $*P < 0.05$ compared with respective activity in saline-instilled animals.

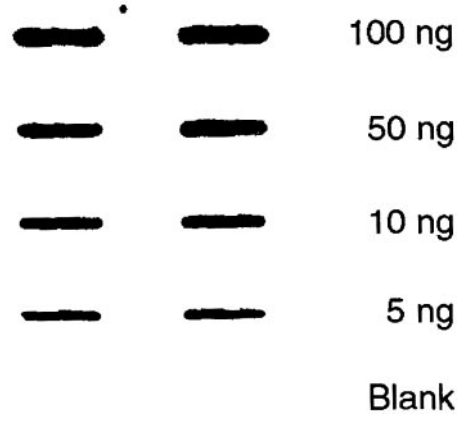

Fig. 8. Sensitivity of immunodetecting antibody for XO. The indicated quantities of bovine XO were blotted onto nitrocellulose. The blot was then blocked with casein and incubated with the antibody followed by a horseradish peroxidase-conjugated goat anti-rabbit antibody. Bands were visualized using enhanced chemiluminescence reagents.

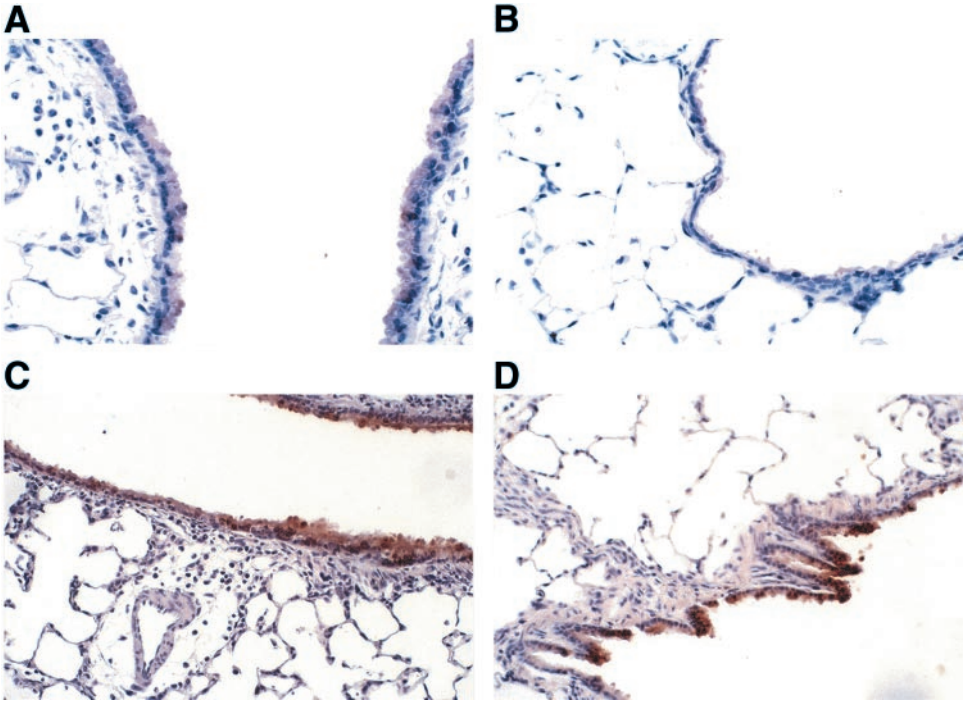

Fig. 9. Intratracheal instillation of iron salts increases immunostainable XOR in lung epithelium. Rats were intratracheally injected with 0.3 ml of saline (*A*) or 500 μM aluminum ammonium sulfate (*B*) or FAS (*C* and *D*) in 0.3 ml of saline. Lung were harvested for XOR immunostaining 2 (*A–C*) or 24 h (*D*) later. Enhanced immunostaining was noted in the alveolar epithelium of iron-treated lungs but was greatest in airway epithelium.

intratracheal treatment with FAS. In lungs administered either FAS or silica-Fe, antibody staining was noted in alveolar epithelium but was greatest in airway epithelium (Fig. 9, *C* and *D*). Similarly, silica-Fe caused markedly enhanced lung xanthine oxidoreductase staining 2 h after intratracheal instillation, whereas immunoreactivity of lungs treated with silica-H or silica-Zn was not different from saline control lungs (data not shown).

Slot blots for lung xanthine oxidoreductase. Twenty-four hours after intratracheal instillation, a significant elevation in protein was evident by slot blot after exposure to FAS relative to saline (Fig. 10). Instillations with aluminum ammonium sulfate were not associated with a similar increased expression of xanthine oxidoreductase in the lung.

Effect of iron salts or silica dusts on levels of uric acid in bronchoalveolar lavage. Urate in bronchoalveolar lavage was dramatically increased after instillation of iron salts or silica-Fe. Compared with lungs instilled with either saline or aluminum ammonium sulfate, bronchoalveolar lavage urate, normalized to protein, was significantly increased 2 h after intratracheal treatment with FAS (Fig. 11*A*). Also, silica-Fe caused marked increase in normalized lavage urate concentration, whereas lavage urate from lungs treated with silica-Zn was only slightly increased from saline control lungs (Fig. 11*B*).

DISCUSSION

The major finding of our studies is that iron appears to regulate the activity of lung xanthine oxidoreductase. Elevating and reducing iron concentrations increases and decreases xanthine oxidoreductase activity, respectively, and, as a consequence, levels of urate in lung epithelial lining fluid (Fig. 11). This is true whether iron is delivered as a soluble ferric salt (Fig. 9) or an environmentally relevant metal chelate (i.e., a silicate). Iron stimulates an increase in total oxidoreductase activity, rather than causing conversion of the dehydrogenase to the oxidase form of the enzyme, since the ratio of XO to total xanthine oxidoreductase

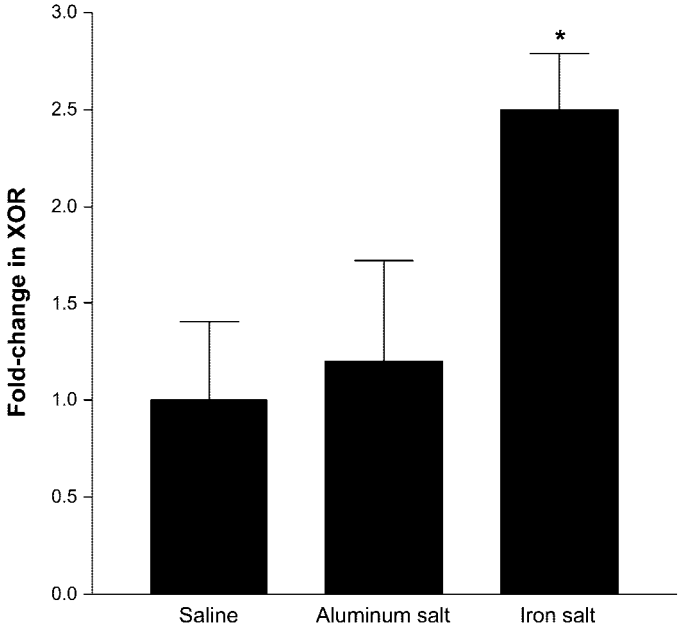

Fig. 10. Lung XOR by slot blot is increased in animals intratracheally instilled with 500 μM FAS relative to those exposed to saline alone. At 24 h, densitometry demonstrates a twofold elevation after instillation with the iron salt relative to saline. Instillation with 500 μM aluminum ammonium sulfate had no significant effect on lung XOR by slot blot. $^*P < 0.05$ compared with saline.

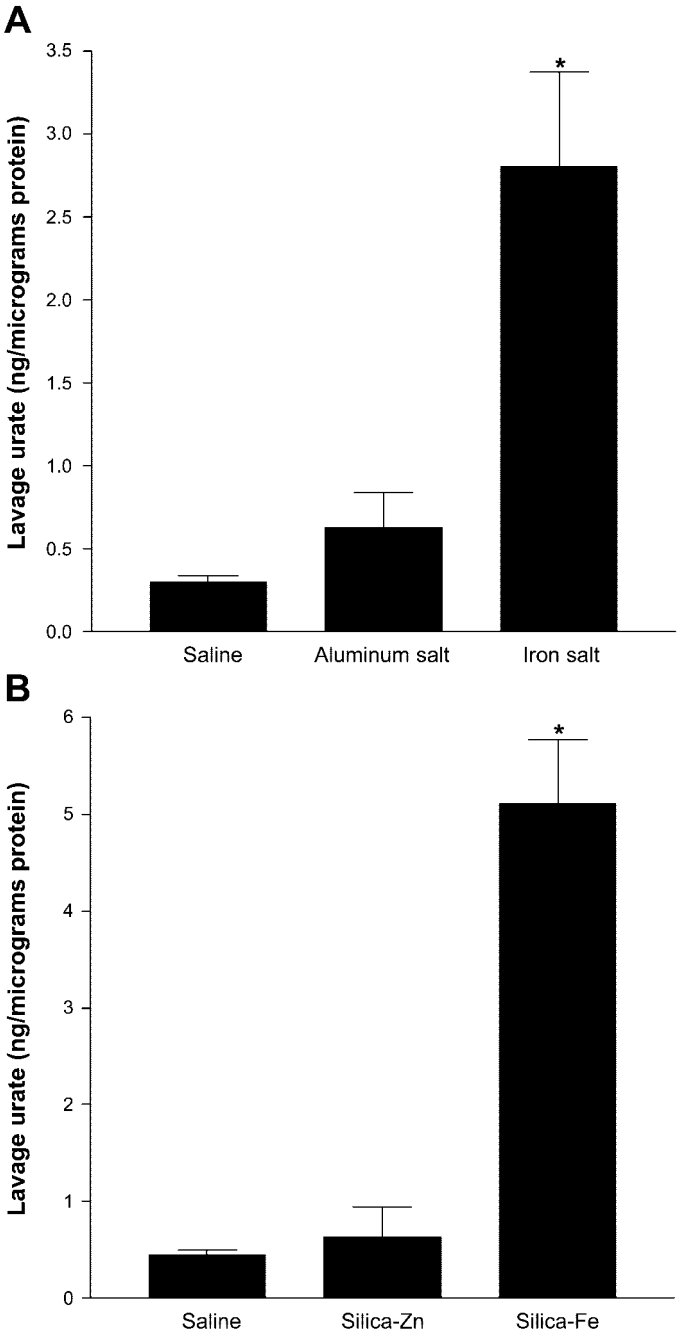

Fig. 11. Intratracheal instillation of iron salts or iron-loaded silica increases concentration of urate in bronchoalveolar lavage. Rats were intratracheally injected with 0.3 ml of saline, 0.3 ml of saline containing 500 μM aluminum ammonium sulfate or FAS (*A*) or 0.3 ml of saline silica-Zn or silica-Fe (*B*). Lungs were lavaged 2 h later and analyzed for urate and protein concentrations. Bronchoalveolar lavage urate was normalized to lavage protein concentration to correct for differences due to exudation of plasma into the airway. $^*P < 0.05$ compared with saline.

activities does not change with iron manipulation. Regulation appears to be exerted at least in part at a translational level (Fig. 3), so that an increase in iron within the local tissue environment results in a rapid, local increase in expression of protein levels of xanthine oxidoreductase (Fig. 9), rather than simply enhanced enzyme activity (Figs. 6 and 7). Oscillations in intestinal XO activity have been previously reported in direct relation with dietary iron (10, 20, 27). Low iron intake decreases and high iron intake increases intestinal XO activity (10, 20, 27). In contrast, hepatic XO increases slightly in response to iron deficiency (27), an effect that may be due to increased protein half-life (7). To our knowledge, no one has previously reported an increase in lung xanthine oxidoreductase activity and protein in response to iron exposure.

If a major purpose of xanthine oxidoreductase teleologically is to produce soluble urate as an iron chelator and antioxidant, a system for enhancing enzyme levels and activity directly in relation to local availability of iron would be logical and elegant. Although iron is necessary for cellular growth and aerobic respiration, this metal can also catalyze formation of reactive oxygen species that cause a potentially detrimental stress to the organism (19). Mammalian cells in general (21, 29) and human airway epithelium in particular (11) have adapted such an iron-responsive system for increasing production of the cellular iron storage protein ferritin in response to enhanced iron in the cellular environment. Such an iron-responsive system would also seem appropriate for control of production of uric acid if this purine degradation product has been evolutionarily harnessed as an antioxidant (2, 4) and iron chelator (8). This would be especially true in the oxygen-rich, lung epithelial environment. Uric acid has been shown to be abundant in bronchoalveolar lavage fluid from humans, with levels two- to eightfold higher in humans than in rats or guinea pigs (44). Uric acid has been identified as a major antioxidant in human nasal airway secretions (37). Human nasal epithelium contains no activity for xanthine oxidoreductase (38). In the nose, uric acid is thought to be taken up secondarily from plasma, concentrated and secreted by nasal glands in response to events such as cholinergic stimulation (38). This could also be true in the lung airway. Compared with studies in pulmonary endothelium, relatively few studies have been performed on XO in lung epithelium. However, XO activity has been identified in bronchoalveolar lavage fluid in mice and guinea pigs (1, 25) and in cultured alveolar epithelial cells (36). We have identified xanthine oxidoreductase protein in airway epithelium from rat lung sections (Fig. 9) and have found cytokine-regulated xanthine oxidoreductase mRNA and activity in BEAS-2B cells, a simian virus-40-transformed human bronchial epithelial cell line (data not shown). This suggests that urate could also be made and secreted locally by respiratory epithelium, regulated in response to an environmentally related increase of iron in lung airway lining fluid.

At present the mechanisms mediating an increase in xanthine oxidoreductase in response to iron are unclear. Iron-mediated generation of reactive oxygen species could promote conversion of the dehydrogenase to the oxidase form of the enzyme by reversible sulfhydryl oxidation (18, 32). However, in our studies, iron supplementation increased and iron depletion decreased total xanthine oxidoreductase activities, without

changing ratios of oxidase to dehydrogenase forms of the enzyme (Figs. 2, 4–7). The reaction of iron with urate could significantly alter the availability of the latter to inhibit xanthine oxidase. This is an alternative explanation of the elevations in enzyme activity, but this would not explain any increased levels of protein. Nitric oxide (NO) binds to iron-sulfur clusters or sulfhydryl groups of xanthine oxidoreductase, inhibiting its activity (24, 40), and this inhibition is reversed by iron chelates such as hemoglobin (24). NO has therefore been proposed as an endogenous regulatory inhibitor of xanthine oxidoreductase activity (24). Increasing the cellular pool of iron could result in scavenging of NO by formation of iron-nitrosyl complexes (24), thereby releasing xanthine oxidoreductase from inhibition. However, such an NO-mediated regulatory mechanism would not account for the iron-induced increase in levels of xanthine oxidoreductase protein suggested by immunohistochemistry of lung sections (Fig. 9). In Madin-Darby bovine kidney renal tubular epithelial cells, xanthine oxidoreductase activity is induced by tumor necrosis factor (TNF), interferon-γ (IFN-γ), interleukin-1 (IL)-1, and IL-6 in a pattern reminiscent of those immunomodulators active in the acute phase response (39). Thus increased xanthine oxidoreductase activity could represent upregulation of protein synthesis in response to an acute phase reaction, induced perhaps by iron-dependent generation of reactive oxygen species. TNF, IFN-γ, IL-1, and IL-6 transcriptionally increase mRNA for xanthine oxidoreductase (39). Cycloheximide alone enhances levels of xanthine oxidoreductase mRNA ninefold, and immunomodulators induce mRNA in the presence of cycloheximide two- to tenfold above stimulation seen in the absence of this inhibitor of protein synthesis (39). This suggests that in both the unstimulated and stimulated state xanthine oxidoreductase regulation may be under additional control by a short-lived repressor protein and/or a labile protein with RNase activity. In this regard, there are a number of the conserved loop sequences C-A-G-U/A-G-U/C/A (10, 20) of potential iron regulatory elements, similar to those posttranscriptionally regulating ferritin synthesis (21, 29), within the published cDNA sequences for human XDH (47).

Our findings have a number of implications. First, by linking xanthine oxidoreductase activity to iron availability, the lung may have evolved a control mechanism to upregulate urate production in response to increased airway concentrations of a potentially harmful metal that must be chelated and sequestered to protect tissues from oxidant stress. This would provide rapid production of urate to complex the iron presented to the lung airway as a consequence of the inhalation of silicates causing pneumoconioses (12–14), coal dust (15), particulate air pollution (9, 16), and even iron complexed to humic acid-like substances abundant in cigarette smoke (17). Organs other than the lung may also express such a control system for xanthine oxidoreductase. Local stereotactic injection of ferric iron greatly increases uric acid concentration within the substantia nigra (46), an observation with relevance for the relationship of oxidant stress to development of Parkinson's disease. Second, if the relationship between iron presentation and xanthine oxidoreductase activity is true not only for the lung but for the organism as a whole, there are possible ramifications for the pathogenesis of gout attacks, the treatment of hyperuricemia, and the prevention of gouty arthritis. Instead of simply presenting an additional purine load, ingestion of red meat, long associated with gout attacks, might also promote uric acid production by offering iron in a particularly absorbable form, the porphyrin chelate. Also, population studies have shown that plasma uric acid levels rise with age and are higher in men than women (26, 30, 43, 45), paralleling age- and sex-related differences in total body iron stores (33). Rather than preventing generation of a remarkably beneficial iron chelator and antioxidant with allopurinol or increasing its urinary excretion with probenecid, perhaps a more rational treatment for hyperuricemia might be not only the avoidance of highly absorbable iron in red meat, but also regular voluntary donation of blood to decrease the relatively elevated body iron stores of men and postmenopausal women, thereby balancing decreased uric acid production with antioxidant needs.

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REFERENCES

1. **Akaike T, Ando M, Oda T, Doi T, Ijiri S, Araki S, and Maeda H.** Dependence on O_2^- generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. *J Clin Invest* 85: 739–745, 1990.
2. **Ames BN, Cathcart R, Schwiers E, and Hochstein P.** Uric acid provides an antioxidant defence in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci USA* 78: 6858–6852, 1981.
3. **Athar M, Elmets CA, Bickers DR, and Muktar H.** A novel mechanism for the generation of superoxide anions in hematoporphyrin derivative-mediated cutaneous photosensitization: activation of the xanthine oxidase pathway. *J Clin Invest* 83: 1137–1143, 1989.
4. **Becker B.** Towards the physiological function of uric acid. *Free Radic Biol Med* 14: 615–631, 1993.
5. **Beckman JS, Parks DA, Pearson JD, Marshall PA, and Freeman BA.** A sensitive fluorometric assay for measuring xanthine dehydrogenase and oxidase in tissues. *Free Radic Biol Med* 6: 607–615, 1989.
6. **Bray RC.** Molybdenum iron-sulfur flavin hydroxylases and related enzymes. In: *The Enzymes,* edited by Boyer PD. New York: Academic, 1975, vol. 12, p. 299–419.
7. **Cherry DM and Amy NK.** Effect of dietary protein and iron on the fractional turnover rate of rat liver xanthine oxidase. *J Nutr* 117: 2054–2060, 1987.
8. **Davies JJA, Sevanian A, Muakkassah-Kelly SF, and Hochstein P.** Uric acid-iron iron complexes. A new aspect of the antioxidant functions of uric acid. *Biochem J* 235: 747–754, 1986.
9. **Dreher KL, Jaskot RH, Lehmann JR, Richards JH, McGee JK, Ghio AJ, and Costa DL.** Soluble transition metals mediate

residual oil fly ash induced acute lung injury. *J Toxicol Environ Health* 50: 285–305, 1997.
10. **Furth-Walker D and Amy NK.** Regulation of xanthine oxidase activity and immunologically detectable protein in rats in response to dietary protein and iron. *J Nutr* 117: 1697–1703, 1987.
11. **Ghio AJ, Carter JD, Samet JM, Quay J, Wortman IA, Richards JH, Kennedy TP, and Devlin RB.** Ferritin expression after in vitro exposures of human alveolar macrophages to silica is iron-dependent. *Am J Respir Cell Mol Biol* 17: 533–540, 1997.
12. **Ghio AJ, Jaskot RH, and Hatch GE.** Lung injury after silica instillation is associated with an accumulation of iron in rats. *Am J Physiol Lung Cell Mol Physiol* 267: L686–L692, 1994.
13. **Ghio AJ, Kennedy TP, Whorton AR, Crumbliss AL, Hatch GE, and Hoidal JR.** Role of surface complexed iron in oxidant generation and lung inflammation induced by silicates. *Am J Physiol Lung Cell Mol Physiol* 263: L511–L518, 1992.
14. **Ghio AJ, Pritchard RJ, Lehman JR, Winsett DW, and Hatch GE.** Lung inflammation after exposure to non-fibrous silicates increases with chelatable [Fe^{3+}]. *J Toxicol Environ Health* 49: 11–28, 1996.
15. **Ghio AJ and Quigley DR.** Complexation of iron by humic-like substances in lung tissue: role in coal workers' pneumoconiosis. *Am J Physiol Lung Cell Mol Physiol* 267: L173–L179, 1994.
16. **Ghio AJ, Stonehuerner J, Pritchard RJ, Piantadosi CA, Quigley DR, Dreher KL, and Costa DL.** Humic-like substances in air pollution particulates correlate with concentrations of transition metals and oxidant generation. *Inhal Toxicol* 8: 479–494, 1996.
17. **Ghio AJ, Stonehuerner J, and Quigley DR.** Humic-like substances in cigarette smoke condensate and lung tissue of smokers. *Am J Physiol Lung Cell Mol Physiol* 266: L382–L388, 1994.
18. **Granger DN.** Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 255: H1269–H1275, 1988.
19. **Halliwell B and Gutteridge JMC.** Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 186: 1–85, 1990.
20. **Hamalainen MM and Makinen KK.** Duodenal xanthine oxidase (EC1.232) and ferroxidase activities in the rat in relation to the increased iron absorption caused by peroral xylitol. *Br J Nutr* 44: 493–498, 1985.
21. **Harrison PM and Arosio P.** The ferritins: molecular properties, iron storage function and cellular regulation. *Biochim Biophys Acta* 1275: 161–203, 1996.
22. **Hasan NM, Cundall RB, and Adams GE.** Effects of hypoxia and reoxygenation on the conversion of xanthine dehydrogenase to oxidase in Chinese hamster V79 cells. *Free Radic Biol Med* 11: 179–185, 1991.
23. **Hassoun PM, Yu FS, Cote CG, Zulueta JJ, Sawhney R, Skinner KA, Skinner HB, Parks DA, and Lanzillo JJ.** Upregulation of xanthine oxidase by lipopolysaccharide, interleukin-1, and hypoxia. Role in acute lung injury. *Am J Respir Crit Care Med* 158: 299–305, 1998.
24. **Hassoun PM, Yu FS, Zulueta JJ, White AC, and Lanzillo JJ.** Effect of nitric oxide and cell redox status on the regulation of endothelial cell xanthine dehydrogenase. *Am J Physiol Lung Cell Mol Physiol* 268: L809–L817, 1995.
25. **Ikuta N, Sugiyama S, Takagi K, Satake T, and Ozawa T.** Implication of oxygen radicals on airway hyperresponsiveness after ovalbumin challenge in guinea pigs. *Am Rev Respir Dis* 145: 561–565, 1992.
26. **Kannel WB.** Metabolic risk factors for coronary heart disease in women: perspective from the Framingham Study. *Am Heart J* 114: 413–419, 1987.
27. **Kelley M and Amy NK.** Effect of molybdenum-deficient and low iron diets on xanthine oxidase activity and iron status in rats. *J Nutr* 114: 1652–1659, 1984.
28. **Kutnink MA, Skala JH, Sauberlich HE, and Omaye ST.** Simultaneous determination of ascorbic acid, isoascorbic acid (erythorbic acid) and uric acid in human plasma by high-performance liquid chromatography with amperometric detection. *J Liq Chromatogr* 8: 31–46, 1985.
29. **Leibold EA and Guo B.** Iron-dependent regulation of ferritin and transferrin receptor expression by the iron-responsive element binding protein. *Annu Rev Nutr* 12: 345–368, 1992.
30. **Loenen HM, Eshuis H, Lowik MR, Schouten EG, Hulshof KF, Odink J, and Kok FJ.** Serum uric acid correlates in elderly men and women with special reference to body composition and dietary intake (Dutch Nutrition Surveillance System). *J Clin Epidemiol* 43: 1297–1303, 1990.
31. **Massey V, Brumby PE, and Koma H.** Studies on milk xanthine oxidase. Some spectral and kinetic properties. *J Biol Chem* 244: 1682–1691, 1969.
32. **McCord JM.** Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* 312: 158–163, 1985.
33. **Milman N.** Serum ferritin in Danes: studies of iron status from infancy to old age, during blood donation and pregnancy. *Int J Hematol* 63: 103–135, 1996.
34. **Miura T, Muraoka S, and Ogiso T.** Inhibitory effect of urate on oxidative damage induced by adriamycin-Fe^{3+} in the presence of H_2O_2. *Res Commun Chem Pathol Pharmacol* 79: 75–85, 1993.
35. **Paller MS and Neumann TV.** Reactive oxygen species and rat renal epithelial cells during hypoxia and reoxygenation. *Kidney Int* 40: 1041–1049, 1991.
36. **Panus PC, Burgess B, and Freeman BA.** Characterization of cultured alveolar epithelial cell xanthine dehydrogenase/oxidase. *Biochim Biophys Acta* 1091: 303–309, 1991.
37. **Peden DB, Hohman R, Brown ME, Mason RT, Berkebile C, Fales HM, and Kaliner MA.** Uric acid is a major antioxidant in human nasal airway secretions. *Proc Natl Acad Sci USA* 87: 7638–7642, 1990.
38. **Peden DB, Swiersz M, Ohkubo K, Hahn B, Emery B, and Kaliner MA.** Nasal secretion of the ozone scavenger uric acid. *Am Rev Respir Dis* 18: 455–461, 1993.
39. **Pfeffer KD, Hueckstead TP, and Hoidal JR.** Xanthine dehydrogenase and xanthine oxidase activity and gene expression in renal epithelial cells. Cytokine and steroid regulation. *J Immunol* 135: 1789–1797, 1994.
40. **Rinaldo JE, Clark M, and Parinello J.** Nitric oxide inactivates xanthine oxidase in interferon stimulated macrophages. *Am J Respir Cell Mol Biol* 11: 625–630, 1994.
41. **Rinaldo JE and Gorry M.** Protection by deferoxamine from endothelial injury: a possible link with inhibition of intracellular xanthine oxidase. *Am J Respir Cell Mol Biol* 3: 525–533, 1990.
42. **Roch-Ramel F and Peters G.** Urinary excretion of uric acid in nonhuman mammalian species. *Hand Exp Pharmacol* 51: 211–255, 1978.
43. **Scott JT, Sturge RA, Kennedy AC, Hart DP, and Buchanan WW.** Serum uric acid levels in England and Scotland. *Adv. Exper Med Biol* 76B: 214–222, 1977.
44. **Slade R, Crissman K, Norwood J, and Hatch G.** Comparison of antioxidant substances in bronchoalveolar lavage cells and fluid from humans, guinea pigs, and rats. *Exp Lung Res* 19: 469–484, 1993.
45. **Thefeld W, Hoffmeister H, Busche EW, Koller PU, and Vollmar J.** Normal values of serum uric acid levels in relation to age and sex as determined using a new enzymatic uric acid color test. *Dtsch Med Wochenschr* 98: 380–383, 1973.
46. **Ward VL, McGinty JF, and Church WH.** Iron (III) chloride injection increases nigral uric acid in guinea-pig. *Neuroreport* 4: 787–790, 1993.
47. **Xu P, Hueckstead TP, Harrison R, and Hoidal JR.** Molecular cloning, tissue expression of human xanthine dehydrogenase. *Biochem Biophys Res Commun* 199: 998–1004, 1994.