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Pharmacology

Gene transfer of endothelial nitric oxide synthase to the penis augments erectile responses in the aged rat

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ABSTRACT **Nitric oxide (NO), a mediator involved in penile erection, is synthesized by the nitric oxide synthase (NOS) family of enzymes. It has been shown that NOS activity decreases with age. To determine whether adenoviral-mediated overexpression of endothelial NOS (eNOS) could enhance erectile responses, we administered a recombinant adenovirus containing the eNOS gene (AdCMVeNOS) into the corpora cavernosum of the aged rat. Adenoviral expression of the β-galactosidase reporter gene was observed in cavernosal tissue 1 day after intracavernosal administration of AdCMVβgal; 1 day after administration of AdCMVeNOS, transgene expression was confirmed by immunoblot staining of eNOS protein, and cGMP levels were increased. The increase in cavernosal pressure in response to cavernosal nerve stimulation was enhanced in animals transfected with eNOS, and erectile responses to acetylcholine and zaprinast were enhanced at a time when the erectile response to the NO donor sodium 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate was not altered. These results suggest that *in vivo* gene transfer of eNOS, alone or in combination with a type V phosphodiesterase inhibitor, may constitute a new therapeutic intervention for the treatment of erectile dysfunction.**

An estimated 20 million to 30 million men in America suffer from erectile dysfunction, and it is estimated that 50–60% of these individuals may have a vascular component to their problem (1, 2). Penile erection is caused by relaxation of the blood vessels and trabecular meshwork of smooth muscle that constitutes the corpora cavernosum (3, 4). Although parasympathetic innervation is involved in erectile function, the erectile response cannot be blocked completely by atropine, suggesting that a nonadrenergic, noncholinergic mechanism may also play a role (5, 6). Nitric oxide (NO) is a radical that serves as a signaling molecule in the central and peripheral nervous systems. NO has vasodilator activity and has an integral role in the physiological regulation of blood flow and pressure (7). Recently, the role of NO in penile erection has been elucidated, and NO has been reported to affect erectile activity profoundly at the level of the central nervous system and at the peripheral level (8–13).

NO is formed from the conversion of L-arginine by nitric oxide synthase (NOS), which exists in three isoforms: endothelial (eNOS, constitutive NOS, or NOS III), neural (nNOS or NOS I), and inducible (iNOS or NOS II). The constitutive forms of the enzyme, eNOS and nNOS, are coupled to calcium and calmodulin. Although iNOS has been shown to be expressed constitutively in tissues, including the penis, it is generally believed to be expressed in cells exposed to cytokines and up-regulated in pathophysiological conditions (14). It is thought that both nNOS, through the release of NO from nerve terminals, and eNOS, through activation by acetylcholine, work together to mediate erection. NO binds to the ferrous heme moiety of soluble guanylate cyclase, resulting in increased cGMP formation and relaxation of cavernosal smooth muscle.

Injection of cDNA encoding *h*Slo, a maxi-K^+ channel, has been shown to increase gap-junction formation and enhance the erectile responses to nerve stimulation in the rat, providing evidence that *in vivo* gene transfer can alter penile erection (15). Although naked cDNA has been shown to alter erectile activity, little if anything is known about the effects of adenoviral gene transfer on erectile responses *in vivo* (15–17). The present study, therefore, was undertaken to investigate the influence of adenoviral gene transfer of eNOS to the corpora cavernosum on nerve- and agonist-induced erectile responses in the rat.

METHODS

Adenovirus Vectors. Two replication-deficient recombinant adenoviruses, serotype 5-encoding nuclear-targeted β-galactosidase (AdCMVβgal) and eNOS (AdCMVeNOS), both driven by a cytomegalovirus (CMV) promoter, were prepared as described (18). Recombinant adenoviruses were plaque-purified, and virus titer was determined by plaque assay on 293 cells. After purification, the virus was suspended in PBS with 3% (vol/vol) sucrose and kept at −80°C until use. Amplification and purification were done by the University of Iowa Gene Transfer Vector Core (Iowa City, IA).

***In Vivo* Gene Delivery to the Corpora Cavernosum.** Male 40-week-old Sprague–Dawley rats weighing 350–400 g were anesthetized with sodium pentobarbital (30 mg/kg i.p.) and placed in a supine position on a thermoregulated surgical table. Body temperature was monitored continuously with a rectal temperature probe (Yellow Springs Instruments) and was maintained at 37°C with a water-jacketed heating blanket. By using a sterile technique, the penis was exposed. By using a 30-gauge needle attached to a microliter syringe, 20 μl of vehicle [3% (vol/vol) sucrose in PBS], AdCMVβgal (1×10^{12} pmol/ml), or AdCMVeNOS (1×10^{12} pmol/ml) was injected into the corpus cavernosum. Immediately before instillation, blood drainage via the dorsal veins was halted by circumferential compression of the penis at the base with an elastic band. Compression was released 5 min after injection of 20 μl of the vehicle or virus.

Expression of β-Galactosidase. The rats were anesthetized with pentobarbital (30 mg/kg i.p.) 1 day after adenovirus administration and placed in a supine position on a surgical table. Rats did not show any overt signs of systemic (fever, dyspnea, tachycardia) or local (purulent discharge, erythema,

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Abbreviations: NOS, nitric oxide synthase; eNOS, endothelial NOS; nNOS, neural NOS; iNOS, inducible NOS; CMV, cytomegalovirus.

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edema) infection when observed 1 day after transfection. The rats were killed with an overdose of pentobarbital (80 mg/kg i.p.), and the penis was removed. Expression of β-galactosidase was evaluated by measurement of β-galactosidase activity in tissue samples and by histochemical staining.

For determination of β-galactosidase activity (Galacto-Light Plus, Tropix, Bedford, MA), the penile cavernosal tissues were minced into small pieces with a scalpel and placed in lysis buffer for 15 min (75 μl per sample; 0.2% Triton X-100/100 nmol potassium phosphate, pH 7.8). Samples were centrifuged (at 12,000 rpm for 10 min; μSpeedfuge, Savant Instruments, Farmingdale, NY), and the supernatant was removed. Original supernatant and dilutions of the supernatant (1:10 and 1:100) were prepared in duplicate for each tissue lysate. Aliquots of tissue lysate were assayed for β-galactosidase activity with 3-(4-methoxyspirol-[1,2-dioxetane-3,2[1]-tricyclo-[3.3.1.1[3,7]]decan]-4-yl)-phenyl β-D-galactopyranoside. Light emission was measured with a luminometer (Luminoscan RS, Labsystems, Franklin, MA) and calibrated with a standard curve generated with the use of purified *Escherichia coli* β-galactosidase. Protein concentrations of the samples were determined (Bio-Rad DC Protein Assay), and normalized β-galactosidase activity was expressed as milliunits of β-galactosidase per milligram of protein.

For histochemical analysis of β-galactosidase localization, the transfected animals were killed as described above, and the penile tissues were cut in 2-mm sagittal sections, incubated in X-Gal stain [PBS, 20 mM $K_4Fe(CN)_6 \cdot {}^3H_2O$, 20 mM $K_3Fe(CN)_6$, 2 mM $MgCl_2$, and 1 mg/ml in DMSO of 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (Sigma)] for 2 h at 24°C, rinsed in PBS, and postfixed in 7% (vol/vol) buffered formalin for 6 h. The sections then were placed in 20% (vol/vol) sucrose for 12 h, overlaid with O.C.T. compound (Sakura Finetek, Torrance, CA), and frozen in liquid nitrogen. Cryostat (7-μm) sections were mounted on poly-L-lysine-coated slides and counterstained with eosin Y. Sections of penis were examined for positive β-galactosidase staining (blue nuclei) by light microscopy.

Expression of eNOS Protein and Activity. Expression of eNOS in rat cavernosal tissue was assessed 24 h after transfection. Animals were killed as described above, and penises were excised and either processed immediately or quick frozen in liquid nitrogen. To extract total protein, cavernosal tissue was homogenized (Polytron, Brinkmann) in ice-cold buffer [5 mM Hepes, pH 7.9/26% (vol/vol) glycerol/1.5 mM $MgCl_2$/0.2 mM EDTA/0.5 mM DTT/0.5 mM phenylmethylsulfonyl fluoride] with NaCl (300 mM final) and incubated on ice for 30 min. After centrifugation twice at 15,000 × *g* at 4°C for 20 min, the supernatant was mixed with an equal volume of 2% (vol/vol) SDS/1% mercaptoethanol and fractionated by using SDS/8% PAGE (70 μg per lane). Proteins were then transferred to a nitrocellulose membrane (Hybond-ECL; Amersham Pharmacia) by semidry electroblotting for 1 h. The membranes were blocked 1 h at room temperature with blotto-Tween [5% (vol/vol) nonfat dry milk/0.1% Tween-20] and incubated with a primary monoclonal rat anti-eNOS IgG antibody (Santa Cruz Biotechnology). Bound antibody was detected with labeled rabbit anti-rat IgG secondary antibody (Santa Cruz Biotechnology) and visualized by using enhanced chemiluminescence.

For the determination of constitutive NOS activity (Calbiochem), L-arginine to L-citrulline conversion was assayed in cavernosal extracts. Briefly, cavernosal samples were homogenized in Tris·HCl (250 mM; pH 7.4), EDTA (10 mM), and EGTA (10 mM) and centrifuged at 12,000 rpm for 10 min (μSpeedfuge) at 4°C. The supernatant was incubated in NADPH (10 mM), L-[^{3}H]arginine (1 μCi/liter), $CaCl_2$ (6 mM), Tris·HCl (50 mM; pH 7.4), tetrahydrobiopterin (6 μM), FAD (2 μM), and FMN (2 μM) for 60 min at 24°C. The reaction was stopped with Hepes (50 μM; pH 5.5) and EDTA (5 mM). The radioactivity of the sample eluate was measured by liquid scintillation counting. Enzyme activity was expressed as citrulline production in picomoles per milligram of protein per hour. In experiments determining the role of iNOS activity in the cavernosal samples, L-[^{3}H]arginine to L-[^{3}H]citrulline conversion was studied under essentially calcium-free conditions.

Measurement of Tissue and Plasma cGMP Levels. Blood was withdrawn, and plasma was removed 24 h after instillation of vehicle, AdCMVβgal, or AdCMVeNOS. The penile tissue was rinsed with PBS, quick frozen in liquid nitrogen, and stored at −70°C until cGMP levels were determined. Plasma samples were added to ethanol, vortexed, and left at room temperature for 5 min. The samples were then centrifuged at 1,500 × *g* for 10 min, and the supernatant was collected, dried by vacuum centrifugation, and resuspended in phosphate buffer. Whole penile tissue was homogenized in 1 ml of ice-cold 6% (vol/vol) trichloroacetic acid (pH 4.0). Each sample was then centrifuged at 1,500 × *g* for 10 min at 4°C; the supernatant was transferred to a 10-ml test tube, and the trichloroacetic acid was extracted with H_2O-saturated diethyl ether. The samples were assayed for cGMP by using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Penile cGMP levels are expressed as femtomoles of cGMP per milligram of protein, and plasma levels are expressed as picomoles per milliliter.

Measurement of Erectile Responses. Rats were anesthetized with sodium pentobarbital (30 mg/kg i.p.) and placed on a thermoregulated surgical table 1 day after vehicle or adenovirus administration. Supplemental doses of pentobarbital were administered as needed to maintain a uniform level of anesthesia. Body temperature was monitored continuously with a rectal temperature probe (Yellow Springs Instruments) and was maintained at 37°C with a water-jacketed heating blanket. The trachea was cannulated (PE 240 polyethylene tubing) to maintain a patent airway, and the animals breathed room air enriched with 95% O_2/5% CO_2. A carotid artery was cannulated (PE 50 tubing) for the measurement of systemic arterial pressure. Systemic arterial pressure was measured continuously with a Viggo-Spectramed transducer (Viggo Spectramed, Oxnard, CA) attached to a polygraph (Grass Instruments Model 7, Quincy, MA). The left jugular vein was cannulated (PE 50 tubing) for the administration of fluids and supplemental anesthesia.

The bladder and prostate were exposed through a midline abdominal incision. The cavernosal nerve was identified posterolateral to the prostate on one side, and an electronic stimulator with a stainless steel bipolar hook was placed around the cavernosal nerve. The skin overlying the penis was incised, and the right crura was exposed by removing part of the overlying ischiocavernous muscle. A 25-gauge needle filled with 250 units/ml heparin and connected to PE-50 tubing was inserted into the right crura. A 30-gauge needle connected to PE-10 tubing was placed in the left corpus cavernosum for administration of drugs into the penis. Systemic arterial and intracavernosal blood pressure was measured with a Statham P23 pressure transducer connected to a polygraph recorder. The cavernosal nerve was stimulated with a square pulse stimulator (Grass Instruments). Electrical field stimulation at a frequency of 15 Hz, pulse width of 30 s, and duration of 1 min was applied to the nerve at 5 V to achieve a consistent erectile response. These procedures have been described (15).

In experiments in which the agonists (diluted to a volume of 50 μl) were administered intracavernosally, injections were made via the 30-gauge needed inserted into the corpus cavernosum. Injections of agonists were made when the cavernosal pressure was at baseline value, and the effect of a single injection of a randomized dose of an agonist on cavernosal pressure and penile length was recorded until cavernosal pressure returned to preinjection level. The next injection was made after a 10- to 15-min period from the end of the

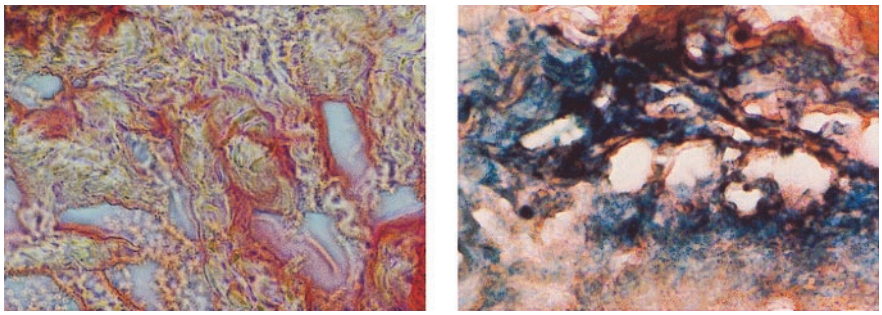

FIG. 1. β-Galactosidase (*Right*; dark stain) expression in the corpus cavernosum of a rat 1 day after transfection with AdCMVβgal. AdCMVβgal-transfected rat penis shows localization of β-galactosidase in the smooth muscle of the corpus cavernosum. Animals treated with vehicle showed no staining for β-galactosidase (*Left*).

preceding response to ensure a stable baseline. Injection of 50 μl of the saline vehicle had no significant effect on cavernosal pressure or penile length.

Arterial blood gases and pH were monitored with a Corning 178 analyzer with a 50-μl blood sample and were within the normal range. P_{O_2}, P_{CO_2}, and pH averaged 126 mmHg (1 mmHg = 133 Pa), 27 mmHg, and 7.41, respectively.

Drugs. Acetylcholine bromide (Sigma) was dissolved in 0.9% NaCl. Sodium 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (Research Biochemicals, Natick, MA) was dissolved in 10 mM NaOH; this alkaline stock solution was then diluted with PBS (pH 7.4), and the dosing solution was administered immediately after preparation. Zaprinast (2-*O*-propoxyphenyl-8-azapurin-6-one; Rhone-Poulenc, Degenham, Essex, U.K.) was dissolved in 0.15 M NaOH in 0.9% NaCl at a concentration of 3 mg/ml and diluted in 0.9% NaCl. The solvents for the agonists used in this study did not alter cavernosal pressure or agonist responses. Stock solutions of acetylcholine and zaprinast were stored in a freezer in 1-ml opaque tubes, and working solutions were prepared daily and kept on crushed ice during the experiment.

Statistics. Data are expressed as means ± SEM and were analyzed by using a one-way ANOVA with repeated measures and a Neumann–Kuels post hoc test for multiple-group comparisons. A *P* value of less than 0.05 was used as the criterion for statistical significance.

RESULTS

***In Vivo* Gene Transfer to the Corpus Cavernosum.** β-Galactosidase activity was quantified in the penis 1 day after intracavernosal administration of vehicle, AdCMVβgal, or AdCMVeNOS by using chemiluminescence. Penile tissue from rats treated with vehicle or AdCMVeNOS showed very low levels of β-galactosidase activity (0.11 ± 0.1 milliunit/mg protein). Penile tissue from rats treated with AdCMVβgal had β-galactosidase activity of 53 ± 4 milliunit/mg protein ($P < 0.05$). β-Galactosidase activity was similar in all areas of the corpus cavernosum of rats treated with AdCMVβgal.

Histochemical analysis of β-galactosidase enzyme transduction was assessed in the penile tissue of rats transfected with AdCMVβgal, and a typical 7-μm section of penis is shown in Fig. 1. β-Galactosidase protein was expressed diffusely throughout the corpus cavernosum 1 day after AdCMVβgal transduction (Fig. 1). β-Galactosidase staining was not observed in the corpus cavernosum of rats treated with vehicle or AdCMVeNOS (Fig. 1).

Tissue eNOS protein levels were measured 1 day after intracavernosal administration of vehicle, AdCMVβgal, or AdCMVeNOS, and these data are shown in Fig. 2. Western immunoblot analysis of whole penile cavernosal tissue with an eNOS-specific antibody detected abundant levels of eNOS (135-kDa) protein in rats transfected with AdCMVeNOS (Fig. 3, lane 3), and levels were greater than those observed in the cavernosal tissue of control (vehicle-treated) rats (Fig. 3, lane 1) and of rats transfected with AdCMVβgal (Fig. 3, lane 2). Levels of α-actin (42-kDa) protein were similar in penile tissue harvested from AdCMVβgal and AdCMVeNOS transfected rats (Fig. 2). When eNOS levels were analyzed by densitometry and expressed per milligram of protein, eNOS protein levels were significantly higher in rats after administration of AdCMVeNOS compared with eNOS protein levels in rats treated with vehicle or AdCMVβgal (Fig. 2).

In experiments determining the activity of the eNOS transgene, the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline was measured in cavernosal tissue from rats treated with vehicle, AdCMVβgal, or AdCMVeNOS. Calcium-dependent L-[^{3}H]arginine conversion in rats treated with vehicle or AdCMVβgal was similar (Fig. 3). Calcium-dependent L-[^{3}H]arginine conversion to L-[^{3}H]citrulline was significantly higher in rats 1 day after transfection with AdCMVeNOS compared with rats treated with vehicle or AdCMVβgal. The role of calcium-independent L-arginine conversion, an indica-

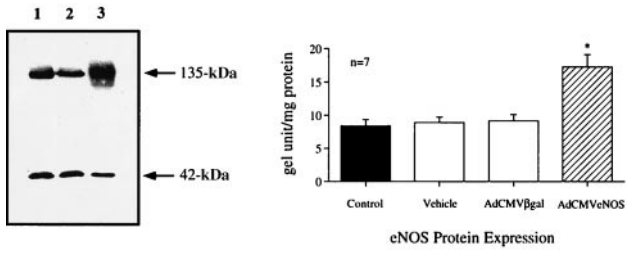

FIG. 2. (*Left*) Western immunoblot analysis of eNOS protein (135-kDa) levels in cavernosal tissue of rats 24 h after administration of vehicle (lane 1), AdCMVβgal (lane 2), or AdCMVeNOS (lane 3). α-Actin (42-kDa) concentrations were used to control for sample loading. (*Right*) Densitometry analysis of eNOS protein (gel unit per milligram of protein) in rat corpus cavernosal tissue 1 day after administration of vehicle, AdCMVβgal, or AdCMVeNOS. *n*, number of experiments; *, response significantly different from AdCMVβgal ($P < 0.05$).

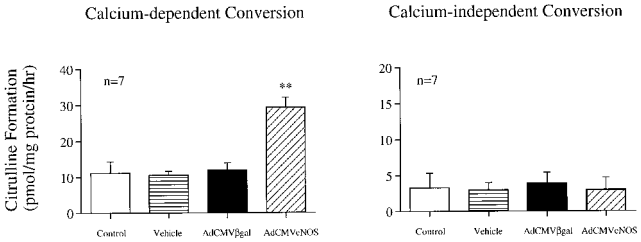

FIG. 3. Comparison of calcium-dependent (*Left*) and calcium-independent (*Right*) L-[^{3}H]arginine conversion to L-[^{3}H]citrulline in homogenates of rat cavernosal tissue 1 day after administration of vehicle, AdCMVβgal, or AdCMVeNOS. Values are picomoles of citrulline per milligrams of protein per hour. $n = 7$; **, response significantly different from AdCMVβgal ($P < 0.05$).

tor of iNOS activity, was studied by measuring the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline in the absence of calcium. Calcium-independent conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline in the corpus cavernosum 1 day after treatment with vehicle, AdCMVβgal, or AdCMVeNOS was not significantly different when the groups were compared (Fig. 3).

Effect of AdCMVeNOS on Penile and Plasma cGMP Levels. Penile tissue and plasma concentrations of cGMP were measured 24 h after intracavernosal administration of vehicle, AdCMVβgal, or AdCMVeNOS, and these data are summarized in Table 1. Cavernosal tissue cGMP and plasma levels were similar in penile tissue of rats treated with vehicle or AdCMVβgal (Table 1). cGMP concentrations were increased significantly in penile tissue of rats transfected with AdCMVeNOS compared with control or animals treated with AdCMVβgal ($P < 0.05$; Table 1). Plasma concentrations of cGMP were not altered significantly in rats treated with AdCMVeNOS (Table 1).

Effect of AdCMVeNOS on Erectile Responses Induced by Nerve Stimulation. The effect of transfection of AdCMVeNOS on erectile responses induced by cavernosal nerve stimulation was studied in the anesthetized rat, and these data are summarized in Fig. 4. The magnitude of the increase in cavernosal pressure in response to nerve stimulation was increased significantly 1 day after treatment with AdCMVeNOS compared with the pressure increase 1 day after administration of vehicle or AdCMVβgal (Fig. 4). The magnitude of the erectile response to cavernosal nerve stimulation in vehicle-treated rats and those treated with AdCMVβgal was similar (data not shown).

Effect of AdCMVeNOS on Agonist-Induced Erectile Responses. The influence of AdCMVeNOS transfection on the erectile response to acetylcholine and zaprinast was investigated, and these data are summarized in Fig. 5. When injected intracavernosally, acetylcholine or zaprinast induced dose-related increases in cavernosal pressure (Fig. 5). Increases in cavernosal pressure were similar in vehicle control and AdCMVβgal-transfected animals (data not shown). In rats treated with AdCMVeNOS, the increase in cavernosal pressure in response to intracavernosal injections of acetylcholine and zaprinast was significantly greater in magnitude and duration than in rats treated with AdCMVβgal (Fig. 5). The increase in cavernosal pressure in response to intracavernosal injection of the NO donor sodium 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate was similar in rats treated with AdCMVβgal or AdCMVeNOS (data not shown).

DISCUSSION

Gene transfer to blood vessels with adenoviral vectors has been accomplished, and it has been shown that gene transfer of eNOS to the pulmonary vascular bed of the rat and mouse can alter pulmonary vascular function (7, 18–23). In this study, we show that adenoviral-mediated transfer of the eNOS gene to the corpus cavernosum of the rat augments the increase in cavernosal pressure in response to cavernosal nerve stimulation and to injections of acetylcholine and zaprinast. Immunoblot studies revealed increased tissue eNOS protein, and eNOS activity and cGMP levels were increased after intracavernosal administration of AdCMVeNOS. These results indicate that the eNOS transgene has biologic activity in the rat penis and suggest that adenoviral transfer of the eNOS gene enhances erectile function in the rat.

Table 1. Influence of AdCMVβgal and AdCMVeNOS on penile tissue and plasma concentration of cGMP in the rat

Group	No. of experiments	Tissue, fmol/mg protein	Plasma, pmol/milliliter
Control	9	6.27 ± 0.9	5.9 ± 1.4
AdCMVβgal	8	6.35 ± 0.8	6.1 ± 1.7
AdCMVeNOS	8	20.4 ± 2.1*	6.8 ± 2.1

Data are shown as means ± SEM. *, $P < 0.05$.

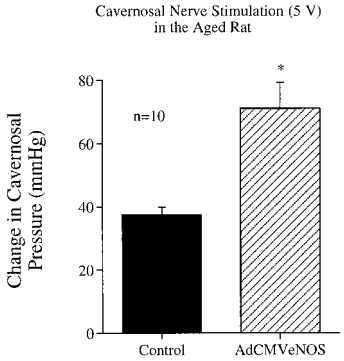

FIG. 4. Increase in cavernosal pressure in response to cavernosal nerve stimulation (5 V) 1 day after intracavernosal administration of AdCMVβgal or AdCMVeNOS. Responses to nerve stimulation 1 day after administration of vehicle were similar to those observed after administration of AdCMVβgal. *n*, number of experiments; *, response significantly different from AdCMVβgal ($P < 0.05$).

It is possible that adenoviral-mediated gene transfer to the penis induces an inflammatory response, increasing levels of iNOS in the corpora cavernosum. However, several observations suggest that an inflammatory response did not account

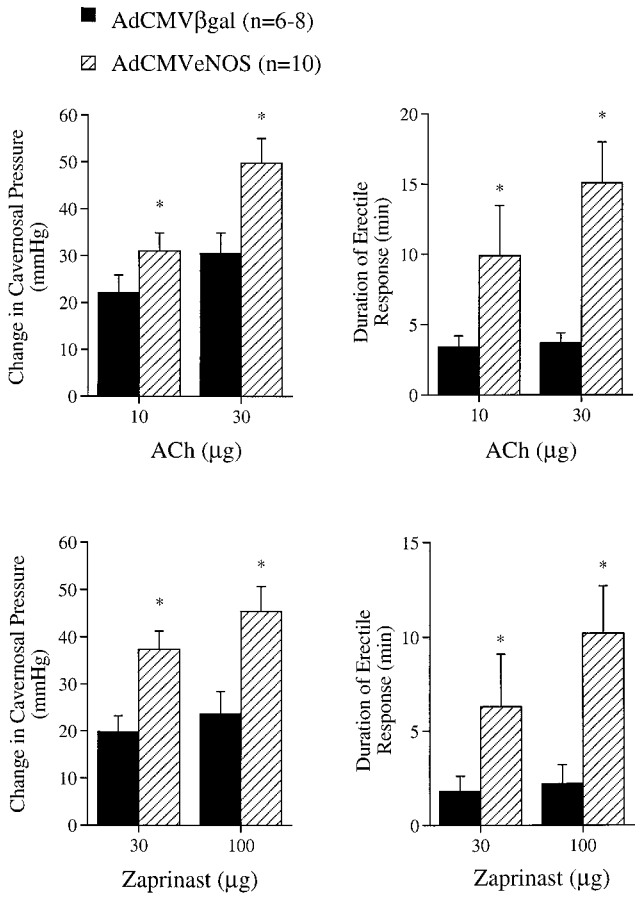

FIG. 5. Magnitude (*Left*) and duration (*Right*) of the increase in cavernosal pressure in response to intracavernosal injection of acetylcholine (ACh; *Upper*) or zaprinast (*Lower*) 1 day after administration of AdCMVβgal or AdCMVeNOS. Responses to acetylcholine and zaprinast 1 day after administration of vehicle were similar to those observed after administration of AdCMVβgal. *n*, number of experiments; *, response significantly different from AdCMVβgal ($P < 0.05$).

for the present findings. First, there was no increase in cGMP levels in corpora transfected with AdCMVβgal, a viral vector devoid of vascular activity. Second, immunohistochemistry for iNOS showed no change in iNOS expression 1 day after transfection with AdCMVβgal or AdCMVeNOS. Third, the calcium-independent conversion of L-arginine to L-citrulline, an indicator of iNOS activity, was not changed at a time when calcium-dependent conversion, an indicator of eNOS activity, was increased. Fourth, there were no overt signs of an inflammatory response in animals treated with AdCMVβgal or AdCMVeNOS under the conditions of the present experiment. Leukocyte counts in blood samples from animals treated with vehicle, AdCMVβgal, or AdCMVeNOS were similar ($P < 0.05$). Histological examination of penile tissue from animals treated with AdCMVβgal and AdCMVeNOS showed few polymorphonuclear leukocytes, which suggests that a local inflammatory response was present. However, this inflammatory response does not seem to alter cavernosal responses 1 day after transfection.

The observation that erectile responses to electrical nerve stimulation and acetylcholine are increased after administration of AdCMVeNOS suggests that penile erection is mediated in part by the release of NO and is consistent with the postulated role of NO in mediating penile erection. These data are consistent with the results of Burnett *et al.* (24) showing that eNOS mediates NO-dependent penile erection in nNOS knockout mice, suggesting an important role for eNOS in the erectile response.

NOS activity is reduced with aging, as well as in diabetic states (25, 26). In addition to showing that the erectile response to acetylcholine is enhanced, the present results show that the response to the type V cGMP phosphodiesterase inhibitor zaprinast is increased. The observation that the erectile response to zaprinast is enhanced after eNOS gene transfer is consistent with the postulated role of cGMP in the erectile response (11, 12). These data are consistent with the concept that the eNOS transgene product is active and suggest that *in vivo* eNOS gene transfer, alone or together with the administration of a cGMP phosphodiesterase inhibitor, may represent a new strategy for the treatment of erectile dysfunction.

In summary, the results of the present study show that adenoviral-mediated transfer of the eNOS gene increases eNOS protein level and activity and elevates cGMP levels in the penis of the rat. Overexpression of eNOS enhances erectile responses to cavernosal nerve stimulation, acetylcholine, and zaprinast without altering erectile responses to a NO donor. These data suggest that adenovirally mediated transfer of the eNOS gene, alone or in combination with the administration of a cGMP phosphodiesterase inhibitor, may represent a new form of therapy for the treatment of erectile dysfunction.

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