Depletion of Intracellular Ascorbate by the Carcinogenic Metals Nickel and Cobalt Results in the Induction of Hypoxic Stress*

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Nickel and cobalt have wide industrial usage, which leads to environmental pollution by these metals and their by-products at all stages of production, recycling, and disposal (1, 2). Additionally, burning of fossil fuels pollutes the air with metal-containing particles, up to 35% of which could be composed of nickel (3). Environmental or occupational exposure to particles containing nickel or cobalt causes various forms of lung injury including pneumonitis, asthma, and fibrosis (4). Both metals are carcinogenic in animals (5, 6), and nickel has been recognized as a human carcinogen (7). The mechanisms of their toxicity and carcinogenicity are not fully understood. An interesting feature of nickel or cobalt exposure is the induction of hypoxia-like stress, which is manifested in cells by the activation of the HIF-1 transcription factor and hypoxia-inducible genes (8–10). It has been suggested that the induction of the hypoxia-like stress by these metals is based on their ability to substitute for an iron atom in an “oxygen sensor” (11). No direct evidence supporting this interesting hypothesis is available, although several studies have demonstrated that Co(II) can inhibit activity of recombinant asparaginyl (12) or prolyl (13) hydroxylase in vitro, presumably because of competition with Fe(II). The induction of hypoxia-like stress by nickel or cobalt has numerous implications. It is well established that HIF-inducible genes are involved in crucial aspects of cancer biology, including angiogenesis, cell survival, glucose metabolism, and invasion (14, 15).

Recently, using HIF-1α normal and knock-out cells, we have shown that nickel promotes soft agar growth via the HIF-1 transcription factor (16). These data indicate that HIF-1 induction may play an important role in nickel-mediated malignant transformation of cells. The HIF-1 transcription factor is a heterodimer composed of α and β subunits (17). The activity of HIF-1 depends on the accumulation of short lived HIFα. Under normoxic conditions, hydroxylation of proline residues 402 and 564 in the ODD of HIF-1α leads to its interaction with the VHL tumor suppressor protein, a part of the ubiquitin-ligase complex, followed by ubiquitylation and rapid proteosomal degradation of HIF-1α (18–20). Under hypoxic conditions, limiting oxygen decreases hydroxylation, which prevents VHL binding and leads to the accumulation of HIF-1α protein. Nickel(II) or cobalt(II) exposure even in the presence of oxygen causes increased accumulation of HIF-1α protein and induction of HIF-1 transcriptional activity (17, 21, 22). The accumulation of HIF-1α protein observed in cells after cobalt(II) exposure resulted from the inability of HIFα to complex with the VHL protein (18). This inability, as we found here, is associated with inhibition of HIF-1α hydroxylation, manifested by both cobalt(II) and nickel(II) exposure. HIF activity can also be affected by the asparaginyl hydroxylation of the C-terminal transactivation domain, which is regulated by FIH-1 (23). Like the prolyl hydroxylase, this enzyme...
is Fe(II)- and ascorbate-dependent and could likewise be affected by metals and contribute to the observed phenotype.

Prolyl hydroxylases are non-heme iron-dependent oxygenases that utilize 2OG as a co-substrate and ascorbic acid as a cofactor (24). It has been proposed that during the enzymatic reaction, in the presence of oxygen and 2OG, the enzyme-bound iron(II) is rapidly converted to iron(III), which causes the inactivation of the enzyme. The role of ascorbate is to reduce iron(III) and thus reactivate the enzyme (25). This scenario implies that depletion or oxidation of iron(II) must lead to the loss of enzymatic activity. In the present paper, we investigated whether nickel(II) and cobalt(II) exposure could deplete cellular iron and/or ascorbate and whether such depletion would result in inhibition of prolyl hydroxylase activity. The results clearly indicate that levels of ascorbate rather than iron limit prolyl hydroxylase activity in metal-exposed cells. Because human cells do not produce ascorbate (26), the intracellular ascorbate level depends on a delicate balance of metabolic consumption, uptake, and efflux. Here we demonstrate that the cellular ascorbate is greatly depleted by exposure to both metals. We propose that ascorbate depletion in nickel(II)- or cobalt(II)-exposed cells favors enzyme-bound iron oxidation, which by itself or followed by metal substitution leads to the inactivation of prolyl hydroxylases.

EXPERIMENTAL PROCEDURES

Reagents—NISO 4H9SO 4 was obtained from Alfa Aesar (Ward Hill, MA). FeSO 4·7H 2O, CoCl 2·6H 2O, and L-ascorbic acid were obtained from Sigma, and deferoxamine mesylate (DFX) was obtained from Calbiochem (La Jolla, CA). Pro tease inhibitor mixture was purchased from Roche Applied Science. Dimethyl sulfoxide (DMSO) was purchased from Frontier Scientific (Logan, UT). 1,1'-2,4-Dinitrophenylhydrazine (DNP) in 30% HClO 4 was purchased from Allied Chemicals (Brea, CA).

Plasmids—The construction of HRE-Luc plasmid is described elsewhere (21). DNA encoding the HIF-1α ODD (amino acids 401–602) was fused in-frame to the 3'-end of the luciferase cDNA in the pcDNA3 vector (Invitrogen) to generate the CMV-Luc-ODD plasmid.

Cell Lines and Culture Conditions—The H1AE0— cell line was obtained from Dr. Grunen et (27). Cells were grown on plastic coated with the mixture of bovine serum albumin (Invitrogen) and collagen (Cohe sion, Palo Alto, CA) in minimum essential medium with Earle’s modified salts (Invitrogen) containing 10% fetal calf serum, 2 mM l-glutamine, 100 μg/ml streptomycin, and 100 units/ml penicillin. 786-0 human renal carcinoma cells with mutant VHL or 786-0 wt4 expressing wild type VHL cells were maintained in Dulbecco’s minimum essential medium (Invitrogen) containing 10% fetal calf serum, 2 mM l-glutamine, 100 μg/ml streptomycin, and 100 units/ml penicillin.

Reporter Assays—20,000 H1AE0— cells/well were plated into 96-well plates in 100 μl of medium. The next day cells were transfected with 50 ng/well CMV-Luc-ODD and 200 ng/well pRL-tk Renilla luciferase (RL-tk) or 100 ng/well HRE-Luc and 200 ng/well RL-tk plasmids with the transfection reagent effecten (Qiagen, Valencia, CA). After transfection, plasmid expression was allowed for 16–24 h followed by exposure to metals or other chemicals for an additional 20 h. The reporter expression was detected using the Dual-Olo luciferase system (Promega, Madison, WI) and the Microplate LB 960 luminometer (Berthold Technolo gies, Bad Wildbad, Germany). The data are presented as mean values ± S.D. of normalized relative luciferase units (RLU), which represent the ratio of luminescence produced by CMV-Luc-ODD or HRE-Luc plasmid to the luminescence of the RL-tk reporter in the same condition. Each condition has been tested in quadruplicate in at least three separate experiments. 

Measurement of Ascorbate Levels—HPLC measurements of total intracellular vitamin C levels were performed by HPLC as described previously (28).

Determination of Prolyl Hydroxylase Activity—Prolyl hydroxylase activity was determined as described elsewhere (29). Briefly, cells were collected by scraping in phosphate-buffered saline. The pellet was resus pended in 6 volumes of 0.15 mM MgCl 2, 10 mM KCl, 10 mM Tris-HCl, pH 6.7, and incubated for 2 min on ice. Cells were then homogenized using Dounce homogenizer (30 strokes). Dextran was added to a final concentration 0.25 M, and the homogenate was centrifuged at 15000 × g for 15 min at 4 °C to remove nuclei. The mitochondrial fraction was separated by the second centrifugation at 6500 × g for 10 min at 4 °C. Protein concentration was measured using a Bio-Rad protein assay kit. The hydroxylase reaction was carried out using 30 μg of protein in the reaction buffer containing 40 mM Tris-HCl, pH 7.5, 50 μM FeSO 4, 0.1 mM L-1,5-[14C]l-2-oxoglutaric acid, 200 μM ODD peptide (amino acids 556–576 of HIF-1α) (Advanced ChemTech, Louisville, KY), 0.25 mM ascorbate, 0.4 mg/ml catalase, 0.5 mM dithiothreitol. Samples were incubated for 1 h at 37 °C in a final volume of 50 μl. After incubation, 25 μl of mixture of 20 mM succinate and 20G was added, followed by the addition of 25 μl of 0.16 mM 2,4-dinitrophenylhydrazine in 30% HClO 4. The samples were allowed to sit for 30 min at room temperature following the addition of 50 μl of 1 M 2OG. Supernatants were separated by spinning at 3000 × g for 5 min, and radioactivity was measured in a liquid scintillation counter.

Western Blot Analysis—Protein extracts were obtained after lysing cells in the lysis buffer (Cell Signaling, Beverly, MA) for 15 min at 4 °C. Equal loading of protein was assured by prior quantitation using the Bio-Rad assay. Protein aliquots were separated by gel electrophoresis in 10 or 12% polyacrylamide gels and transferred onto a polyvinylidene difluoride membrane (Roche Applied Science). Western blot analysis of hypoxia-inducible NDRG-1/Cap43 protein levels was performed using rabbit polyclonal antibody as described previously (30). Antibodies against α-tubulin were purchased from Sigma. Immunoreactive bands were detected using horseradish peroxidase-linked secondary antibody and enhanced chemiluminescence (PerkinElmer Life Sciences).

RESULTS

Effect of Nickel(II) or Cobalt(II) on Hydroxylation of Pro-402/564 in HIF-1α- and HIF-dependent Transcription—The hypothetical mechanism of induction of the hypoxia-like stress by metals suggests substitution for iron in an oxygen sensor (11). To find whether nickel can directly substitute for iron in prolyl hydroxylases, H1AE0— cells were fractionated, and ODD-dependent activity (Pro-564) was tested in the nuclear, mitochondrial, and microsomal fraction. No ODD-dependent activity was found in the nuclear fraction. A high level of ODD-independent activity was observed in the mitochondrial fraction. The ODD-dependent prolyl hydroxylase activity in the microosomal fraction was tested using 0–400 μM ODD substrate (Fig. 1A). 200 μM ODD peptide was selected for reactions in the presence of NiSO 4 or CoCl 2. Addition of different concentrations of NiSO 4 or CoCl 2 from 0.05 to 0.5 mM to cell extracts revealed 15–25% inhibition of prolyl hydroxylase activity (Fig. 1B). This inhibition may result from partial substitution of iron in the active center, from a direct interaction of the enzyme with metals, not necessarily at the active center, or from interaction of metals with other components of reaction.

It was well established that hydroxylations of Pro-402/564 located in the ODD of HIF-1α is required for HIF-1α interaction with the VHL tumor suppressor protein. To test whether hydroxylations of Pro-402/564 is affected by nickel(II) or cobalt(II) in living cells, we designed a reporter plasmid expressing a protein containing the HIF1α ODD fragment fused with luciferase (CMV-Luc-ODD). It was expected that similar to the HIF-1α protein, the half-life of CMV-Luc-ODD reporter protein would depend on the hydroxylation status of Pro-402/564. To test this, we investigated whether CMV-Luc-ODD would undergo the VHL-dependent degradation in a prolyl hydroxylation-dependent manner. The CMV-Luc-ODD reporter was transfected into renal carcinoma 786-0 cells lacking functional VHL and into the same cells that were stably transfected with normal VHL (786-0 wt4 cells). No increase in CMV-Luc-ODD expression was found when the CMV-Luc-ODD plasmid-transfected 786-0 cells were exposed to 0.5 mM NiSO 4, 0.25 mM CoCl 2, 0.25 mM DFX, or 1 mM DMOG for 2 h (Fig. 2A). In contrast, significant signal induction/reporter stabilization was found in 786-0 cells containing wild type VHL following exposure to the same agents (Fig. 2B). The level of expression of the reporter plasmid in unexposed cells was higher in
786-0 cells with mutated VHL than in cells with normal VHL. These data indicated that the CMV-Luc-ODD reporter protein is normally degraded via the VHL pathway but can be stabilized by exposure to prolyl hydroxylase inhibitors such as DMOG, iron chelator DFX, and metals like nickel(II) or cobalt(II).

Because the inhalation of metal-containing particles is known to induce inflammation and cancer in the lung, we next studied the effect of both metals on Pro-402/564 hydroxylation and HIF-1-dependent transcription in human lung epithelial cells (1HAEo/H11002). It was expected that the VHL-mediated HIFα degradation pathway is functional in these cells and that the CMV-Luc-ODD reporter protein, in a manner similar to HIFα, would be preferentially degraded under normoxic conditions by the proteasome. Indeed, the addition of proteasomal inhibitor lactacystin stabilized the reporter in human 1HAEo/H11002 cells, providing evidence that the reporter normally is not accumulated and is indeed degraded by proteasomes in these cells (Fig. 3A). Essentially the same results as obtained in 786-0 wt4 cells were obtained in 1HAEo—cells transfected with CMV-Luc-ODD and exposed for 20 h to nickel(II) or cobalt(II) (Fig. 3, A and C), DFX, or DMOG (not shown). The stabilization of the reporter protein by both nickel(II) and cobalt(II) was dose-dependent starting at 0.1 mM NiSO4 or 0.05 mM CoCl2. The level of signal caused by 0.25 mM NiSO4 was comparable with the signal produced by 0.25 mM CoCl2 at 0.5 mM NiSO4, the signal was almost doubled. It was shown previously that exposure to nickel(II) or cobalt(II) stabilizes HIFα and increases HIF-1-dependent transcription (10). Indeed, transfection of the HRE-Luc reporter showed a dose-dependent induction of HIF-dependent transcription in nickel(II)- and cobalt(II)-exposed 1HAEo—cells (Fig. 3, B and D). These data are in good agreement with those obtained for the CMV-Luc-ODD reporter.

Nickel(II)- or Cobalt(II)-induced Hypoxic Stress Is Not Prevented by Iron(II)—Prolyl hydroxylases are iron-containing enzymes. Iron, nickel, and cobalt could be delivered to cells via the divalent metal transporter DMT1 (31). To test whether the inhibition of hydroxylation by nickel(II) or cobalt(II) was because of a competitive inhibition of iron uptake, we supplemented the cell culture medium with iron(II). The addition of ferrous iron alone had no effect on the control CMV-Luc-ODD signal. Co-exposure of cells to equal amounts of iron(II) and nickel(II) or cobalt(II) also did not change the signal produced by the non-iron metals alone (Fig. 3, A and C). As it was found for the CMV-Luc-ODD reporter, the addition of iron(II) also had no effect on the expression of the nickel(II)- or cobalt(II)-induced HIF-dependent reporter (Fig. 3, B and D). Finally, using the same conditions, we analyzed the expression of the
endogenous hypoxia-inducible NDRG-1/Cap43 gene (22) in 1HAEo/H11002 cells. We have found that nickel(II) and cobalt(II) strongly induced NDRG-1/Cap43, whereas the simultaneous addition of these metals with ferrous or ferric iron showed no effect on NDRG-1/Cap43 expression (32). This finding is in full concordance with the results showing a lack of an iron(II) effect on the expression of the Luc reporters.

Nickel(II) and Cobalt(II) Deplete Intracellular Ascorbate—Prolyl hydroxylases require both 2OG and ascorbic acid for full activity. Because 2OG is a part of the tricarboxylic acid cycle, we expected that it would be present in cells in sufficient amounts. However, ascorbate, a well known reducing agent and an antioxidant, can be easily exhausted. Determination of intracellular ascorbate levels following exposure of 1HAEo/H11002 cells to nickel(II) and cobalt(II) for 20 h showed substantial depletion of intracellular ascorbate (Fig. 4A).

Effect of Nickel(II) or Cobalt(II) on Ascorbate Uptake and Efflux—The intracellular level of ascorbate is maintained as a balance of uptake and efflux. To understand why exposure to nickel(II) or cobalt(II) depletes intracellular ascorbate, we investigated both uptake and efflux of 14C-labeled ascorbate in the presence of both metals. Fig. 4C shows that the uptake of ascorbate is blocked by nickel or cobalt, whereas the efflux is only slightly affected (Fig. 4, B and D). Compared with that in the control cells, the initial rapid uptake of ascorbate in both nickel(II)- and cobalt(II)-exposed cells came to a halt 2–4 h after the addition of the metals to the cell culture medium. Then, within the next 16–18 h, the cellular [14C]ascorbate decreased gradually to barely detectable levels (Fig. 4C). This time course could be the result of a relatively slow interaction of the metals (or metal complexes) with critical target molecules (ligands) in the medium and/or cells, including ascorbic acid. To test this possibility, [14C]ascorbate in the complete medium was preincubated for 4 h with metals and then added to cells. As expected, much less 14C-labeled ascorbate was found in cells that received ascorbate preincubated with nickel(II) or cobalt(II) (Fig. 4C).

Ascorbate Prevents the Hypoxia-like Effects of Nickel(II) and Cobalt(II)—The addition of 100 μM ascorbate to the culture medium restored intracellular ascorbate levels in metal-exposed cells as determined by HPLC (not shown). This increase in the intracellular ascorbate level correlated well with the diminished stability of the CMV-Luc-ODD reporter (Fig. 5, A and C) and the activity of the HRE-Luc reporter (Fig. 5, B and D) as well as the hypoxia-inducible protein NDRG-1/Cap43 (Fig. 5E), indicating that the “hypoxia-like stress” produced by both metals was prevented and that prolyl hydroxylase activity was restored by ascorbate complementation.

To confirm that ascorbate specifically alleviates the effect of metals, cells were exposed to metals in the presence of another antioxidant and reducing agent, 2-mercaptoethanol. No effect of 2-mercaptoethanol on reporter hydroxylation or HIF-dependent transcription was found (Fig. 5, A, B, and D). The depletion of glutathione using buthionine sulfoximine, a specific inhibitor of γ-glutamylcysteine synthase, did not stabilize the CMV-Luc-ODD reporter or activate HIF-dependent transcription (not shown). These data confirmed again the importance of ascorbate, rather than glutathione or other reducing molecules, in the regulation of hypoxia-inducible response.
Molecular Basis for Metal-induced Hypoxia

Enzymatic hydroxylation reactions offer an attractive model for the oxygen sensor in the hypoxic response pathway (19, 20). In the course of the hydroxylation reaction, an oxygen molecule is split into two atoms, one of which can be attached in the form of a hydroxyl group to amino acids. This posttranslational modification changes protein-protein interactions. Under normal oxygen conditions, hydroxylation of Pro-402/564 located in the HIF-1α ODD allows for interaction of HIF-1α with the tumor suppressor protein VHL (19, 20), whereas hydroxylation of Asn-803 prevents interaction of the C-terminal transactivation domain of HIF-1α with the CH1 pocket of the P300 protein (23). Exposure to nickel(II) or cobalt(II) results in accumulation of Asn-803 prevents interaction of the C-terminal transactivation domain of HIF-1α with the CH1 pocket of the P300 protein (23). Exposure to nickel(II) or cobalt(II) results in accumulation of HIF-1α and up-regulation of hypoxia-inducible genes in cells even in the presence of oxygen (8, 17, 21, 22). This phenomenon has been described as “metal-induced hypoxia” (10). We investigated the mechanism of inhibition of prolyl hydroxylase activity and HIF-1 induction in human lung cells following nickel(II) or cobalt(II) exposure. For this, we created the CMV-Luc-ODD reporter, which allowed us to evaluate the hydroxylation status of HIF-1α in cells. These results confirmed the involvement of VHL in the degradation of the reporter. It has been established that three prolyl hydroxylases, PHD1, PHD2, and PHD3, can each hydroxylate the HIFα subunit (13, 33). These enzymes belong to the superfamily of iron(II)- and 2OG-dependent dioxygenases (34). To function, they all require ascorbate, which is bound to the enzyme and serves to reduce iron following the hydroxylation reaction (35).

Using an in vitro prolyl hydroxylase assay we assessed nickel(II)- or cobalt(II)-inhibiting activity. The low level of inhibition of proline hydroxylation by these metals in cell extracts and strong induction of the Luc-ODD reporter and HIF-dependent transcription in living cells suggested that enzyme inhibition in cells by nickel(II) or cobalt(II) might be indirect. It was conceivable that other critical components of the prolyl hydroxylation reaction, such as iron, ascorbate, or 2OG, might be affected by metal exposure in cell culture. Ferrous iron, for example, could be transported into cells via a divalent metal transporter DMT1. Nickel(II) or cobalt(II) can be transported by the same transporter (31); therefore, they could, by competition, prevent iron entry into cells. To assess this possibility we exposed cells to nickel(II) or cobalt(II) in the presence of iron(II) or iron(III). The co-exposure had very little effect on either the CMV-Luc-ODD reporter stability or HIF-dependent transcription. The induction of the hypoxia-inducible NDRG1/Cap43 gene by nickel(II) or cobalt(II) was also not affected (32). Moreover, measurements of intracellular iron revealed that the level was not significantly changed following exposure to the hypoxia-inducing metals.

In contrast, measurement of intracellular ascorbate in cells exposed to nickel(II) or cobalt(II) revealed very low ascorbate level. Because human cells are unable to synthesize ascorbate,
it must be provided in the diet and then transported into tissues (26, 36). In a cell culture, serum is the only source of ascorbate because most types of media do not contain ascorbate. The concentration of ascorbate determined in our medium was between 1 and 5 \( \mu \text{M} \). Ascorbate was accumulated in cells in a concentrative manner via uptake of both dehydroascorbic acid (DHA) and ascorbic acid (Fig. 6). DHA is transported into and out of cells by sodium-independent glucose transporters (GLUT 1 and GLUT 3) (37, 38), whereas ascorbic acid enters cells through sodium-dependent ascorbic acid transporters (39–41). Inside the cell, DHA is immediately reduced to ascorbate (42). The intracellular level of ascorbate is therefore regulated by its oxidation status, uptake, and efflux. The exposure of cells to nickel(II) or cobalt(II) only slightly affected the efflux of ascorbate; at the same time it greatly diminished the intracellular accumulation of ascorbic acid. Because both oxidized and reduced forms of ascorbic acid can be delivered into cells, we postulate that the observed decrease in intracellular accumulation of ascorbate is not because of its oxidation to DHA but rather because of metal binding, oxidation, and hydrolytic reactions involving ascorbic acid and other ligands, as well as ambient oxygen (Fig. 6). Such reactions lead to irreversible degradation of ascorbate to smaller products (43, 44). The reactions are known to be greatly accelerated by nickel(II) or cobalt(II) and accelerated even more by their amino acid complexes that are easily formed in a

Fig. 5. Addition of ascorbate induces proline hydroxylation and inhibits HIF-dependent transcription. 1HAEo− cells were transiently transfected with the CMV-ODD-Luc (A and C) or HRE-Luc (B and D). Cells were exposed to 0.5 mM NiSO4 (A and B) or 0.25 mM CoCl2 (C and D) for 24 h after transfection alone or with 10, 20, 50, 100, or 200 \( \mu \text{M} \) ascorbate (Asc). Relative luciferase activity was detected 20 h after exposure to chemicals and expressed as -fold increase over control. The data are presented as mean values ± S.D. 2-ME, 2-mercaptoethanol. E, addition of ascorbate suppresses induction of hypoxia-inducible protein NDRG-1/Cap43. The 1HAEo− cells were exposed to 0.25 mM NiSO4, 0.25 mM CoCl2, or 1 mM DMOG for 20 h alone or with 100 \( \mu \text{M} \) ascorbate. 40 \( \mu \text{g} \) of total protein was resolved over 12% SDS-PAGE. Western blot analysis was performed on the same membrane using antibodies against hypoxia-inducible protein NDRG-1/Cap43 (upper panel) and \( \alpha \)-tubulin (lower panel).
biological environment (45–47). The preincubation of ascorbate with metals in cell culture medium markedly inhibited ascorbate uptake; cobalt(II) exhibited a stronger effect than nickel(II). These data show that cobalt(II) is much more active than nickel(II) in mediating ascorbate destruction. This is consistent with its higher redox activity, oxygen binding capability, and faster complex formation kinetics as compared with nickel(II) (48). The exact mechanism of this effect and identification of the postulated ascorbate degradation products awaits further physicochemical studies.

The above results led us to assume that increased concentrations of ascorbate in the cell culture medium should abolish, or at least alleviate, the effect of metals. Indeed, as we found, addition of ascorbate to cell cultures prevented the inhibition of prolyl hydroxylase and suppressed the HIF-1-dependent gene suppression of HIF-1α transcription. These results are similar to the recently reported suppression of HIF-1α protein induction by cobalt following the addition of ascorbate (49).

The depletion of intracellular ascorbate by nickel(II) and cobalt(II) will inevitably lead to the inactivation of prolyl hydroxylases because iron cannot be maintained in the reduced state. Such inactivation could alternatively/additionally be accomplished through metal substitution in the enzyme. Some inhibition of hydroxylase activity observed in our experiments and data published on isolated recombinant enzymes support this possibility (12). It remains to be determined whether nickel(II) or cobalt(II) in situ can replace iron in prolyl hydroxylases and inactivate the enzymes. It is conceivable, however, that the depletion of ascorbate is by itself sufficient for the inactivation of prolyl hydroxylases and the induction of hypoxia-like stress in cells.

Our data reveal a new mechanism by which nickel(II) or cobalt(II) can produce hypoxia-like stress in cells. They provide insight for understanding the molecular mechanisms of nickel(II)- or cobalt(II)-induced carcinogenesis that may lead to the design of preventive strategies for protecting workers in metal-related industries from lung injuries and lung cancers.

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40344

Molecular Basis for Metal-induced Hypoxia

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