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Gene Transfer of Prepro-Calcitonin Gene-Related Peptide Restores Erectile Function in the Aged Rat[1]

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ABSTRACT

Erectile dysfunction in the aging male is caused, in part, by inadequate relaxation of the corpora cavernosal smooth musculature. Calcitonin gene-related peptide (CGRP), a peptide neurotrasmitter localized in the corpora cavernosa, is down-regulated in the aging rat penis. We examined the hypothesis that this reduction in CGRP may contribute to decreased cavernosal smooth muscle relaxation. Therefore, we sought to determine whether adenoviral-mediated gene transfer of prepro-CGRP (AdRSVCGRP) could enhance erectile responses in aged rats. We found a significant decrease in CGRP concentrations and in cAMP and cGMP levels in aged rat cavernosal tissue compared to younger rats. Aged rats also had significantly lower erectile function as determined by cavernosal nerve stimulation compared to younger rats. Five days after transfection with AdRSVCGRP, these aged rats had an approximately threefold increase in cavernosal CGRP levels compared to animals transfected with adenoviruses encoding nuclear-targeted β-galactosidase (AdRSVβgal). The AdRSVCGRP-transfected animals also demonstrated an increase in CGRP mRNA and immunohistochemical localization of CGRP in the smooth muscle of the corpora cavernosa. In addition, cAMP levels in the corpora cavernosa were significantly increased, whereas cGMP levels remained unchanged. Adenoviral transduction efficiency of β-galactosidase reporter gene was measured by chemiluminescence and was observed in cavernosal tissue 5 days after transfection with AdRSVβgal. More importantly, 5 days after administration of AdRSVCGRP, a significant increase was observed in the erectile response to cavernosal nerve stimulation in the aged rat, similar to the response observed in younger rats. These data suggest that in vivo adenoviral gene transfer of CGRP can physiologically improve erectile function in the aged rat.

aging, cyclic adenosine monophosphate, cyclic guanosine monophosphate, male sexual function, penis

INTRODUCTION

Male erectile dysfunction (ED) has been defined as the inability to attain and/or maintain penile erection sufficient for satisfactory sexual intercourse. It afflicts approximately 20–30 million men in the United States and is caused, in part, by inadequate relaxation of the corpora cavernosal smooth musculature of the penis [1–3]. Erectile dysfunction is considered to be a natural process of aging and normally affects older men. In 1995, it was estimated that approximately 152 million men worldwide suffered from ED, with projections for 2025 growing to 322 million men suffering from some degree of sexual dysfunction [4].

Penile erection is a hemodynamic process involving three synergistic and simultaneous events: increase of arterial inflow into the sinusoids under the control of systemic arterial pressure, relaxation of the cavernosal smooth muscle, and veno-occlusion or restriction of venous outflow from the penis. This process is generally accepted to be under neuroregulatory control. Nitric oxide (NO) is considered to be the principle stimulator of cavernosal smooth muscle relaxation and penile erection [5–7]. In the penis, NO is released from nonadrenergic/noncholinergic nerves and from vascular and sinusoidal endothelium. It binds to guanylate cyclase to increase intracellular levels of 3′,5′-cGMP, resulting in corporal smooth muscle relaxation. However, vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), and prostaglandin-mediated pathways, which produce an increase in intracellular cAMP and a reduction in intracellular Ca^{2+}, also contribute to smooth muscle relaxation in the penis [8–12]. Such diverse mediators as VIP and CGRP have been evaluated for their possible roles as neurotransmitters in regulating the process of penile erection.

In 1982, CGRP was first cloned and isolated from the gene encoding calcitonin [13, 14]. Alternate splicing of the calcitonin gene leads to the production of the 37-amino acid peptide CGRP, which has a characteristic seven-amino acid ring, linked by a disulfide bridge between positions 2 and 7, and an amidated N-terminus [13, 14]. The CGRP is a potent vasodilator in a number of peripheral vascular beds, whereas in the penis, it relaxes the smooth muscle cells of the corpora cavernosa by hyperpolarization via K^+-channel opening and activation of adenylate cyclase, with subsequent increases in intracellular 3′,5′-cAMP leading to erection [8, 15, 16]. When CGRP is administered intracavernosally in patients suffering from age-related ED, a dose-related increase occurs in penile arterial inflow and erection [17, 18]. Recently, immunoreactive CGRP has been shown to be down-regulated in the penis of aged rats [19]. Therefore, the aim of the present study was to determine whether adenoviral-mediated overexpression of prepro-CGRP could enhance erectile responses in aged rats to closely mimic those found in younger animals.

MATERIALS AND METHODS

Adenovirus Vectors

Two replication-deficient recombinant adenoviruses encoding nuclear-targeted β-galactosidase (AdRSVβgal) and prepro-CGRP (AdRSVCGRP), both driven by a rous sarcoma (RSV) promoter, were generated by standard methods of the University of Iowa Gene Transfer Vector Core Laboratory (Iowa City, IA) as previously described [20, 21]. Briefly, human

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TABLE 1. Primer sequences used for PCR.

Gene	Oligonucleotide primers	Size of PCR product (bp)
CGRP (sense)	5′-TCCTGCAACACCGCCACCTG-3′	
CGRP (antisense)	5′-GGTGGGCACAAAGTTGTCCT-3′	90
β-Actin (sense)	5′-AACCGCGAGAAGATGACCCAGATCATGTTT-3′	
β-Actin (antisense)	5′-AGCAGCCGTGGCCATCTCTTGCTCGAAGTC-3′	350

prepro-CGRP was cloned by blunt-end ligation into pAdRSV4. The resultant plasmid and adenovirus backbone sequences restricted of prostaglandin E_1 were transfected into HEK 293 cells, and plaques were isolated and amplified for analysis of CGRP expression. Recombinant adenoviruses were triple-plaque purified to ensure that viral suspensions were free of wild-type virus, and viral titers were determined by plaque assay on HEK 293 cells. After purification, the virus was suspended in PBS with 3% (w/v) sucrose and kept at −70°C until use. The AdRSVβgal was used as a control virus in the present study. Amplification and purification were performed by the University of Iowa Gene Transfer Vector Core Laboratory.

In Vivo Gene Delivery to the Corpora Cavernosa

Three groups of rats were utilized in the following study: young rats (12 wk), aged rats (60 wk) transfected with AdRSVβgal, and aged rats (60 wk) transfected with AdRSVCGRP. The 12- and 60-wk-old male Brown Norway rats (young rats, 225–300 g; aged rats, 450–550 g) were purchased from the NIH/NIA colony (Harlan Sprague-Dawley, San Diego, CA), maintained under controlled temperature and lighting, and treated according to National Institutes of Health regulations. The aged rats were anesthetized with sodium pentobarbital (30 mg/kg i.p.) and placed in a supine position on a thermoregulated surgical table. Using sterile technique, the penis was exposed. Then, 20 μl of vehicle (3% sucrose in PBS), AdRSVβgal (1×10^{12} parts/ml), or AdRSVCGRP (1×10^{12} parts/ml) were injected into the corpus cavernosum with a 30-gauge needle attached to a microliter syringe as previously described [22, 23]. Rats did not show any overt signs of systemic (fever, dyspnea, tachycardia) or local (purulent discharge, erythema, edema) infection when observed any day after transfection.

Expression of β-Galactosidase in Cavernosal Tissue

Five days after adenovirus administration of vehicle and AdRSVβgal, the aged rats were killed with an overdose of pentobarbital (80 mg/kg i.p.), and their penile shafts were removed. Expression of β-galactosidase was evaluated by measurement of β-galactosidase activity in cavernosal tissue samples using a β-galactosidase reporter gene assay (Galacto-Light Plus; Tropix, Bedford, MA) as previously described [23]. Protein concentrations of the samples were determined (Pierce Protein Assay; Pierce Endogen, Rockford, IL), and normalized β-galactosidase activity was expressed as relative light units of β-galactosidase per milligram of protein.

Measurement of Immunoreactive Cavernosal CGRP Levels

Cavernosal CGRP levels were measured in 12-wk-old (young) and 60-wk-old (aged) Brown Norway rat penile shaft cavernosal tissue 5 days after instillation of vehicle, AdRSVβgal, or AdRSVCGRP into the corpus cavernosum. Animals were killed, and the penises were excised and processed immediately or quick-frozen in liquid nitrogen. Cavernosal tissues were homogenized (Polytron; Brinkmann Instruments, Westbury, NY), tissue peptides extracted by boiling of homogenates in saline for 15 min and centrifugation (−4°C, $1500 \times g$ for 30 min), and supernatants collected. Cavernosal pellet boiling was repeated in 0.5 mol/L of acetic acid for 15 min, followed by centrifugation. For each penis, the combined supernatants were stored lyophilized until assay. Radioimmunoassays were performed on cavernosal extracts as previously described [24]. The CGRP levels were expressed as picomoles per 100 mg of protein.

RNA Extraction and Reverse Transcription-Polymerase Chain Reaction Amplification

Total RNA was isolated from the penile shaft corpus cavernosum of aged animals 5 days after transfection with vehicle, AdRSVβgal, and AdRSVCGRP using 1 ml of TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH) and prepared for reverse transcription-polymerase chain reaction (RT-PCR) as previously described [23]. The PCR reactions for β-actin were cycled 35 times at 94°C (denaturation) for 1 min, 60°C (annealing) for 1 min, and 72°C (extension) for 1 min. The PCR reactions for CGRP were cycled 33 times at 94°C (denaturation) for 1 min, 58°C (annealing) for 1 min, and 72°C (extension) for 1 min. Samples were incubated at 72°C for an additional 7 min after the last cycle was completed. All nucleotide primers were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). The primer pairs were chosen from the published cDNA sequences of rat CGRP and human β-actin as previously described [25, 26]. The primer sequences for CGRP and β-actin are shown in Table 1. The PCR products were separated on a 2% (w/v) agarose gel in 0.5× Tris-borate-EDTA buffer, stained with ethidium bromide (0.5 μg/ml), visualized under ultraviolet light, and quantitated by densitometric analysis.

Immunohistochemical Localization of CGRP

Immunohistochemical localization of CGRP was performed in the penises of young rats and 5 days after transfection of AdRSVβgal and AdRSVCGRP into the corpus cavernosum of aged rats. The penile shaft was separated from the crura, cut in cross-sections, fixed in 10% (w/v) formalin, and embedded in paraffin. Sections were deparaffinized in xylenes and hydrated through graded alcohols. Endogenous peroxidases were quenched with 3% H_2O_2, and sections were washed with PBS. Nonspecific binding of immunoglobulin G was blocked using normal horse serum diluted 1:50 in 0.1% (w/v) bovine serum albumin in PBS. The sections were incubated overnight with a rabbit monoclonal antibody for anti-CGRP (1:250; Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C, washed in PBS, and incubated for an additional 30 min with a biotinylated secondary antibody. Following a 30-min incubation with ABC horse radish peroxidase (Dako, Carpinteria, CA), the substrate (diaminobenzidine; Vectastain; Vector Laboratories, Peterborough, UK) was added for 5 min. This resulted in positive cells being labeled brown. Sections were then stained with hematoxylin.

Measurement of Cavernosal Tissue cAMP and cGMP Levels

Cavernosal cAMP and cGMP levels were measured in young rats and 5 days after instillation of AdRSVβgal and AdRSVCGRP into the corpus cavernosum of aged rats. Cavernosal tissue was rinsed with PBS, quick-frozen in liquid nitrogen, and stored at −70°C until determination of cAMP and cGMP levels. The samples were assayed for cAMP and cGMP using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) as previously described [23]. Cavernosal cAMP and cGMP levels are expressed as picomoles per 100 mg of protein.

Measurement of Erectile Responses

The 12-wk-old rats were anesthetized with sodium pentobarbital (30 mg/kg i.p.) and placed on a thermoregulated surgical table. Five days after vehicle or adenovirus administration, 60-wk-old rats were also anesthetized with sodium pentobarbital (30 mg/kg i.p.) and placed on a thermoregulated surgical table. 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 transducer (Viggo Spectramed, Oxnard, CA) attached to a computerized system for data acquisition (DATAQ; Data Systems International, St. Paul, MN). The left jugular vein was cannulated (PE-50 tubing) for fluid administration and supplemental anesthesia.

The bladder and prostate were exposed through a midline abdominal incision. The cavernosal nerve was identified posterolateral to the prostate

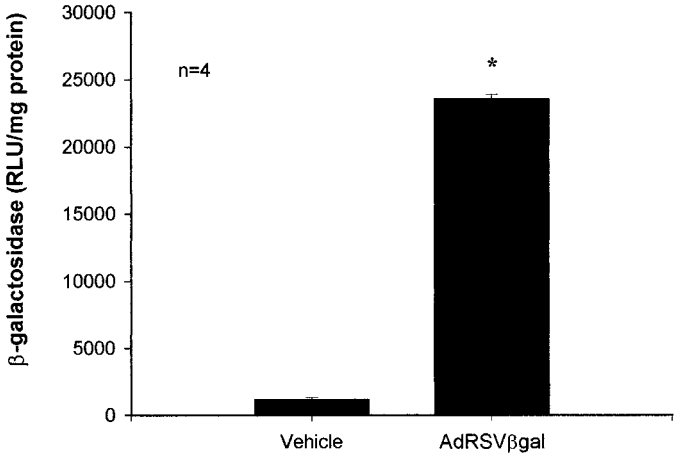

FIG. 1. β-Galactosidase activity in the corpus cavernosum of the aged rat 5 days after intracavernosal administration of vehicle and AdRSVβgal. The letter n indicates number of experiments. An asterisk ($P < 0.05$) indicates that β-galactosidase expression is significantly different from vehicle-treated animals.

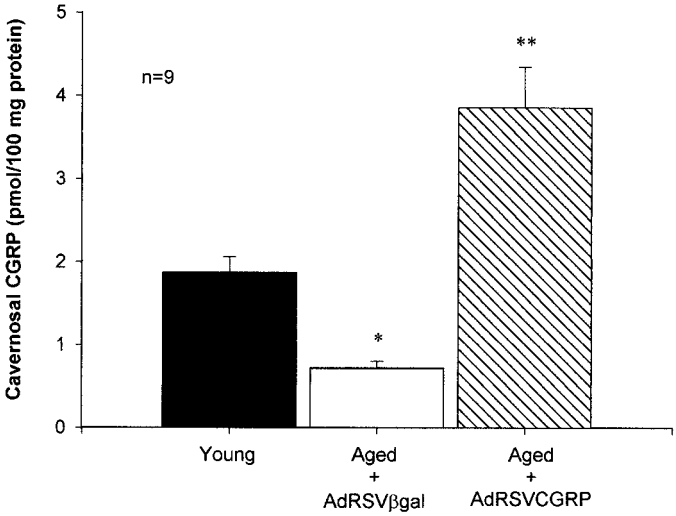

FIG. 2. Changes in cavernosal CGRP concentrations in young rats, aged rats transfected with AdRSVβgal, and aged rats transfected with AdRSVCGRP. The letter n indicates the number of animals. An asterisk indicates a significant difference ($P < 0.05$) from CGRP concentrations in younger rats; a double asterisk indicates a significant difference ($P < 0.05$) from young and aged rats treated with AdRSVβgal.

on one side, and an electrical stimulator with a stainless steel bipolar hook was placed around the cavernosal nerve. A 25-gauge needle filled with 250 U/ml of heparin and connected to PE-50 tubing was inserted into the right crura. Systemic arterial and intracavernosal blood pressure were measured with a statham P23 pressure transducer (Grass Instruments, Quincy, MA) connected to a computerized system for data acquisition (DATAQ). The cavernosal nerve was stimulated with a square-pulse stimulator (Grass Instruments). Each rat underwent electrical field stimulation at a frequency of 15 Hz, pulse width of 30 sec, and duration of 1 min. The application of 2.5, 5, and 7.5 V was used in the current protocol to achieve a significant and consistent erectile response. These procedures have been previously described [23, 27–29]. The Tulane University Animal Care and Use Committee approved all procedures used in the present study.

Statistics

Data are expressed as the mean ± SEM and were analyzed using a one-way ANOVA with repeated measures and Neumann-Keuls post-hoc test for multiple-group comparisons (Statview; Abacus Concepts, Inc., Berkeley, CA). A P value of less than 0.05 was used as the criterion for statistical significance.

RESULTS

β-Galactosidase Activity in Cavernosal Tissue

Five days after intracavernosal administration of vehicle or AdRSVβgal, β-galactosidase activity was quantified in the penis using chemiluminescence to detect transgene expression. Cavernosal tissue from rats transfected with vehicle showed very low levels of β-galactosidase, whereas cavernosal tissue from rats transfected with AdRSVβgal had significantly increased β-galactosidase activity ($P < 0.05$) (Fig. 1).

Immunoreactive Cavernosal CGRP Levels

Immunoreactive CGRP levels were measured in the cavernosal tissue of young rats and 5 days after intracavernosal administration of vehicle, AdRSVβgal, or AdRSVCGRP in aged rats, and these data are summarized in Figure 2. Cavernosal CGRP levels were significantly reduced in the aged rats compared to the younger animals ($P < 0.05$) (Fig. 2). The CGRP concentrations were increased threefold in the cavernosal tissue of aged rats transfected with AdRSVCGRP compared to concentrations in aged rats transfected with AdRSVβgal ($P < 0.05$) (Fig. 2). No statistical difference was found in cavernosal CGRP levels between the vehicle- and AdRSVβgal-transfected aged rats (data not shown).

Semiquantitative RT-PCR for CGRP

Five days after transfection with vehicle, AdRSVβgal, or AdRSVCGRP, CGRP and β-actin mRNA expression was determined in cavernosal tissue from all groups of aged rats (Fig. 3). The mRNA for CGRP (90 base pairs [bp]) was expressed in cavernosal tissue from all three groups of animals and was significantly higher in animals transfected with AdRSVCGRP compared to those transfected with vehicle or AdRSVβgal (Fig. 3A). As a positive control for RNA extraction and the RT reaction, the β-actin product was detected in cavernosal tissue from all three groups with the predicted size of 350 bp (Fig. 3A). When CGRP mRNA levels were analyzed by densitometry and normalized by dividing each integrated density value (I.D.V.) by the value of the β-actin I.D.V. band, CGRP gene expression was significantly higher in rats transfected with AdRSVCGRP compared to rats transfected with vehicle or AdRSVβgal (Fig. 3B). These results with mRNA expression for CGRP and β-actin are typical of four independent observations.

Immunohistochemical Localization of CGRP

To determine the location of CGRP after adenoviral transfection with CGRP in the aged rat penis, immunohistochemical localization of CGRP was performed in the rat penises 5 days after transfection with AdRSVβgal or AdRSVCGRP and is summarized in Figure 4. An increase was found in the immunohistochemical staining of CGRP in the sinusoidal spaces and the cavernosal smooth muscle cells in the AdRSVCGRP-treated aged rats (Fig. 4, B and E) compared to the corpus cavernosum of AdRSVβgal-treated rats (Fig. 4, A and D). The staining for CGRP was found predominately, but not exclusively, in the corpus cavernosum on the side of the penis in which the adenovirus was injected.

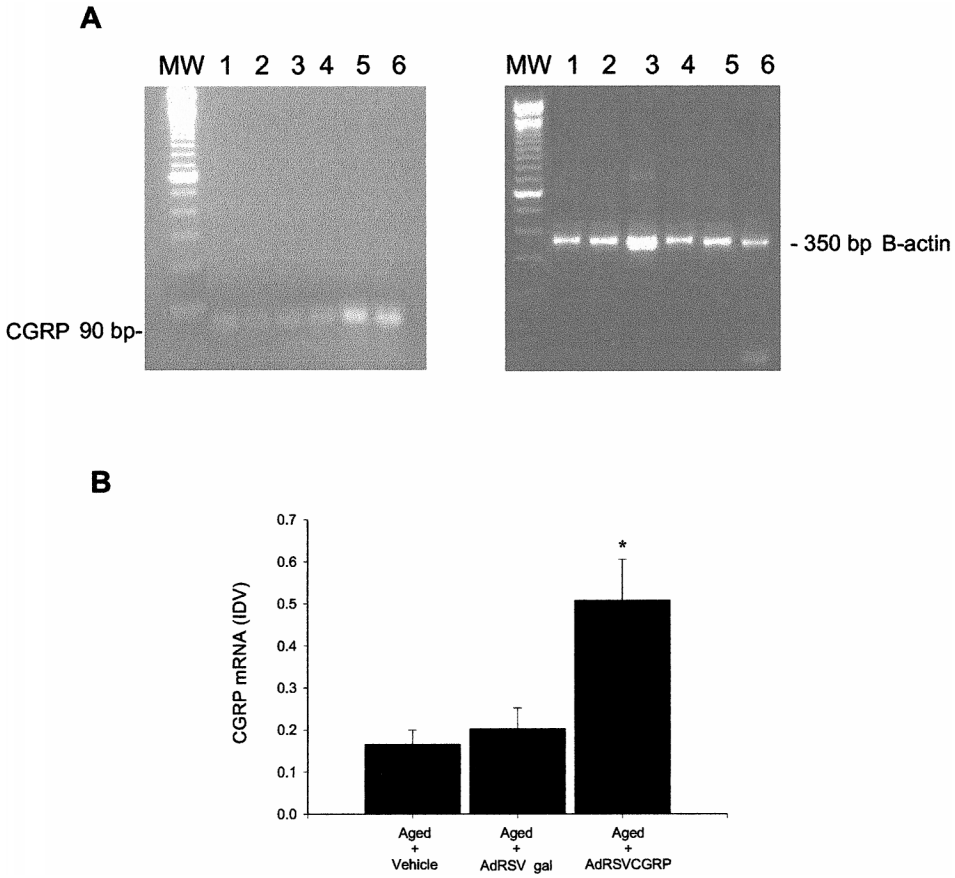

FIG. 3. The RT-PCR products of CGRP and β-actin (**A**) in cavernosal tissue of rats transfected with vehicle (lanes 1 and 2), AdRSVβgal (lanes 3 and 4), and AdRSVCGRP (lanes 5 and 6) and the mean of the normalized I.D.V. for each mRNA band (**B**) obtained by densitometric analysis (mean ± SEM). Normalization was performed by dividing each I.D.V. value by the value of the β-actin I.D.V. band in the same sample. Sizes of the RT-PCR products of CGRP and β-actin were 90 and 350 bp, respectively. These results are typical of four independent observations. An asterisk indicates a significant difference ($P < 0.05$) from vehicle- and AdRSVβgal-treated cavernosal tissue.

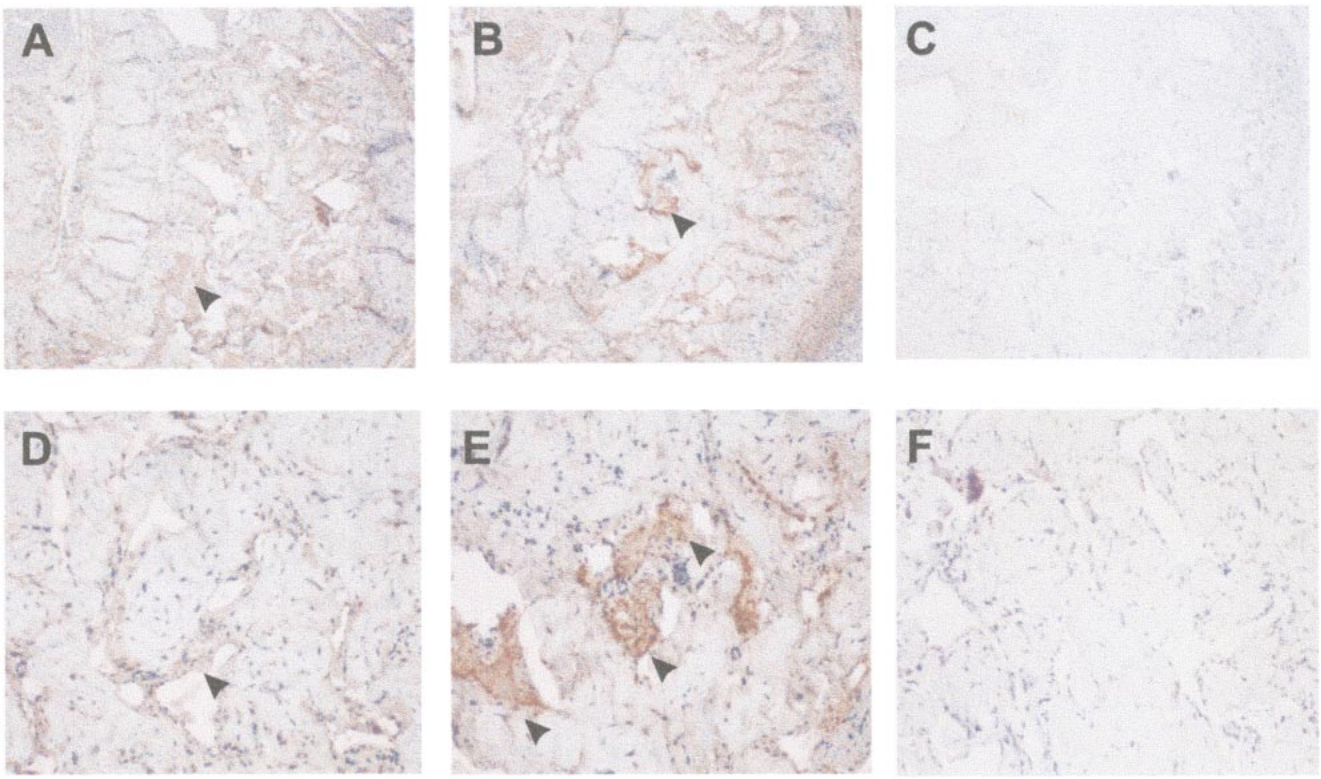

FIG. 4. Immunohistochemical localization of CGRP in rats transfected with AdRSVβgal (**A** and **D**) and AdRSVCGRP (**B** and **E**). An increase was observed in immunohistochemical staining of CGRP in the sinusoidal spaces and the cavernosal smooth muscle cells in the AdRSVCGRP-treated aged rat (**B** and **E**) corpus cavernosum. The brown staining and arrowhead denote the presence of CGRP. Negative controls (**C** and **F**) are also shown. Magnification ×40 (**A**–**C**) and ×100 (**D**–**F**).

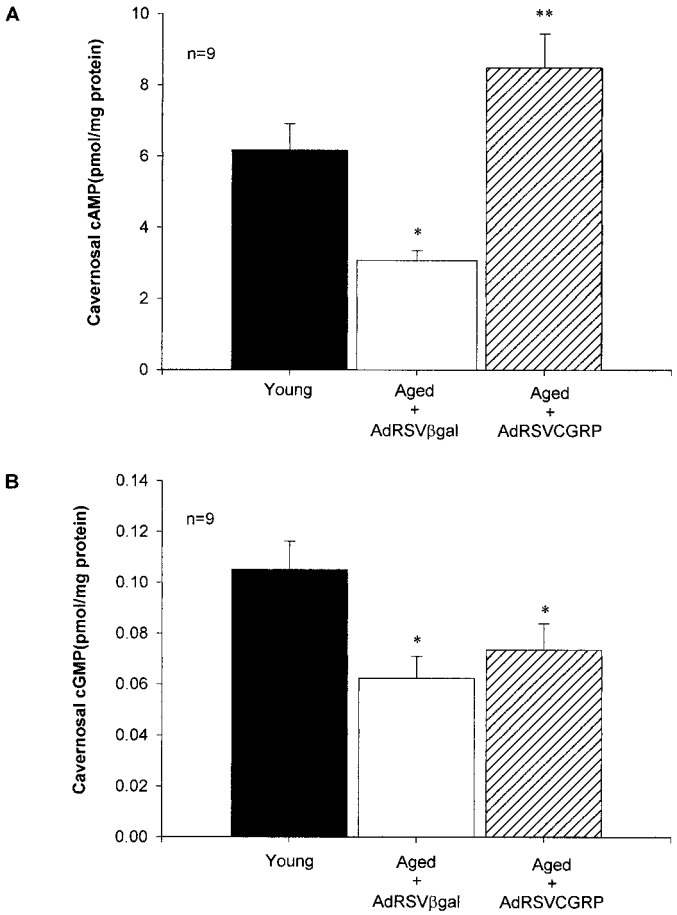

FIG. 5. Changes in cavernosal cAMP (**A**) and cGMP (**B**) concentrations in young and aged rats transfected with AdRSVβgal and AdRSVCGRP. The letter n indicates the number of experiments. An asterisk indicates a significant difference ($P < 0.05$) from concentrations in young rats; a double asterisk indicates a significant difference ($P < 0.05$) from concentrations in aged rats transfected with AdRSVβgal.

Cavernosal cAMP and cGMP Levels

Cavernosal tissue concentrations of cAMP and cGMP were measured in young rats and in aged rats treated with vehicle, AdRSVβgal, and AdRSVCGRP, and these data are summarized in Figure 5. Both cavernosal cAMP and cGMP were markedly reduced ($P < 0.05$) in the aged rats compared to the younger rats (Fig. 5). The cAMP concentrations were significantly increased ($P < 0.05$) in the cavernosal tissue of aged rats transfected with AdRSVCGRP compared to animals transfected with AdRSVβgal ($P < 0.05$) (Fig. 5). The cGMP concentrations were similar in the cavernosal tissue of aged rats transfected with AdRSVβgal and with AdRSVCGRP (Fig. 5). The cAMP and cGMP levels were similar in aged rat cavernosal tissue transfected with vehicle and with AdRSVβgal (data not shown).

In Vivo Gene Transfer of AdRSVCGRP and Erectile Function

The effect of cavernosal nerve stimulation on erectile function in vivo was measured to evaluate the physiological relevance of overexpression of the CGRP gene via adenoviral gene transfer of CGRP to the corpus cavernosum of aged rats. A significant ($P < 0.05$), voltage-dependent decrease was found in cavernosal nerve-induced erectile responses in aged animals compared to younger rats (Fig. 6). The magnitude of the increase in cavernosal pressure after cavernosal nerve stimulation in aged rats transfected with AdRSVβgal was significantly lower ($P < 0.05$) than in young rats, whereas aged rats transfected with the adenoviral gene encoding for CGRP had an increase in cavernosal pressure after cavernosal nerve stimulation similar to that in younger rats (Fig. 7). The magnitude of erectile responses to cavernosal nerve stimulation in aged rats treated with vehicle and those treated with AdRSVβgal were sim-

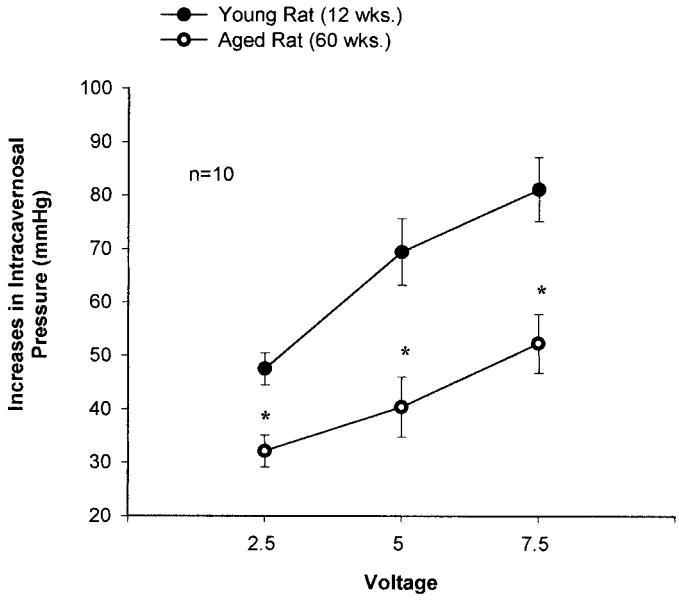

FIG. 6. Increases in intracavernosal pressure after cavernosal nerve stimulation in young and aged rats. The letter n indicates the number of experiments; an asterisk indicates a response significantly different ($P < 0.05$) from that of younger animals.

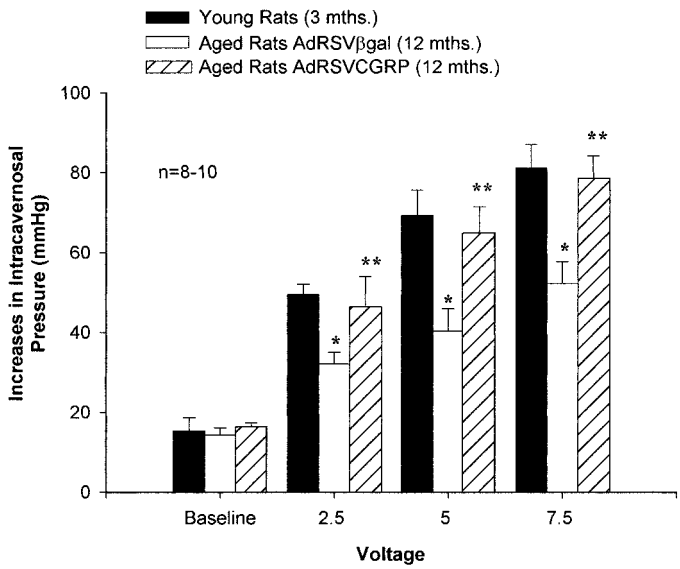

FIG. 7. Increase in intracavernosal pressure in response to cavernosal nerve stimulation in young rats and aged rats transfected with AdRSVβgal and AdRSVCGRP. In vivo erection experiments were conducted 5 days after transfection with adenoviruses. The letter n indicates the number of experiments. An asterisk indicates a response significantly different ($P < 0.05$) from that of young rats; a double asterisk indicates a response significantly different ($P < 0.05$) from that of aged rats transfected with AdRSVβgal.

ilar (data not shown). The increase in cavernosal pressure in the AdRSVCGRP-transfected group was similar to that in the younger control rats at all voltage settings (2.5, 5, and 7.5 V) (Fig. 7). The responses were reproducible 30 min after the initial stimulation.

No detectable difference ($P > 0.05$) was found in resting intracavernosal pressure (mm Hg) and systemic arterial pressure (mm Hg) among the three experimental groups. The mean values for intracavernosal pressure and systemic arterial pressure, respectively, were 15.1 ± 2.7 and 111 ± 6.7 mm Hg for the young rats (n = 8), 14.6 ± 1.9 and 102 ± 4.7 mm Hg for the AdRSVβgal-transfected aged rats (n = 10), and 15.7 ± 0.7 and 109 ± 8.9 mm Hg for the AdRSVCGRP-transfected aged rats (n = 10).

DISCUSSION

This study demonstrates that direct injection of an adenovirus encoding the prepro-CGRP gene to the aged rat penis increases cavernosal levels of CGRP, CGRP mRNA, and cAMP, which subsequently cause physiologically relevant changes in erectile function as determined by cavernosal nerve stimulation. These data imply that the CGRP transgene has biological activity in the rat penis and can reverse the age-related ED seen in the aged rat population. We also found a decrease in CGRP levels as well as cAMP and cGMP concentrations in the aged rat cavernosal tissue compared to younger animals.

The CGRP is a vasoactive neuropeptide, predominantly located in the nervous system, that influences a number of physiological actions, such as smooth muscle relaxation and regulation of vascular tone [15, 30]. It is a potent endogenous vasodilatory peptide, and its receptor is widely distributed throughout the body. Afferent nerves immunoreactive for CGRP are prevalent in the penis of the rat and have been localized in the cavernosal smooth muscle of the penis [31–33]. CGRP has been shown to induce penile erection in primates and cats [10, 34]; clinically, CGRP can induce erections when administered intracavernosally in men [10, 17]. The mechanism by which CGRP causes vasodilation or relaxation varies among the species and vascular beds studied. Whereas CGRP-induced vasodilation has been shown in some systems to be mediated by the NO/cGMP second-messenger system, others have shown its actions to be mediated in a cAMP-dependent manner [35]. In the penis, the predominant second-messenger system is the NO pathway. However, the cAMP second-messenger system does play a role in mediating penile erection and has been exploited in the pharmacological management of ED.

Aging is the strongest predictor of ED. Most authorities believe that this decreased erectile capacity in aging is associated with decreased NO synthase (NOS) activity and NO/cGMP synthesis in the corpus cavernosum of the penis, resulting in reduced smooth muscle relaxation [36, 37]. However, CGRP levels are also decreased in the aged rat penis, which may also contribute to this diminished cavernosal smooth muscle relaxation [19]. In the present study, we sought to determine the concentrations of CGRP and the second-messenger molecules, cGMP and cAMP, in the corpus cavernosum of the aged rat and to compare these values with those of younger rats. We found a significant decrease in immunoreactive CGRP levels at a time when decreased concentrations of cGMP and cAMP were observed in the corpus cavernosum of the aged rat penis, suggesting that this may contribute to the ED seen in these older animals. The decreased cGMP and cAMP levels may be due, in part, to less NO and CGRP synthesis, or it may be due to increased collagen deposition and less endothelial or vascular smooth muscle cells in the aged corpus cavernosum, as described by Ferrini et al. [38].

Recently, our laboratory and other investigators reported that gene transfer of endothelial NOS, penile inducible NOS, and hSlo Maxi K genes can reverse age-related ED in rats [22, 23, 28, 39]. Therefore, our aim was to determine if overexpression of the CGRP gene could restore erectile function in the aged rat population. Five days after intracavernosal injection of AdRSVCGRP into the rat corpora, an age-related reversal of ED was observed. Aged rats (60 wk) transfected with AdRSVβgal had significantly lower erectile function than younger rats (12 wk). However, 5 days after transfection with AdRSVCGRP, the aged rats showed increased intracavernosal pressure, as determined by cavernosal nerve stimulation, to a level similar to that of younger rats at all voltage settings. Consistent with this observation, increases were observed in CGRP protein, gene expression, and immunohistochemical localization of CGRP in the smooth muscle cells of the corpora, as well as an increase in cavernosal cAMP at a time when cavernosal nerve-stimulated erectile responses were increased, indicating that the increase in erectile response was due to increased CGRP expression and subsequent increases in intracellular cAMP in the corpus cavernosum of the rat. No change was found in cavernosal cGMP levels, suggesting that CGRP does not cause penile erection in the rat via a NO/cGMP-dependent mechanism. These data provide strong evidence that adenoviral gene transfer of CGRP can cause physiologically relevant changes in erectile responses in the aged rat through a cAMP-dependent mechanism, suggesting that second-messenger systems other than NO/cGMP can regulate the vascular tone of the penis.

In summary, our findings suggest a possible role for CGRP as a mediator of cavernosal smooth muscle relaxation and penile erection, which may contribute to the pathophysiology of age-related ED. These results demonstrate that adenoviral-mediated transfer of the CGRP gene can increase CGRP protein, cAMP, and CGRP mRNA in the corpora cavernosa of the aged rat penis. Moreover, overexpression of CGRP enhances the erectile response to cavernosal nerve stimulation in the aged rat. Additionally, CGRP exerts its pro-erectile effects via a cAMP-dependent mechanism in the penis of the rat. These results support the hypothesis that in vivo gene transfer of targeted genes can have physiological benefits on erectile function when administered intracavernosally. These data also suggest that adenoviral-mediated transfer of the CGRP gene or other genes reduced in the aging penis may represent an exciting new form of therapy for the treatment of male ED.

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