Dexamethasone suppresses eNOS and CAT-1 and induces oxidative stress in mouse resistance arterioles


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Schäfer, S. C., T. Wallerath, E. I. Closs, C. Schmidt, P. M. Schwarz, U. Förstermann, and H.-A. Lehr. Dexamethasone suppresses eNOS and CAT-1 and induces oxidative stress in mouse resistance arterioles. Am J Physiol Heart Circ Physiol 288: H436–H444, 2005; doi:10.1152/ajpheart.00587.2004.—Long-term treatment with glucocorticoids is associated with mild to moderate hypertension. We reported previously that downregulation of endothelial NO synthase (eNOS) expression and activity is likely to contribute to this increase in blood pressure. In the present study, we tested the effects of dexamethasone on the vasodilation of microvascular arterioles using implanted dorsal skin-fold chambers in anesthetized C57BL/6J mice. Experiments were performed on control mice or on mice treated with dexamethasone (0.1–3 mg/kg of body wt). Endothelium-dependent vasodilation in response to ACh (0.1–10 μM) was reduced by dexamethasone in a dose-dependent fashion. Comparable inhibition was seen in tissues superfused with 30 μM Nω-nitro-L-arginine methyl ester. In contrast, endothelium-independent vasodilation in response to S-nitroso-N-acetyl-d,l-penicillamine (10 μM) was not influenced by either dexamethasone or Nω-nitro-L-arginine methyl ester. Levels of eNOS mRNA in murine hearts and NO3-/NO2− in serum were suppressed by dexamethasone (down to 63 and 50% of control values, respectively, at 3 mg/kg of body wt) along with a reduction in eNOS protein to 85.6%. Dexamethasone also concentration dependently reduced the expression of the cationic amino acid transporter-1 in murine hearts and cultured endothelial cells. The suppression of dexamethasone of the ACh-induced vasodilation could be partially reversed by dietary l-arginine (50 mg/kg of body wt) and by dietary vitamin C (10 g/kg of diet). We conclude that suppression by dexamethasone of the endothelium-mediated microvascular vasodilation involves several mechanisms including 1) downregulation of eNOS, 2) downregulation of cationic amino acid transporter-1, and 3) generation of reactive oxygen species. The demonstration that l-arginine and vitamin C can partially offset the effects of dexamethasone on microvascular arterioles suggests the potential clinical usefulness of these agents for the reduction of glucocorticoid-induced hypertension.

Methodological considerations for the study of the in vivo expression of eNOS and cationic amino acid transporter-1 (CAT-1), which is the major l-arginine transporter in endothelial cells (ECs; Ref. 7). In addition, we also examined whether dexamethasone-induced endothelial dysfunction may be associated with the production and action of oxygen species (19). To test the subsequent vasoregulation of resistance vessels, we applied intravitral microscopy in a dorsal skin-fold chamber implanted in striated skin muscle of C57BL/6J mice. This technique allows the study of an intact microcirculation in nonanesthetized, awake mice (30).

METHODS

Skin-fold chamber model in C57BL/6J mice. C57BL/6J mice were housed at 22°C with a 12:12-h day-night rhythm and were fed rodent maintenance diet that contained 100 mg/kg vitamin E (Altromin; Lage, Germany) and tap water ad libitum. Weight gains were comparable in all groups of animals. The daily water intake was measured and found to be identical for all animals in the four experimental groups regardless of dietary supplement (data not shown). For intravitral microscopy, we used the dorsal skin-fold chamber preparation. This model permits the microscopic investigation of pre- and post-capillary microvessels and of nutritional capillary perfusion in a fine, striated skin muscle in nonanesthetized animals (24, 25). C57BL/6J mice (10 wk old; body wt, 28–32 g) were anesthetized with xylazine-ketamine, and titanium observation chambers were implanted into the dorsal skinfold. For this purpose, the back of each animal was shaved, and two titanium frames were implanted to sandwich the extended double layer of the skin. In a circular area (18 mm in diameter), one layer was completely removed, and the remaining layer, which consisted of epidermis, subcutaneous tissue, and a thin striated skin

Long-term administration of glucocorticoids for the treatment of rheumatoid arthritis, autoimmune disorders, organ transplantation, and other indications eventually elicits a moderate and sometimes severe increase in systemic blood pressure in treated patients (40). Similarly, patients with Cushing’s syndrome exhibit systemic hypertension (40). Glucocorticoid-induced hypertension is largely independent of mineralocorticoid effects and sodium retention, because the mineralocorticoid receptor antagonist spironolactone is unable to prevent glucocorticoid-induced hypertension (20, 50). Kelly et al. (20) proposed the concept that steroid-induced hypertension may involve the dysregulation of nitric oxide (NO) generation and action on the tone of resistance vessels. This assumption is founded on the facts that 1) hypertension is linked functionally to increased peripheral vascular disease, 2) inhibition of endothelial NO synthase (eNOS) increases blood pressure (38, 39), and 3) eNOS-knockout mice are hypertensive (18). In a previous study, we provided experimental proof for this notion by demonstrating significant inhibition of eNOS expression in various organs of dexamethasone-fed rats as well as significant reduction of NO3−/NO2− (the oxidation products of NO) in the plasma of these animals (50).

In the present study, we investigated whether dexamethasone treatment affects the in vivo expression of eNOS and cationic amino acid transporter-1 (CAT-1), which is the major l-arginine transporter in endothelial cells (Ref. 7). In addition, we also examined whether dexamethasone-induced endothelial dysfunction may be associated with the production and action of oxygen species (19). To test the subsequent vasoregulation of resistance vessels, we applied intravitral microscopy in a dorsal skin-fold chamber implanted in striated skin muscle of C57BL/6J mice. This technique allows the study of an intact microcirculation in nonanesthetized, awake mice (30).

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muscle, was covered with a coverslip that was incorporated in one of the frames (25). The animals tolerated the dorsal skin-fold chambers well and showed no signs of discomfort. In particular, no adverse effects on sleeping, cleaning, or feeding habits were observed. The experiments were approved by the local ethics committee (AZ 177-07/971-6, Bezirksregierung Rheinhessen-Pfalz).

**Intravital microscopy.** To eliminate the effects of anesthesia and direct surgical trauma on the microvasculature, a recovery period of 72–96 h was allowed between observation-chamber implantation and microscopic investigations. For intravital microscopy, the awake mice were placed in a Plexiglas tube fixed to a Plexiglas platform under a standard diagnostic microscope. Then the coverslip was gently removed. During the observation period, the tissue was superfused at a continuous flow rate of 2 ml/min (roller pump Reglo Analog MS-4/6 with Tygon MH2075 silicone tubes; both from Ismatek) with buffered Krebs-Ringer solution [pH 7.4; that included (in mM) 120.0 NaCl, 4.7 KCl, 1.2 KH2PO4, 2.5 CaCl2•H2O, 1.2 MgSO4•7H2O, 25.0 NaHCO3, and 11.0 glucose, at 34°C]. For 30 min before and during the entire experiments, 95% O2-5% CO2 was bubbled through the solution at a pressure of 1 bar. This superfusion protocol was adopted from a previous study on hamsters (8). To prevent the visual distortion of the microscopic image, a semicircular coverslip fragment was loosely placed onto the superfusion medium. For the intravital microscopic studies, two or three arteriolar segments (with diameter from 20 to 60 μm) were chosen from the immediate proximity of bifurcations (to facilitate identification) as well as >300 μm away from bifurcations. After an equilibration period of 30 min, the arteriolar segments were observed at 500 magnification for 1-min intervals, and the sequences were recorded on videotape using a 1-chip, color, charge-couple-device camera (TK-C1381; JVC) and a S-VHS tape recorder (NV-HS900; Panasonic). The same blood vessel segments were observed again after a 5-min superfusion period with ACh (0.1–10 μM) and, after a 10-min recovery period, after a 5-min superfusion period with 5-nitroso-N-acytetyl-
\( \text{L-arginine methyl ester} \) (L-NAME, 30 μM). In a subset of experiments, an initial ACh superfusion period was followed by a period in which \( N^2 \)-nitro-L-arginine methyl ester (L-NAME, 30 μM) was superfused onto the chamber tissue for 30 min. The above-described superfusion protocol was repeated in the presence of 30 μM L-NAME. The same protocol was subsequently performed with animals that had received a dietary supplement of vitamin C for 1 wk in a dose (10 g/kg of diet) that increases serum vitamin levels by a factor of three (from 16.4 ± 5.7 to 55.6 ± 22.2 μM; Ref. 23); this is comparable to the increase in vitamin C levels observed in human subjects taking vitamin C supplements of 1 g/day and hence reaching levels considered relevant for protection from cardiovascular disease (40–50 μM; Ref. 12).

**Drug administration.** Dexamethasone (Fortecortin Mono 100; Merck; Darmstadt, Germany) was administered for 1 wk before the experiments in doses that yielded 0.1, 0.3, or 3.0 mg/kg body wt. Likewise, L-arginine (Sigma; Deisenhofen, Germany) was administered for 2 wk before the experiments to yield a dose of 50 mg/kg body wt. Doses were based on a daily water intake of 5 ml in a 30-g mouse (as assessed in pilot experiments). Both substances were added to the drinking water in bottles covered with aluminum foil to prevent exposure to light.

**Image analysis.** For quantification of the intravital microscopic findings, video still images were imported at a resolution of 72 dpi using the S-VHS port of a Macintosh computer (Power Macintosh G3; Apple Computer; Cupertino, CA) equipped with an inbuilt graphic capture board using the acquire command within Photoshop (Photoshop 5.0; Adobe Systems; San Jose, CA). Assessment of microvessel diameters was performed using Photoshop-based image analysis as described previously (2).

**Determination of NO\(_2\)/NO\(_3\) in mouse serum.** Mice were fasted for 24 h before blood collection to minimize the influence of dietary NO\(_2\)/NO\(_3\). Then serum NO\(_2\)/NO\(_3\) was measured as NO\(_2\) after enzymatic reduction with NO\(_3\) reductase (50). NO\(_2\) was determined by chemiluminescence after chemical reduction to NO using an NOA 280 nitric oxide analyzer (Sievers; Boulder, CO).

**Cloning of murine eNOS cDNA fragment.** The murine eNOS cDNA fragment was generated by reverse transcription of mRNA from mouse aorta. The fragment was amplified by PCR using the following oligonucleotide primers: 5′-GACATTGAGACGAAAGGCGCTGC-3′ (sense) and 5′-CGGCTTTGACCTCCTGG-3′ (antisense) corresponding to positions 3111–3132 and 3518–3535 of the murine eNOS gene. The cDNA fragment (425 bp) was cloned into the EcoR V site of vector pCR-Script to generate the cDNA clone pCR-eNOS-mouse. The DNA sequence of the cloned PCR product was determined from the plasmid template using the dyeoxy chain-termination method with a T7-sequencing Kit (Amersham Biosciences; Freiburg, Germany). The plasmid was linearized with Asp718, extracted with phenol-chloroform, and concentrated by ethanol precipitation.

**eNOS protein preparation and Western blotting.** Protein isolation and Western blotting were carried as previously described (50). Briefly, a 3′-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) extract was prepared from the different types of ECs and separated by SDS-PAGE (on 7.5% gels). The proteins were transferred to nitrocellulose membranes (Schleicher and Schuell; Dassel, Germany) by electroblotting (Bio-Rad). Proteins were detected using a 1:500 dilution of a polyclonal anti-eNOS antibody and a monoclonal anti-β-tubulin antibody. After incubation with the appropriate secondary antibodies conjugated to alkaline phosphatase as described, densitometric analyses were performed with a Video Imager (Bio-Rad). The eNOS protein bands were normalized using the respective β-tubulin protein bands (50).

**Cell culture.** Human umbilical vein EC (HUVEC)-derived EA.hy 926 cells were grown in Dulbecco’s modified Eagle’s medium as described previously (50). The cells were incubated for 24 h with dexamethasone (1–1,000 nM; Sigma) or were left untreated.

**RNase protection assay of eNOS and CAT-1 mRNA.** Total RNA was isolated from human ECs or mouse tissues by guanidinium thiocyanate-phenol-chloroform extraction (50). The eNOS and CAT-1 mRNA were measured by RNase protection assay using specific radiolabeled antisense RNA probes, which were described previously: murine CAT-1, CAT-2A, CAT-2B, and β-actin, human CAT-1 (7), and human eNOS and β-actin (50). Densitometric analyses of the RNase protection gels were performed using a Phospho-Imager (Bio-Rad; Munich, Germany). The density of each eNOS or CAT-1 band was normalized to the corresponding β-actin band.

**Analysis of plasma arginine content.** To 100 μl of plasma, 33 nmol homoserine (as internal standard) and 250 μl of ethanol were added. After centrifugation at 14,000 g for 10 min, supernatants were extracted twice with 0.5 ml of petrolether each and then dried by vacuum centrifugation. Pellets were resuspended in 400 μl of borate buffer (pH 10), and 150 μl were used for precolumn derivatization with o-phthalaldehyde (OPA; Autosampler L7250; Merck). To verify the authenticity of the arginine peaks, some aliquots were spiked with 5 mmol arginine and others were treated with arginase (7 U/150 μl; Sigma) before precolumn derivatization. Amino acid derivatives were separated on a Nova-Pak C18 column (particle size, 4 μm; 3.9 × 300 mm; Waters; Eschborn, Germany) using a two-solvent gradient (acetoniitrile and 50 mmol/l sodium acetate, pH 7.0). The flow rate was 0.8 ml/min. Fluorescence (excitation wavelength, 330 nm; emission wavelength, 450 nm) was monitored with a Shimadzu RF-530 fluorimeter and was quantified using the D-7000 HSM analysis program (Merck).

**Statistical analysis.** All data given in the text are means ± SE. Statistical differences between mean values were determined by the Wilcoxon rank sum test (Figs. 1, 2, 5, and 6) or by ANOVA followed by the Fisher protected least-significant-difference test (Figs. 3 and 4).
RESULTS

Dexamethasone attenuated ACh-induced, EC-dependent vasodilation. Superfusion of the chamber tissue with 10 μM ACh induced pronounced vasodilation of resistance arterioles (Fig. 1). This vasodilation was attenuated significantly in a dose-dependent fashion in animals that had received oral administration of dexamethasone for 1 wk (0.1–3.0 mg/kg body wt; Fig. 1A). In contrast, the endothelium-independent vasodilation by the NO donor SNAP was not affected significantly by dexamethasone at any of the tested doses (Fig. 1B). In animals treated with dexamethasone (0.3 or 3.0 mg/kg body wt), the vasodilation induced by lower concentrations of ACh (0.1 and 1 μM) was also significantly reduced (data not shown). To test the relative importance of NO generation for the ACh-induced vasodilation, the chamber tissue was preincubated for 30 min with the NO synthase inhibitor l-NAME (30 μM). The ACh-induced vasodilation was significantly reduced in l-NAME-pretreated arterioles (Fig. 2). In contrast, the SNAP-induced vasodilation was not affected (Fig. 2).

Dexamethasone decreased eNOS expression as well as serum NO₂/NO₃ levels. To estimate eNOS expression in vivo, we analyzed eNOS mRNA levels in heart as a model for microcirculation. RNase protection analyses revealed a significant reduction of eNOS mRNA in hearts of dexamethasone-treated mice compared with control animals (down to 63 ± 2.6%; Fig. 3, A and B). The eNOS protein levels decreased to 85.6 ± 5.2% of control animals (n = 7 dexamethasone treated vs. 10 control animals; data not shown). NO production was assessed by measuring serum levels of NO₂/NO₃, which are the oxidation products of NO. Oral dexamethasone treatment decreased serum NO₂/NO₃ levels significantly in a dose-dependent manner (Fig. 3C).

Dexamethasone decreased CAT-1 expression in murine hearts and human ECs. In hearts of dexamethasone-treated mice, the expression of CAT-1 mRNA was also reduced significantly as shown by RNase protection analyses (down to 70 ± 3.5% of control mice; Fig. 4A). Incubation of human EA.hy 926 ECs with increasing concentrations of dexamethasone (1–1,000 nM) also decreased CAT-1 mRNA expression in a concentration-dependent fashion (down to 71 ± 3.7% of control animals; Fig. 4B). Because CAT-2 has been identified and found upregulated (particularly in heart and aortic endothelium of rat and bovine origin) by treatment with BH₄, transforming growth factor-β, as well as with combinations of interferon-γ and endotoxin, interferon-γ and interleukin-1, endotoxin and tumor necrosis factor-α, and endotoxin and BH₄ (9, 14, 33, 41, 42), and because CAT-2 transcripts have previously been identified in murine tissues [in particular in mouse hepatocytes (6)], we used an RNase protection assay that detects both CAT-2A and CAT-2B. However, in heart tissues of dexamethasone-treated and control mice alike, CAT-2 transcript levels were well below the detection limit of our assay (n = 6) with convincing positive controls in liver tissues and activated macrophages (n = 6; Ref. 7).

Dexamethasone-induced suppression of EC-dependent vasodilation was improved by pretreatment of animals with L-arginine and vitamin C. Addition of L-arginine to the drinking water for 2 wk before the experiments (50 mg/kg of body wt) partially restored the ACh-induced vasodilation in dexamethasone-treated animals (Fig. 5). L-Arginine was without effect on ACh-induced vasodilatation in control animals not treated with dexamethasone (Fig. 5). These changes in ACh-induced vasodilation in dexamethasone-treated animals were associated with a 40% increase in L-arginine plasma levels.
4) and that ACh-induced vasorelaxation could be partially inhibited by methasone both in mice in vivo and in cultured ECs (see Fig. 3). In addition, we demonstrated that the suppression of eNOS expression reported previously (50) and in the present study (see Fig. 3) is in agreement with the inhibitory action of dexamethasone on eNOS mRNA levels found in hearts of dexamethasone-treated animals and in patients (20, 40, 50).

The relatively moderate individual inhibitions of CAT-1 and eNOS mRNA levels found in hearts of dexamethasone-treated mice (15–40% inhibitions, see Figs. 3 and 4) cannot fully account for the almost complete suppression of NO-mediated (l-NAME inhibited) endothelial vasorelaxation (see Figs. 1 and 2). Along these lines of evidence, the restoration of dexamethasone-inhibited, EC-dependent vasorelaxation by arginine treatment was likewise only incomplete, leaving quite some room for an additional restorative action of vitamin C. Although these two means of restoring EC function (as well as their underlying pathophysiology) are not necessarily exclusive, we speculate that through the combined dysfunction of NO synthesis on one side and enhanced generation of reactive oxygen species on the other side, dexamethasone may leave NO-mediated, EC-dependent dysfunction completely suppressed. Whether an additional pathomechanism may have contributed to this orchestra of events is well beyond the realm of speculation at this point.

In previous reports, dexamethasone, when given acutely, failed to inhibit endothelium-dependent vasodilation in acutely treated isolated rings of conductance vessels in rats (31) as well as in chronically treated rabbits (49). This is not in contrast to our present observations if the mechanism of action of dexamethasone is an attenuation of eNOS and CAT-1 gene expression. A downregulation of gene expression requires time to take effect and thus would not be seen after an acute administration of a steroid. Furthermore, large-caliber conductance arteries (such as aortic or carotid arterial rings) may differ in their response from the small resistance arterioles investigated here, not only in terms of anatomic structure (32) but also in terms of functionality including endothelial control of vascular tone. Based on their findings in mouse coronary vessels, Guillot et al. (13) identified vascular-bed-specific pathways of eNOS regulation with striking differences in eNOS gene expression between small arterioles and larger conductance vessels. The relatively moderate individual inhibitions of CAT-1 and eNOS mRNA levels found in hearts of dexamethasone-treated mice (15–40% inhibitions, see Figs. 3 and 4) cannot fully account for the almost complete suppression of NO-mediated (l-NAME inhibited) endothelial vasorelaxation (see Figs. 1 and 2). Along these lines of evidence, the restoration of dexamethasone-inhibited, EC-dependent vasorelaxation by arginine treatment was likewise only incomplete, leaving quite some room for an additional restorative action of vitamin C. Although these two means of restoring EC function (as well as their underlying pathophysiology) are not necessarily exclusive, we speculate that through the combined dysfunction of NO synthesis on one side and enhanced generation of reactive oxygen species on the other side, dexamethasone may leave NO-mediated, EC-dependent dysfunction completely suppressed. Whether an additional pathomechanism may have contributed to this orchestra of events is well beyond the realm of speculation at this point.

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Besides NO, endothelium-derived hyperpolarizing factor(s) represents an additional vasorelaxing principle whose relative importance compared with NO varies among vascular beds investigated (3). In the present study, a moderate concentration (30 μM) of the NOS inhibitor L-NAME inhibited the ACh-induced vasodilation of resistance arterioles by 60–70% but had no effect on the endothelium-independent relaxation by SNAP (see Fig. 2). This indicates that NO plays a significant role as an endothelium-dependent vasodilator in this model. De Wit et al. (8) made similar observations on chronically instrumented, nonanesthetized hamsters, where 30 μM L-N(G)-nitro-L-arginine reduced ACh-induced vasodilation by about two-thirds. The residual ACh-induced vasodilation was largely blocked by charybdotoxin and potassium depolarization, which suggests that endothelium-derived hyperpolarizing factor(s) were responsible for the remainder of the ACh-induced response (8).

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Of particular interest in the present study was the observation that dexamethasone reduced the expression of CAT-1 (see Fig. 4) and that the dexamethasone-induced suppression of endothelium-derived vasodilation was partially reversed by...
pretreatment of the mice with the NO synthase substrate l-arginine (see Fig. 5). CAT-2A and CAT-2B are unlikely to contribute significantly to l-arginine transport in our model, because their expression was undetectable in murine heart tissue and thus markedly lower than that of CAT-1. L-Arginine has been shown to improve endothelium-dependent vasodilation in various pathophysiological situations in both animals and humans (26), but it usually does not do so in the absence of vascular disease. In this respect, dexamethasone treatment seems to mimic vascular pathology, because l-arginine enhanced the ACh-induced vasodilation in dexamethasone-treated animals but did not change the ACh response in control animals (see Fig. 5). Also, Li et al. (28) demonstrated a normalization of dexamethasone-induced hypertension after administration of l-arginine, whereas Chen and coworkers (4) did not see a reversal by l-arginine of the effect of dexamethasone on NO synthesis and blood pressure in hypertensive rats.

Under healthy conditions and with normal serum concentrations of l-arginine (50–100 μM; Ref. 1), eNOS (whose $K_m$ for l-arginine is $\sim$3 μM; Ref. 36) should be saturated with substrate. It is therefore surprising that l-arginine administration should reverse endothelial dysfunction (see Fig. 5). However, Suzuki et al. (45), using intravital fluorescence microscopy on rat mesenteric arteries, reported a dramatic increase in oxidative stress in response to dexamethasone. The extent of oxidative stress correlated well with the vascular tone of mesenteric arterioles. These authors suggested that the increased oxidative stress in dexamethasone-treated animals may promote oxidative breakdown of NO (45). In addition, in the presence of oxidative stress, eNOS itself can become dysfunctional and produce reactive oxygen species at the expense of NO (28). This condition can in part be reversed by excess substrate and thus could serve as an explanation for the effect of l-arginine. To test this effect in our model, we administered a dietary vitamin C supplement to the animals and found that this partially restored endothelium-dependent vasorelaxation to roughly the same extent as administration of l-arginine (see Fig. 6). The finding is well in line with several previous publications initially presented by Suzuki et al. (45) and more recently by Iuchi et al. (19). These authors used electron-spin

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Fig. 4. Reduction of mRNA expression of the mouse cationic amino acid transporter 1 (mCAT-1). Mice were either left untreated (control) or were treated for 1 wk with dexamethasone (3.0 mg/kg of body wt). The mRNA was isolated from hearts and analyzed by RNase protection assay. An autoradiograph of a representative gel (A) shows tRNA control (T), undigested murine CAT-1 antisense probe (C), and undigested murine β-actin antisense probe (A). In densitometric analysis of three different gels (B), columns represent means ± SE. **$P < 0.01$, significant differences from untreated mice. Human EA.hy 926 endothelial cells (C) were left untreated (control) or were incubated for 24 h with dexamethasone in increasing concentrations (1–1,000 nM). RNase protection analyses were performed using antisense RNA probes to human CAT-1 (hCAT-1) and human β-actin (for standardization). All data are means ± SE ($n = 6$). *$P < 0.05$; **$P < 0.01$, significant differences from untreated cells.
resonance to identify the source of dexamethasone-induced reactive oxygen species in the mitochondrial transport chain, NAD(P)H oxidase, and xanthine oxidase. In agreement with our own data, the attenuated reactive hyperemia (an endothelium-dependent vasorelaxing response) in dexamethasone-treated human subjects was effectively reconstituted by oral vitamin C (37).

Another factor possibly contributing to a relative L-arginine deficiency of endothelial NO synthesis after dexamethasone administration is the steroid-induced decrease in CAT-1 expression. Murine CAT-1 has an apparent $K_m$ for L-arginine of $\sim 100–200 \mu M$ (5) and thus operates under subsaturating conditions at normal plasma concentrations. The downregulation of its expression would reduce the overall transport capacity for L-arginine. Increased extracellular L-arginine concentrations could, in turn, stimulate the transport velocity of the remaining CAT-1 protein and thereby increase the substrate supply to eNOS.

Translating these observations into the clinical situation, there are several reports worth noting. For one, arginine has been given to patients with hypertension, including one patient with Cushing’s syndrome, with obvious benefit (15). Of interest, indicators of NO release such as plasma concentrations of L-citrulline and urinary excretion of nitrite/nitrate increased simultaneously during administration of arginine (16). Likewise, vitamin C has been given to patients with endothelium-dependent vasodysregulation such as during steroid-resistant nephrosis, and this treatment has also led to significant lowering of blood pressure (10).

In conclusion, we have demonstrated in a mouse microcirculation model under quasi-physiological conditions that dexamethasone reduces the capacity for endothelium-dependent, NO-mediated vasodilation. Mechanisms contributing to this dexamethasone-induced endothelial dysfunction include the downregulation of the gene expression of eNOS and CAT-1. The finding that L-arginine and vitamin C can partially reverse the effect of dexamethasone on microvascular arterioles suggests a potential clinical usefulness of these agents for the treatment of glucocorticoid-induced hypertension.

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