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In Vivo Gene Transfer of Nitric Oxide Synthase Enhances Vasomotor Function in Carotid Arteries From Normal and Cholesterol-Fed Rabbits

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Background—The vascular endothelium is anatomically intact but functionally abnormal in preatherosclerotic states, and an early deficit in the bioavailability of nitric oxide (NO) or related molecules has been described in both humans and animal models. We hypothesized that the targeted gene transfer of NO synthase (NOS) isoforms might ameliorate or reverse the deficit.

Methods and Results—We constructed a recombinant adenovirus, Ad.nNOS, that expresses the neuronal isoform of NOS (nNOS) and used it for in vivo endovascular gene transfer to carotid arteries (CA) from normal and cholesterol-fed rabbits. Vessels were harvested 3 days after gene transfer. In CA from normal rabbits, Ad.nNOS generated high levels of functional nNOS protein predominantly in endothelial cells and increased vascular NOS activity by 3.4-fold relative to sham-infected control CA. Ad.nNOS gene transfer also significantly enhanced endothelium-dependent vascular relaxation to acetylcholine; at 3 μmol/L acetylcholine, Ad.nNOS-treated arteries showed an 86±4% reduction in precontracted tension, whereas control CA showed a 47±6% reduction in tension. Contraction in response to phenylephrine and relaxation in response to nitroprusside were unaffected in both control and Ad.nNOS-treated CA. To determine the effect of Ad.nNOS in atherosclerotic arteries, 10 male New Zealand White rabbits maintained on a 1% cholesterol diet for 10 to 12 weeks underwent gene transfer according to the same protocol used in normal rabbits. Ad.nNOS-treated arteries showed a 2-fold increase in NADPH-diaphorase staining intensity relative to sham-infected and Ad.βGal-treated arteries. The CA from cholesterol-fed rabbits showed impaired acetylcholine-induced relaxation, but this abnormality was almost entirely corrected by Ad.nNOS gene transfer.

Conclusions—In vivo adenovirus-mediated endovascular delivery of nNOS markedly enhances vascular NOS activity and can favorably influence endothelial physiology in the intact and atherosclerotic vessel wall. **(*Circulation*. 1998;98:1905-1911.)**

Key Words: genes ■ adenovirus ■ endothelium ■ nitric oxide ■ atherosclerosis

The free radical second messenger nitric oxide (NO) and related bioactive molecules[1] may have a significant role in atherogenesis. Reduced endothelium-dependent vascular relaxation is an early event in atherosclerosis[2,3]; in addition, NO inhibits many key atherogenic processes, including platelet adhesion and aggregation,[4,5] adhesion molecule and chemokine expression,[6,7] and smooth muscle cell migration and proliferation.[8,9] Thus, early endothelial NO deficiency may promote progression to more advanced vascular lesions.

Because of their potential importance in vascular diseases, the NO synthase (NOS) isoforms represent attractive targets for vascular gene transfer. Plasmid-liposome gene transfer of the endothelial isoform of NOS (eNOS) restored NO production and inhibited intimal hyperplasia in balloon-injured rat carotid arteries.[10] More recently, ex vivo[11] and adventitial[12] deliveries of eNOS-expressing adenoviruses were shown to favorably affect vasomotor function. However, no study to date has shown that gene transfer can reverse hyperlipidemia-induced vascular dysfunction.

We recently developed an adenoviral vector for gene transfer of the neuronal isoform of NOS (Ad.nNOS).[13] This vector generates high-level nNOS protein expression and augments agonist-stimulated NO production in cultured vascular smooth muscle and endothelial cells. In the present study, we use Ad.nNOS (with some of its control elements modified to enhance in vivo expression) for carotid artery gene transfer in normal and cholesterol-fed rabbits. We demonstrate in vivo expression of functional recombinant nNOS in the vessel wall,

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predominantly in endothelial cells. More importantly, we show that Ad.nNOS gene transfer enhances endothelium-dependent vasomotor function in the normal CA and substantially restores the vasomotor deficit in CA from cholesterol-fed rabbits.

Methods

Construction and Purification of Recombinant Adenoviruses

A recombinant adenovirus encoding β-galactosidase (β-Gal), Ad.Pacβ Gal, served as a control virus. We generated Ad.nNOS, containing a cDNA encoding the rat neuronal NOS isoform, driven by the cytomegalovirus immediate-early enhancer/promoter, as previously described.[13] For in vivo studies, Ad.nNOS was modified by removing the 5′-untranslated region from the nNOS cDNA and replacing the Simian virus 40 polyadenylation signal (pA) with a bovine growth hormone pA derived from the plasmid pcDNA3 (InVitrogen), which enhanced in vivo expression.

In Vivo Carotid Artery Gene Transfer

All animal care and procedures were approved by the Duke University Institutional Animal Care and Use Committee and complied with the "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 80–23, revised 1985). Male New Zealand White rabbits (weight, 2 to 2.5 kg) were maintained on a normal diet or were fed a 1% cholesterol diet for 12 weeks before surgery. Vascular surgery, gene transfer to the carotid arteries, and vessel harvest were performed essentially as described previously.[14]

NOS Protein Analysis by Western Immunoblotting

Freshly harvested vessels were immediately frozen at −80°C for storage. For analysis, thawed vessels were weighed and then sonicated in 3 vol of ice-cold lysis buffer (20 mmol/L Tris, pH 7.4, 0.1% Triton X-100, containing 0.2 mg/mL phenylmethylsulfonyl fluoride and 0.5 μg/mL leupeptin). Tissue debris was pelleted at 14 000*g*, and total protein concentration in the lysate was determined. In addition to analysis of NOS protein in the crude lysate, NOS protein was enriched by binding the crude lysate for 1 hour at 4°C with 25 μL/mL of 2′,5′-ADP–agarose (Sigma Chemical). After binding, the beads were pelleted, washed in lysis buffer to remove residual unbound protein, and then boiled in SDS-PAGE loading buffer to release the bound proteins. Samples were analyzed by Western blotting using an isoform-specific mouse monoclonal anti-NOS peptide antibody (Transduction Laboratories).

Immunohistochemistry and Staining for NADPH-Diaphorase Activity

Vessel segments were briefly equilibrated in 30% sucrose in PBS at 4°C, embedded in optimal cutting temperature compound (OCT; Miles Scientific), frozen in liquid nitrogen, and sectioned (6-μm sections) onto silane-coated glass microscope slides. Immunohistochemistry to identify nNOS protein expression was performed using a monoclonal anti-nNOS peptide antibody (Transduction Laboratories). Immunostaining of smooth muscle cells (HHF 35; DAKO) and endothelial cells (anti–von Willebrand factor; Atlantic Antibodies) also was performed. Immune complexes were localized with the use of chromogenic alkaline phosphatase substrate Vector red (Vector Laboratories), and sections were lightly counterstained with hematoxylin.

Vessels also were stained for NADPH-diaphorase activity.[13] This stain demonstrates the presence of functional NOS protein but is not isoform specific.[15] Briefly, vessel cryosections were fixed for staining in 4% paraformaldehyde for 30 minutes; permeabilized for 30 minutes in 0.1 mol/L Tris, pH 7.2, and 0.2% Triton X-100; and then stained with 1 mmol/L NADPH and 0.2 mmol/L nitroblue tetrazolium in the same buffer at 37°C for ≈20 minutes, until the development of blue-purple staining was observed. Staining intensity was quantified using an image analysis system (Olympus IX70 inverted microscope, Optronics DEI-750 image-capturing hardware; PowerTowerPro 180 CPU). Images were captured using Adobe Premiere and quantified using NIH Image software.

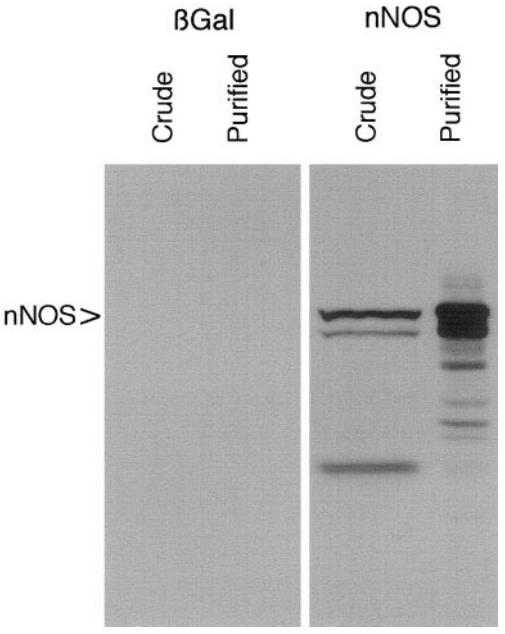

Figure 1. In vivo expression of recombinant nNOS protein after adenoviral vascular gene transfer. Rabbit carotid arteries were harvested 3 days after gene transfer with Ad.βGal or Ad.nNOS, sonicated in lysis buffer, and analyzed directly by SDS-PAGE (Crude) or partially purified by binding to 2′,5′-ADP–agarose before SDS-PAGE (Purified). Western immunoblots from representative pair of arteries are shown. Extra bands present are believed to result primarily from low-level proteolysis. Experiment was repeated 6 times with similar results. Marker shows position of nNOS standard.

Determination of Vascular NOS Activity

The conversion of [^{3}H]arginine to [^{3}H]citrulline was used to measure NO production from intact vessel rings according to modifications of existing methods.[13] Briefly, freshly harvested vessels were weighed, cut into 2-mm rings, equilibrated for 1 hour in 1 mL of Krebs-Henseleit buffer (KHB) (containing [in mmol/L] NaCl 120, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 mm, glucose 5.5 mm, pH 7.4), and gassed with 95% O_2/5% CO_2 in miniature organ baths maintained at 37°C. After equilibration, KHB was replaced with 0.6 mL of fresh, warm KHB containing [^{3}H]arginine (5 μCi/mL, 63 Ci/mmol; New England Nuclear) and 1 μmol/L calcium ionophore (A23187; Sigma). Incubation was continued for 4 hours at 37°C, with 95% O_2/5% CO_2 gassing. The conditioned KHB (0.6 mL) was added to 1 mL of NOS assay stop solution (20 mmol/L HEPES, pH 5.5, 2 mmol/L EDTA), and the organ chambers were washed out with 0.4 mL of fresh stop solution. [^{3}H]citrulline was separated from [^{3}H]arginine by ion-exchange chromatography of the pooled KHB/stop solution over a 1-mL column of AG 50w-X8 resin (Na form; Bio-Rad), prewashed with 2 mL of stop solution, and quantified by liquid scintillation counting. NO production was calculated from the proportion of total arginine counts converted to citrulline, with the background counts having been subtracted in a blank sample, and from the known specific activity of the [^{3}H]arginine.

Vasomotor Studies

The vasomotor studies were conducted as described previously.[14] Briefly, freshly harvested vessel rings (5 mm) were maintained in oxygenated KHB at 37°C; cumulative dose-response curves to phenylephrine (PE; 10^{-9} to 10^{-4} mol/L) were established; the vessels were submaximally precontracted with PE (typically 1×10^{-6} mol/L); and

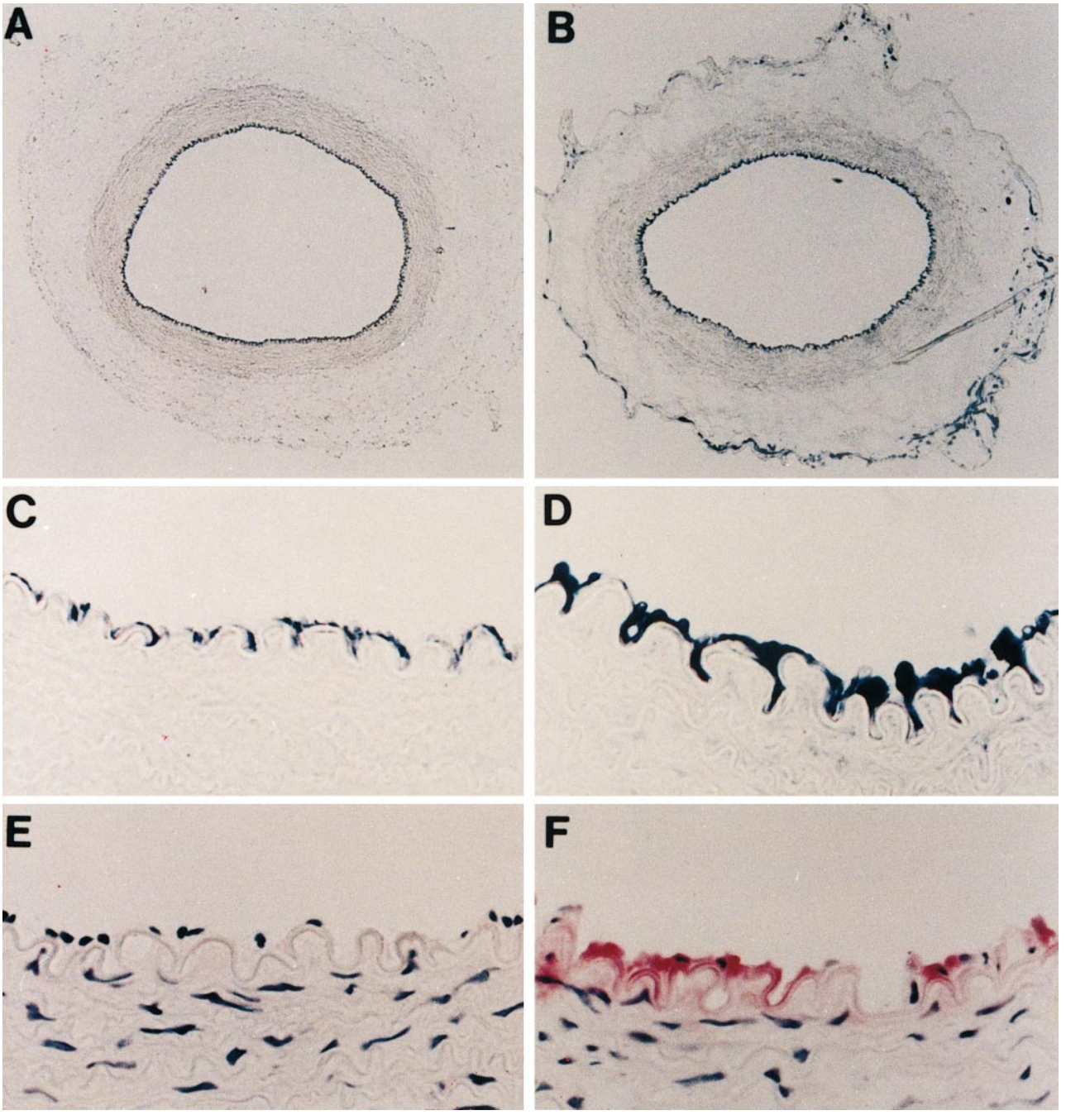

Figure 2. Localization of nNOS expression by immunostaining and NADPH-diaphorase staining. Control arteries (infected with Ad.PacβGal; A, C, and E) or arteries infected with Ad.nNOS (B, D, and F) were stained for NADPH-diaphorase activity (A through D) or for nNOS protein expression by immunohistochemistry (E and F). Original magnification, $\times$150. In control arteries, moderate diaphorase staining is seen on endothelial surface. Ad.nNOS-treated arteries show enhanced endothelial staining and adventitial staining (B). Isoform-specific immunostaining for nNOS shows staining in endothelium of Ad.nNOS-infected arteries only (E and F).

endothelial function was evaluated by vascular relaxation to acetylcholine (ACh; 10^{-8} to 3×10^{-5} mol/L). Endothelium-independent relaxation responses to sodium nitroprusside (SNP; 10^{-8} to 10^{-4} mol/L) also were determined. Statistical significance was assessed by ANOVA.

Results

In Vivo Adenoviral Gene Transfer Augments Endothelial NOS Activity

In Western immunoblots (Figure 1), no nNOS protein was seen in either native carotid arteries or arteries after β-Gal gene transfer. In contrast, nNOS protein was clearly detectable in crude vessel lysate after Ad.nNOS infection and was greatly enriched by 2′,5′-ADP affinity purification.

To investigate the distribution of NOS activity, frozen tissue sections were stained for NADPH-diaphorase activity (Figure 2). Because development of the blue color requires active NOS, NADPH-diaphorase staining accurately reflects the distribution of functional NOS enzyme.[15] Control carotid arteries (both Ad.βGal infected, shown in Figure 2, and

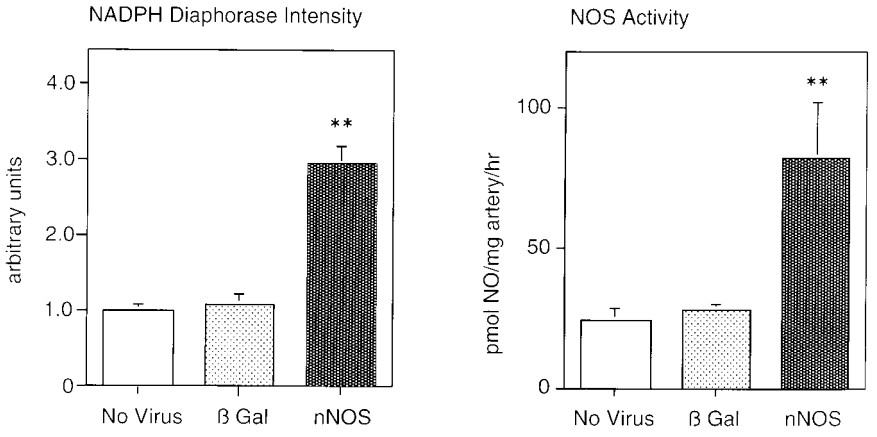

Figure 3. NADPH-diaphorase staining intensity and vascular NO production in vessel rings. Rabbit carotid arteries were infected with Ad.nNOS (nNOS; n=6) or Ad.βGal (β Gal; n=4) or underwent sham infection (No Virus; n=6). Vessels were harvested after 3 days. Left, Frozen sections were stained for NADPH-diaphorase (shown in Figure 2); staining intensity was then determined by blinded observer in two randomly selected sections from each vessel using image analysis (presented as mean±SD, in arbitrary units). Left, Sham-infected (1.0±0.1) and Ad.βGal-infected arteries (1.1±0.2) did not differ in staining intensity; Ad.nNOS-treated arteries showed 3-fold increase over sham-infected arteries (3.0±0.2). Right, NO production was determined by quantifying [^{3}H]arginine–to–[^{3}H]citrulline conversion in vessel rings stimulated with the calcium ionophore A23187 (1 μmol/L). The figure shows mean±SD NO production in pmol of citrulline produced · h^{-1} · mg of vessel $weight^{-1}$. Ad.nNOS-treated arteries produced 3.4-fold more [^{3}H]citrulline than did sham-infected arteries. $^{**}P<0.001$ calculated with an unpaired 2-tailed Student's *t* test.

sham-infected, not shown) had moderate diaphorase staining confined to endothelial cells, reflecting native eNOS activity. In arteries infected with Ad.nNOS, however, endothelial diaphorase staining was clearly more intense; in addition, significant blue staining was observed in the adventitia. To confirm the NADPH-diaphorase findings and to determine the extent of nNOS expression, we also performed immunohistochemical staining for nNOS protein (Figure 2). The areas of nNOS expression corresponded to the areas of enhanced NADPH-diaphorase activity. In control arteries, no nNOS immunostaining was observed.

We quantified the degree of endothelial NADPH-diaphorase staining by computer-assisted image analysis (Figure 3, left). This revealed that the endothelial layer of Ad.nNOS-treated vessels had 3-fold higher diaphorase staining intensity than sham- or Ad.βGal-infected vessels. We next assessed NOS activity in vessels stimulated with 1 μmol/L A23187. Mean NOS activity after nNOS gene transfer was increased by 3.4-fold compared with control arteries (Figure 3; $P<0.001$). Thus, Ad.nNOS gene transfer produces a quantitatively similar increase in NADPH-diaphorase staining intensity and vascular NO production (a relationship that is also present across a broad range of infecting titers in cultured vascular cells; H.S. Qian, V. Neplioueva, and S.E. George, unpublished data).

In Vivo NOS Gene Transfer Enhances Endothelial Vasomotor Function

The responses of arteries to vasoactive agonists were determined using isometric tension studies. Responses to cumulative doses of PE, ACh, and SNP were recorded (Figure 4). All vessels contracted similarly in response to PE. In Ad.βGal- or sham-infected arteries, acetylcholine reduced tension in PE-precontracted arteries to ≈25% of precontracted values. Ad.nNOS gene transfer significantly augmented ACh-dependent relaxation. Relaxation at every concentration of ACh was greater than that in controls ($P<0.05$), and maximal relaxation was greatly increased, to almost complete relaxation (5% of precontracted tension). Finally, Ad.nNOS-infected arteries showed a 20-fold increase in sensitivity to ACh relative to sham-infected arteries, as judged by reduction in EC_{50} value (0.22×10^{-6} mol/L versus 4.5×10^{-6} mol/L; $P<0.01$). The difference in ACh-induced relaxation was not due to differences in sensitivity to NO because all vessels relaxed in a similar manner to that in response to SNP (Figure 4C).

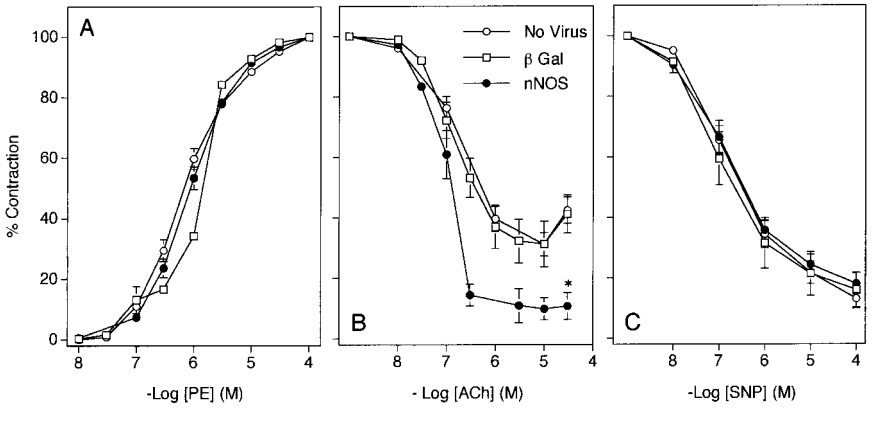

Figure 4. In vivo Ad.nNOS gene transfer to normal rabbit carotid arteries enhances endothelial vasomotor function. Gene transfer to rabbit carotid arteries was carried out as described. Vessels were harvested after 3 days, and 5-mm rings were suspended in organ baths for isometric vasomotor tension studies. Contraction responses to cumulative concentrations of PE (A) were similar in control arteries that had undergone mock infection (No Virus), in arteries infected with Ad.PacβGal (β Gal), and in arteries infected with Ad.nNOS (nNOS). In contrast, endothelium-dependent relaxation in response to ACh (B) after precontraction with PE was significantly enhanced in arteries after Ad.nNOS gene transfer. No differences were observed in endothelium-independent relaxation in response to SNP (C). Error bars show SEM values. $*P<0.05$ for comparison between ACh-induced relaxations in β Gal or No Virus arteries with nNOS arteries, calculated by ANOVA.

In Vivo NOS Gene Transfer Substantially Reverses Vasomotor Impairment in Cholesterol-Fed Rabbits

We next sought to determine whether NOS gene transfer could reverse the vasomotor impairment known to occur in cholesterol-fed rabbits. Ten male New Zealand White rabbits (weight, 2 to 2.5 kg) were fed a 1% cholesterol diet for 11 to 12 weeks. Serum cholesterol levels were monitored at 4 and 8 weeks and at the time of gene transfer, and levels were markedly elevated in all rabbits (mean±SEM cholesterol levels in mg/dL: baseline, 67±8; 4 weeks, 1260±259; 8 weeks, 1141±334; 12 weeks, 2279±320). The rabbits underwent carotid artery gene transfer with either Ad.nNOS (10 arteries) or Ad.PacβGal (4 arteries) or were mock-transferred (6 arteries) according to the protocol used in normal rabbits. At harvest, segments of the arteries were stained for NADPH-diaphorase activity, and the staining intensity was quantified by image analysis (Figure 5). Arteries receiving Ad.nNOS showed >2-fold higher diaphorase staining intensity than either Ad.PacβGal or sham-transfected arteries, directly reflecting higher NOS activity in the Ad.nNOS-treated arteries.

As expected, ACh-induced relaxation was significantly impaired in arteries from cholesterol-fed rabbits. In sham-infected carotid arteries, ACh reduced tension to 69±5% of precontracted values (Figure 5C) (compare with values for normal carotids in Figure 4, where ACh relaxes normal carotids to 25% of precontracted tension). Ad.βGal-infected arteries yielded similar results. In contrast, Ad.nNOS substantially reversed the vasomotor impairment associated with cholesterol feeding; Ad.nNOS-treated vessels relaxed to 43±5% of precontracted tension ($P<0.01$ versus sham- and Ad.βGal-infected arteries). The degree of contraction in response to PE and relaxation in response to SNP were not affected by Ad.nNOS, Ad.PacβGal, or sham infection (data not shown). In summary, the results in cholesterol-fed rabbits show that Ad.nNOS gene transfer approximately doubles the amount of functional NOS in the vascular wall and substantially reverses the vasomotor deficit associated with cholesterol feeding.

Discussion

Although eNOS is the only NOS isoform found in the normal vessel wall, other NOS isoforms may be effective for vascular gene therapy in diseased or injured vessels. Our studies establish that Ad.nNOS expresses a functional enzyme that augments vascular NO production and enhances endothelial vasomotor function in arteries from both normal and cholesterol-fed rabbits. This adds to recent evidence suggesting that the calcium/calmodulin–regulated NOSs can functionally complement each other.[16] In support of this view, Shears et al[17] recently showed that an adenovirus expressing the immunological isoform of NOS (iNOS) reduced vasculopathy in rat aortic allografts. Thus, all three major NOS isoforms appear to be reasonable candidates for vascular gene therapy applications.

In this study, we sought to avoid the confounding effects of virus-induced inflammation. First-generation, E1-deleted adenoviral vectors have a well known propensity to provoke a chronic inflammatory response in infected tissues.[18] In the

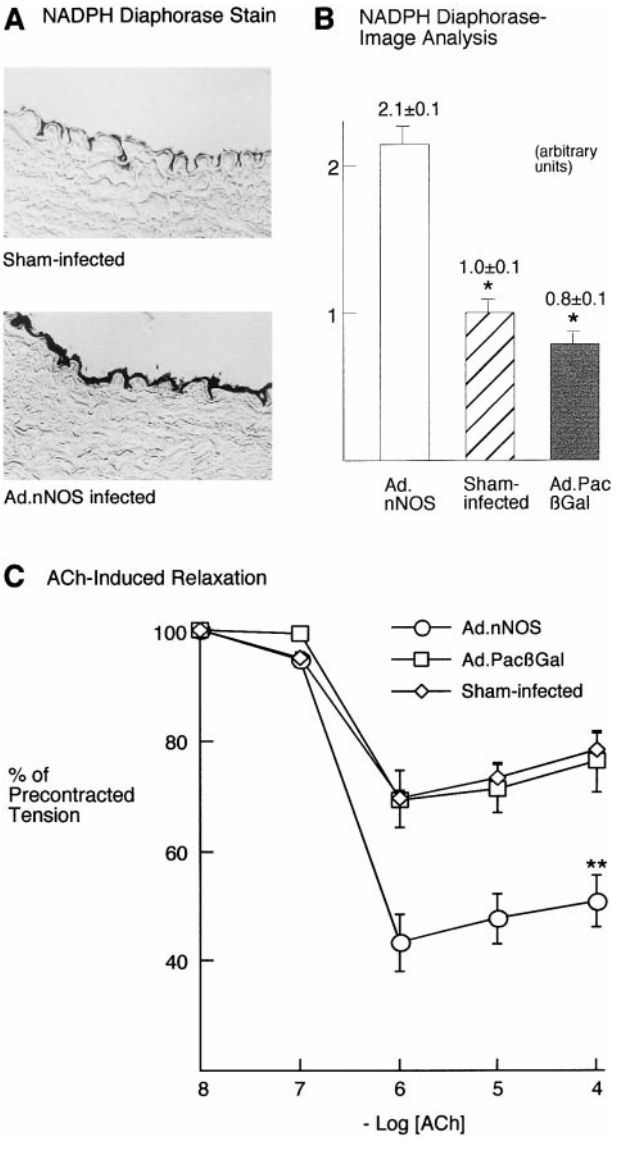

Figure 5. In vivo Ad.nNOS gene transfer to carotid arteries from cholesterol-fed rabbits. Ten male New Zealand White rabbits were maintained on a 1% cholesterol diet for 11 to 12 weeks and then either received Ad.nNOS (10 arteries) or Ad.βGal (6 arteries) or were sham-transferred (4 arteries), with the same protocol and infectious doses used for normal arteries. Arteries were harvested 3 days after gene transfer, and 5-mm rings were either snap-frozen for tissue cryosections or immediately suspended in organ baths for isometric vasomotor tension studies. A, Representative cryosections of arteries transformed with Ad.nNOS or Ad.βGal and stained for NADPH-diaphorase activity. Sham-transferred arteries (not shown) had modest endothelial staining that was visually indistinguishable from that of Ad.βGal-transferred arteries. B, Image analysis of NADPH-diaphorase–stained arteries. Staining intensity of sham-transferred arteries was arbitrarily set at 1.0. There was no significant difference in staining intensity between sham-transferred (1.0±0.1) and Ad.βGal-transferred arteries (0.8±0.1). Ad.nNOS-transferred arteries showed significantly higher staining intensity (2.1±0.1). *$P<0.01$ versus sham-transferred and Ad.βGal-transferred arteries. C, ACh-induced relaxation of vessels precontracted with PE. There was no significant difference between sham-transferred and Ad.βGal-transferred arteries. **$P<0.01$ versus sham-transferred and Ad.βGal-transferred arteries.

vasculature, this response characterized by a viral antigen–specific cytotoxic T lymphocyte response beginning ≈7 days after infection, with loss of transgene expression and intimal hyperplasia in the ensuing days and weeks.[19] Accordingly, we chose to study the vessels at an early time point, 3 days after infection, when adenovirus-mediated transgene expression is near-maximal but no chronic inflammatory response has developed. In addition, adenoviral infection can cause a dose-dependent acute inflammatory injury that can impair endothelial vasomotor function.[14] To avoid this, we conducted our studies at a relatively low infectious titer (3×10^9 pfu/mL), a dose that we have shown yields maximal transgene expression without apparent vascular injury. Although others have reported medial inflammation and contractile dysfunction after higher-titer infection rabbit arteries,[20] we observed no such impairment here. The difference is likely attributable to the fact that we used a 10-fold lower adenovirus titer.

Prior published studies have shown that eNOS gene transfer has favorable vascular effects in normal and balloon-injured arteries.

First, Von der Leyen et al[10] used liposome–Sendai virus hemagglutinin protein complexes to deliver an eNOS-expressing plasmid to balloon-denuded rat carotids. This approach restored NOS activity to near-normal levels and significantly limited the subsequent development of neointimal hyperplasia.

Second, Janssens et al[21] delivered recombinant adenovirus expressing eNOS (Ad.eNOS) to rat lungs via aerosol; these experiments showed that Ad.eNOS significantly attenuated the rise in pulmonary artery pressure induced by acute hypoxia.

Third, Kullo et al[12] showed that the adventitial delivery of an eNOS-expressing adenovirus markedly enhanced vascular NOS enzymatic activity and basal vascular cGMP levels in rabbit carotid arteries. The effects on vasomotor function, however, were more modest: reduced contractile force in response to PE and ≈2-fold enhanced sensitivity to A23187 and ACh. Although comparisons should be made with caution, we observe substantially greater enhancement of endothelium-dependent relaxation: for example, Ad.nNOS-infected arteries have 20-fold increased sensitivity to ACh. One possible explanation is that endovascular delivery is more effective than adventitial delivery; the somewhat higher maximal velocity and specific activity of the nNOS isoform relative to eNOS also may contribute.[22,23] However, the precise reason for the differences cannot be stated in the absence of direct experimental comparisons.

NOS Gene Transfer Restores Vasomotor Function in Cholesterol-Fed Rabbits

Although normal arteries represent a significant proving ground for NOS gene transfer, an important next step is to determine whether NOS gene transfer can reverse the vasomotor deficit known to occur in arteries from hypercholesterolemic rabbits. Reduced NO bioavailability occurs early in the atherosclerotic process in animal models; for example, hypercholesterolemia impairs endothelium-dependent relaxation in rabbits,[24] pigs,[25] and primates.[26] Similarly, a number of studies in humans document decreased vasomotor responsiveness in preatherosclerotic conditions such as hypercholesterolemia,[27] hypertension,[28] and cigarette smoking.[29]

The mechanism for the NO deficit has not been firmly established in any model of atherogenesis. Possibilities include (1) impaired NO production due to reduced NOS expression, reduced substrate availability (eg, L-arginine), reduced cofactor availability (eg, tetrahydrobiopterin),[30] or an effect of endogenous inhibitors (eg, asymmetric dimethylargine)[31]; (2) enhanced NO destruction, due to increased production of reactive oxygen species such as superoxide radical[32]; and (3) deleterious effects on downstream mediators of NO activity. The effect of NOS gene transfer in atherosclerosis models would depend on the underlying mechanism. To the extent that NO deficit is due to decreased expression, forced overexpression of NOS should have a favorable impact. However, in cholesterol-fed rabbits, eNOS expression appears to be well preserved, and the NO deficit appears to be due to increased production of reactive oxygen species.[33] In this circumstance, nNOS overexpression might increase NO bioavailability, but it also might increase the production of other reactive nitrogen species with an uncertain effect on vasomotor function.[34] However, if the observed deficit is due to NOS substrate or cofactor deficiency, nNOS overexpression should have a limited impact on NO production and indeed may merely enhance the production of reactive oxygen species.[35,36] That Ad.nNOS gene transfer substantially improved vasomotor function in arteries from cholesterol-fed rabbits is compatible with NOS deficiency, enhanced NO destruction, or impaired downstream signaling, but it suggests that NOS substrate or cofactor deficiency is not the dominant mechanism in this model.

The impact of NOS gene transfer on other indexes of atherosclerosis remains to be investigated. Ad.nNOS roughly doubles NO bioavailability in arteries from hypercholesterolemic rabbits, which may have favorable effects on arterial platelet deposition,[4] expression of chemokines such as monocyte chemoattractant protein-1,[7] expression of adhesion molecules,[37] and inflammatory cell accumulation. These possibilities are currently under investigation.

Acknowledgments

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