

## Intracorporal injection of *hSlo* cDNA restores erectile capacity in STZ-diabetic F-344 rats in vivo

George J. Christ,<sup>1,2</sup> Nancy Day,<sup>1</sup> Cristian Santizo,<sup>1</sup> Yoshi Sato,<sup>1</sup> Weixin Zhao,<sup>1</sup> Theresa Sclafani,<sup>1</sup> Ron Bakal,<sup>1</sup> Masha Salman,<sup>1</sup> Kelvin Davies,<sup>1</sup> and Arnold Melman<sup>1</sup>

Departments of <sup>1</sup>Urology and <sup>2</sup>Physiology and Biophysics, Institute for Smooth Muscle Biology, Albert Einstein College of Medicine, Bronx, New York 10461

Submitted 21 August 2003; accepted in final form 2 June 2004

**Christ, George J., Nancy Day, Cristian Santizo, Yoshi Sato, Weixin Zhao, Theresa Sclafani, Ron Bakal, Masha Salman, Kelvin Davies, and Arnold Melman.** Intracorporal injection of *hSlo* cDNA restores erectile capacity in STZ-diabetic F-344 rats in vivo. *Am J Physiol Heart Circ Physiol* 287: H1544–H1553, 2004; 10.1152/ajpheart.00792.2003.—The ability of gene transfer with the pore-forming subunit of the human maxi-K channel (*hSlo*) to ameliorate the decline in erectile capacity commensurate with 12–24 wk of streptozotocin (STZ)-diabetes was examined in 181 Fischer-344 rats. A 2-mo period of STZ-diabetes was induced before gene transfer, and erectile capacity was evaluated by measuring the intracavernous pressure response (ICP) to cavernous nerve (CN) stimulation (ranging from 0.5 to 10 mA). In the first series of experiments, ANOVA revealed increased CN-stimulated ICP responses at 1 and 2 mo postinjection of 100 µg pcDNA-*hSlo* compared with control values. A second series of experiments further examined the dose dependence and duration of gene transfer. The ICP response to submaximal (0.5 mA) and maximal (10 mA) nerve stimulation was evaluated 3 or 4 mo postinjection of a single dose of pcDNA-*hSlo* ranging from 10 to 1,000 µg. ANOVA again revealed that *hSlo* overexpression was associated with increased CN-stimulated ICP responses compared with responses in corresponding control animals. Histological studies revealed no immune response to the presence of *hSlo*. PCR analysis documented that expression of both plasmid and transcript were largely confined to the corporal tissue. In the third series of pharmacological experiments, *hSlo* gene transfer in vivo was associated with iberiotoxin-sensitive relaxation responses to sodium nitroprusside in corporal tissue strips in vitro. The latter data indicate that gene transfer produces functional maxi-K channels that participate in the modulation of corporal smooth muscle cell tone. Taken together, these observations suggest a fundamental diabetes-related change in corporal myocyte maxi-K channel regulation, expression, or function that may be corrected by expression of recombinant *hSlo*.

gene transfer; maxi-K channel; corporal smooth muscle; myocytes; erectile dysfunction

DIABETES MELLITUS is a multifactorial disease involving neural, metabolic, endocrine, and vascular changes, ultimately culminating in a host of well-documented clinical sequelae. Among the prominent end-organ complications of diabetes is erectile dysfunction, with an incidence of >50% (3, 17, 18, 22, 31). In fact, in a majority of patients it appears that the etiology of erectile dysfunction is related to heightened contractility and/or impaired relaxation of the corporal smooth muscle and penile vasculature (2, 4, 30). The main physiological implication is that endogenous relaxing mechanisms are no longer able to

elicit a degree of smooth muscle relaxation that is adequate to support the increase in blood flow and enhanced intracavernous pressure (ICP) required for the initiation and maintenance of penile erection.

Although effective treatments are now available for many patients with erectile dysfunction, undeniably, diabetic patients are always among the most refractory to any given pharmacotherapy. As such, more effective nonsurgical treatment options are still required for erectile dysfunction in the diabetic patient population. The postgenomic age presents enormous opportunities to this end by virtue of the numerous molecular targets now available for the development of improved therapies; both small molecule pharmacology approaches as well as novel gene-based strategies are possible. One such possibility is gene therapy/transfer (5, 11, 13–15).

Several groups have recently published distinct gene transfer strategies for the treatment of erectile dysfunction (4–7, 12, 16, 28, 32, 34, 37). These approaches take advantage of the fact that enhancing corporal smooth muscle relaxation per se is both necessary and sufficient to restore erectile capacity in the majority of impotent men. Cells, enzymes, growth factors, or proteins that are major modulators of corporal smooth muscle tone have all been targeted. The ultimate goal is to create an “on demand” endogenous signal adequate to restore sufficient relaxation of corporal smooth muscle to permit erection.

This report builds on previous work that has shown that gene transfer with the large-conductance, Ca-sensitive K channel subtype (i.e., *hSlo*) restores the age-related decline in erectile capacity in rats in vivo (16, 32). In fact, a single intracavernous injection of the “naked” pcDNA-*hSlo* (100 µg), which encodes the  $\alpha$ -subunit of the human maxi-K channel, was associated with physiologically significant increases in the magnitude of the cavernous nerve (CN)-stimulated ICP response that lasts for up to 6 mo after a single intracavernous injection (16, 32). The mechanism of action was presumably related to the proximal importance of K channel hyperpolarizing currents to relaxation of corporal smooth muscle and penile erection. In this scenario, increased expression of *hSlo* on a subpopulation of corporal myocytes resulted in an enhanced sensitivity to nerve stimulation.

In this regard, recent evidence indicates that altered smooth muscle cell K channel function may contribute to the etiology of vascular complications of diabetes mellitus (3, 24, 26, 36), including erectile dysfunction (26, 27, 30, 38). Thus the goal of the present study was to determine whether a similar *hSlo* gene

Address for reprint requests and other correspondence: G. J. Christ, Depts. of Urology and Physiology and Biophysics, Institute for Smooth Muscle Biology, Rm. 744, Forchheimer Bldg., Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461 (E-mail: christ@aecom.yu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

transfer approach would be sufficient to ameliorate the decrease in erectile capacity associated with peripheral neuropathy in an established rat model of experimental diabetes (7, 10, 33). These initial studies provide further evidence for the potential utility of K channel gene transfer for the treatment of erectile dysfunction.

## MATERIALS AND METHODS

**Induction of streptozotocin-diabetes.** All experimental studies were conducted according to a protocol approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine.

A 2-mo period of diabetes mellitus was induced in experimental animals (~8 wk old at the time of injection) via a single intraperitoneal injection of streptozotocin (STZ; 35 mg/kg) dissolved in citrate buffer (60 ml of 0.1 M citric acid and 40 ml of 0.2 M Na<sub>2</sub>HPO<sub>4</sub>, pH 4.6), as described elsewhere (33). Table 1 lists the exact demographics of the animal population. Age-matched control animals received an injection of vehicle only. Diabetic rats were entered into the study when they were confirmed to be diabetic for 1 wk (i.e., blood glucose levels  $\geq$ 250 mg/dl and urine glucose levels  $\geq$ 1,000 mg/dl). Urine glucose levels were monitored weekly, and blood glucose levels were determined at the end of the terminal cavernosometry experiment. Diabetic rats had blood glucose levels  $\geq$ 250 mg/dl at the time of euthanization.

Anesthesia was induced in all animals by the intraperitoneal injection (35 mg/kg) of pentobarbital sodium (Anpro Pharmaceuticals). Anesthesia was maintained during the course of the experimental protocol (2–3 h), as necessary, by the subsequent injection of pentobarbital (5–10 mg/kg) every 45–60 min as required.

**Placement of systemic pressure and corporal pressure monitoring cannulae and dissection of the CN.** As previously described (16, 32, 33), animals were placed in the supine position, and the bladder and prostate were exposed through a midline abdominal incision. The inferior hypogastric plexus (i.e., the pelvic plexus or major pelvic ganglia), pelvic nerves, and CN were identified posterolateral to the prostate on both sides, and stainless steel bipolar wire electrodes were placed around these structures for electrical stimulation. The penis was denuded of skin; both crura (corpus cavernosa) were exposed by removing part of the overlying ischiocavernosus muscles. To monitor ICP, a 23-gauge cannula was filled with 250 U/ml heparin solution, connected to polyethylene-50 tubing (Intramedic, Becton Dickinson),

and inserted into the right corpus cavernosum (crura). Another 23-gauge cannula preconnected to a 1-ml syringe was inserted into left corpus cavernosum for intracavernous drug injection (if necessary). Systemic arterial blood pressure was monitored via a 25-gauge cannula placed into the carotid artery. A second cannula was placed in the external jugular vein for intravenous administration of the medication/test substance.

Both pressure lines were then connected to a pressure transducer, which was, in turn, connected via a Transducer amplifier (ETH 400, CB Sciences) to a data-acquisition board (Mac Lab/8e, AD Instruments). Real-time display and recording of pressure measurements was performed on a Macintosh computer (Mac Lab software V3.4, AD Instruments). The pressure transducers and analog-to-digital board were calibrated (in cmH<sub>2</sub>O) before each experiment.

**Neurostimulation of the CN.** Direct electrostimulation of the CN was performed with a delicate stainless steel bipolar hook electrode attached to a multijointed clamp. Each probe was 0.2 mm in diameter; the two poles were separated by 1 mm. Monophasic rectangular pulses were delivered by a signal generator (custom made and with a built-in constant current amplifier). Stimulation parameters were as follows: frequency, 20 Hz; pulse width, 0.22 ms; and duration, 1 min. The experimental protocol always used increasing current increments of 0.5 and then 6 mA.

**Gene therapy experiments.** Microinjection of vectors/plasmids into rat corporal tissue was achieved as described elsewhere (16, 32). Briefly, animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (35 mg/kg). An incision was made through the perineum, the corpus spongiosum was identified, and a window was made in the corpus spongiosum for identification of the corpus cavernosum. All microinjections consisted of a single bolus injection of naked cDNA into the corporal tissue, made using an insulin syringe. The final volume of all microinjections was 200  $\mu$ l.

**Plasmid preparation and transfection of rat corporal smooth muscle in vivo.** The strategy used was identical to that previously described for hSlo cDNA (17, 38). Briefly, the hSlo cDNA was inserted into the XhoI-XbaI cloning site of the pcDNA3 vector, where expression is driven off of the cytomegalovirus promoter; 10-, 100-, or 1,000- $\mu$ g doses in 200  $\mu$ l PBS (containing 20% sucrose) were injected into the corpus cavernosum of anesthetized Fischer-344 rats. Control rats consisted of sham-operated animals given an intracorporal injection of 200  $\mu$ l PBS containing 20% sucrose as well as rats given 1,000  $\mu$ g pcDNA. Seven additional Sprague-Dawley retired breeder rats

Table 1. Mean body weights for experimental animals during the time course of gene therapy studies

	hSlo					pcDNA (1,000 $\mu$ g)	PBS (Vehicle)	Age-Matched Control (Sham-Operated)
	10 $\mu$ g	30 $\mu$ g	100 $\mu$ g	300 $\mu$ g	1,000 $\mu$ g			
Initial	210 $\pm$ 2.6 (n=12)	210 $\pm$ 1.9 (n=14)	217 $\pm$ 3.2 (n=40)	208 $\pm$ 1.2 (n=9)	221 $\pm$ 2.0 (n=13)	226 $\pm$ 1.1 (n=29)	212 $\pm$ 2.1 (n=27)	238 $\pm$ 1.4 (n=27)
Injection	235 $\pm$ 6.8 (n=12)	229 $\pm$ 6.7 (n=14)	231 $\pm$ 12.7 (n=40)	230 $\pm$ 9.3 (n=9)	246 $\pm$ 21.4 (n=13)	257 $\pm$ 7.7 (n=29)	183 $\pm$ 15.7 (n=27)	342 $\pm$ 8.0 (n=27)
Postinjection								
1 mo	NA	NA	202 $\pm$ 12.3 (n=12)	NA	NA	185 $\pm$ 9.8 (n=11)	169 $\pm$ 9.0 (n=15)	350 $\pm$ 3.8 (n=6)
2 mo	NA	NA	189 $\pm$ 17.4 (n=16)	NA	NA	235 $\pm$ 2.2 (n=8)	206 $\pm$ 3.0 (n=8)	372 $\pm$ 8.9 (n=7)
3 mo	211 $\pm$ 14.6 (n=7)	233 $\pm$ 11.5 (n=9)	189 $\pm$ 11.3 (n=8)	215 $\pm$ 20.0 (n=4)	275 $\pm$ 28.3 (n=8)	258 $\pm$ 12.0* (n=10)	248 $\pm$ 20.0† (n=4)	390 $\pm$ 0.3 (n=6)
4 mo	169 $\pm$ 19.0 (n=5)	241 $\pm$ 13.0 (n=5)	191 $\pm$ 20.7 (n=4)	323 $\pm$ 48.0 (n=5)	221 $\pm$ 2.0 (n=5)	258 $\pm$ 12.0* (n=10)	248 $\pm$ 20.0† (n=4)	390 $\pm$ 2.8 (n=8)

Values are means  $\pm$  SE (in g); n, no. of observations. Initial values are those of animal weights when rats were entered into the study, that is, at the time of injection of streptozotocin (STZ) or vehicle. Injection values are those of animal weights after 2 mo of STZ-diabetes, that is, at the time of intracorporal injection as indicated in the various treatment groups. The number of months after injection of the various treatments at which the corresponding cohort of animals was studied is shown. NA, not applicable; hSlo, human slo gene. \*pcDNA-treated rats from 20–24 wk of diabetes were not significantly different with respect to the intracavernous pressure/blood pressure (CP/BP) response to cavernous nerve stimulation, and, therefore, they were pooled into a single group (n = 10). †Vehicle-treated rats were almost homogeneous with respect to their ICP responses to cavernous nerve stimulation, and, therefore, they were also pooled (n = 4).

were injected with 1,000  $\mu\text{g}$  recombinant *hSlo*, with five age-matched control Sprague-Dawley rats receiving injection of vehicle only.

**Pharmacological studies in vitro.** For these studies, we examined the impact of the maxi-K channel-selective blocker (9, 20, 29) iberiotoxin (IBTX; 200 nM, Sigma; St. Louis, MO) on the sodium nitroprusside (SNP)-induced relaxation response in phenylephrine (PE)-precontracted corporal tissue strips from retired breeder Sprague-Dawley rats. The retired breeder rats all weighed  $\geq 600$  g and, as described elsewhere (32), have a documented decline in erectile capacity. One corporal tissue strip was prepared from each crura in 11 of 12 rats; in 1 animal, the dissection of 1 strip resulted in significant trauma and, therefore, loss of robust contractile activity (contractile responses of  $\geq 500$  mg were required for inclusion in the study). One strip from each rat was preincubated with 200 nM IBTX for 10–15 min before the addition of 10  $\mu\text{M}$  PE; the other strip served as a control. Upon establishment of a steady-state contractile response, a cumulative concentration-response curve (CRC) to SNP was constructed at 0.5-log increments. In this fashion, a single SNP CRC was performed on each corporal tissue strip in either the absence or presence of 200 nM IBTX.

**Detection of steady-state levels of *hSlo* transcript in rat corpora.** Corporal tissue was harvested and flash frozen in liquid nitrogen. Total RNA was extracted from frozen tissue by TRIzol reagent (GIBCO-BRL; Grand Island, NY) as described (16, 32). RT-PCR was performed with oligonucleotide primers specific to the first six amino acids of *hSlo* (3' primer, 5'-GCCGCCACCATTTGCCAT-3') and T7 promoter of plasmid sequences (5' primer, 5'-CCCTATAGTGAGTCGTATTA-3') as described (13, 32). The DNA sequences between the T7 promoter and the *Xenopus laevis*  $\beta$ -globin 5'-untranslated region (UTR) correspond to the polylinker of the pcDNA3 vector. The *X. laevis*  $\beta$ -globin 5'-UTR was present to enhance the expression level of *hSlo*. The *hSlo* transcripts were confirmed by transblotting the RT-PCR products onto nylon membranes followed by ethidium bromide detection. The use of these primers revealed an expected 229-bp DNA fragment. The identity of the 229-bp RT-PCR products and the RT-PCR fragments were further confirmed by DNA sequencing in our Albert Einstein College of Medicine core sequencing facility. *Slo* transcript expression levels were evaluated in parallel with ribosomal protein large subunit 19 (RPL19; molecular mass: 19 kDa); RPL19 is an integral part of the ribosome and can be considered a "housekeeping gene." The use of RPL19 permits normalization of the RT-PCR reactions so that the expression of *hSlo* can be compared in STZ-diabetic and age-matched control corporal tissues.

**Biodistribution studies.** Rats received a single intracavernous injection of naked pcDNA-*hSlo* cDNA (100  $\mu\text{g}$  in 200  $\mu\text{l}$  final volume). Tissues were harvested at various time points postinjection. Steady-state levels of the recombinant *hSlo* transcripts were evaluated using PCR and RT-PCR. The primer sets for PCR amplification were the code for the first six amino acids of *hSlo* (5'-GCCGCCACCATTTGCCAT-3') and the T7 promoter (5'-CCCTATAGTGAGTCGTATTA-3').

**Histological evaluation.** Corpora were excised, and sections were obtained from the crura and junction of the crura and the cavernous bodies. For hematoxylin and eosin histological evaluation, the tissue was fixed in neutral buffered formalin and, after being embedded in paraffin, sectioned at 4  $\mu\text{m}$ . For phase-contrast evaluation, the tissue was fresh and flash frozen in liquid nitrogen and sectioned in a cryostat at 7–10  $\mu\text{m}$ . Synaptophysin, a presynaptic fusion terminal protein, was used to assess autonomic efferent innervation (see Ref. 31 for details). Immunostaining was done on fresh frozen tissue cut at 7–10  $\mu\text{m}$ , dried onto slides, and stored at  $-20^\circ\text{C}$  until they were stained. For staining, the sections were rehydrated in PBS with 0.3% Triton X-100 and then incubated with nonspecific goat serum (1:75) for 1 h at room temperature. The slides were then drained, and prediluted rabbit primary antisera to synaptophysin (Zymed Laboratories; San Francisco, CA) were applied to the sections. Within each batch of slides, one or two slides were not drained of the goat serum

and served as control slides for the specificity of the primary antisera. All slides were incubated overnight at room temperature in a humid chamber. They were then washed several times for 0.5 h each with PBS-Triton X-100 and then exposed to FITC-labeled goat anti-rabbit antisera (Cappel, Organon Technical; Durham, NC) for 1 h at room temperature. Sections were then washed for at least 1 h in the PBS-Triton X-100 and then coverslipped with either gelmount or vectashield. All sections were examined with a Nikon epifluorescence microscope with conventional optics. Images were captured with a color charge-coupled device camera and stored on a Pentium computer.

**Statistical analysis.** Unless otherwise stated, data are expressed as means  $\pm$  SE. Most data were analyzed using the SigmaStat software package (SigmaStat version 2.03, SPSS), e.g., one-way ANOVA analyses were used to evaluate treatment effects, with a post hoc protected Fisher's least-significant difference test used for pairwise comparisons of group mean values when the ANOVA was significant. For analysis of the pharmacological data, a Student's *t*-test was used to evaluate the effect of IBTX on the contractile response at each PE concentration.  $P < 0.05$  was considered significant in all cases.

## RESULTS

**Induction of experimental diabetes, demographic profile, and mean body weights.** STZ-injected rats were diabetic for 2 mo before the intracavernous injection of pcDNA-*hSlo*, vector alone, or vehicle. Table 1 illustrates the demographic profile for all rats on which cavernosometry was performed. As previously reported (4, 9, 31), diabetic cohorts had lower body weights than their age-matched control counterparts. At death, diabetic rats had blood glucose levels  $\geq 250$  mg/dl and urine glucose levels  $\geq 1,000$  mg/dl, whereas age-matched control rats had blood glucose levels  $\leq 90$  mg/dl.

**Effects of gene transfer with a single injection of 100  $\mu\text{g}$  pcDNA/*hSlo* on CN-stimulated ICP response 1–2 mo postinjection.** Figure 1 compares representative examples of the

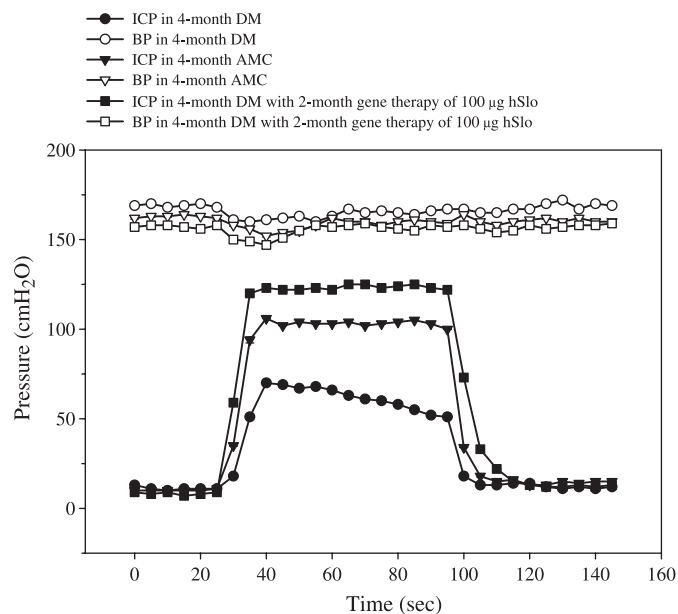


Fig. 1. Representative original records of digitized data from cavernosometry experiments performed at 4 mA current stimulation on 3 distinct rats 2 mo postinjection or after 4 mo of streptozotocin (STZ)-diabetes. ICP, intracavernous pressure; BP, blood pressure; DM, diabetes mellitus; *hSlo*, human *Slo* gene.

CN-stimulated ICP response (i.e., cavernosometry) among the various treatment groups 4 mo after the induction of STZ-diabetes and 2 mo after a single intracavernous injection of 100  $\mu\text{g}$  pcDNA-hSlo. Consistent with previous studies, STZ-diabetes (i.e., 3–4 mo) was associated with a significant decline in the mean amplitude of the CN-stimulated ICP response (7, 10, 33). Moreover, the diminished STZ-induced ICP response exhibited a characteristic further decline in value during the presence of continuous stimulation of the CN (see Fig. 1). In sharp contrast, gene transfer with 100  $\mu\text{g}$  pcDNA-hSlo was sufficient to produce a significant and physiologically relevant increase in the mean amplitude of the CN-stimulated ICP response in the absence of detectable effects on mean arterial pressure [i.e., blood pressure (BP)]. The increased ICP response lasted the entire 60-s duration of nerve stimulation and returned quickly to resting levels after the termination of nerve stimulation.

Average values for the mean ICP-to-BP ratio on all STZ-diabetic animals, from all treatment groups, at both the 1 and 2 mo time points (i.e., 3–4 mo of STZ-diabetes) are graphically depicted in Fig. 2. As illustrated, at the 1-mo time point, the gene transfer group had significantly greater ICP-to-BP ratios than all other groups at the highest levels of stimulation (6 and 10 mA). Statistical analysis also revealed that at 2 mo postinjection, the 100  $\mu\text{g}$  gene transfer group had a significantly greater ICP-to-BP ratio than all other treatment groups at all levels of CN stimulation. The corresponding mean resting ICP and BP values in those same animals are summarized in Table 2.

**Evaluation of the dose dependence and duration of gene transfer with pcDNA-hSlo.** To further evaluate the dose dependence and duration of this gene transfer technique, a second series of experiments was conducted in which the amount of pcDNA-hSlo injected was varied over two log orders of magnitude, ranging from 10 to 1,000  $\mu\text{g}$ . For these experiments, the number of neurostimulations evaluated was reduced, and the ICP-to-BP ratio was examined at one submaximal (0.5 mA) and one maximal (10 mA) level of nerve stimulation. As described elsewhere, the rationale for this approach was related to the relatively shallow relationship observed between CN stimulation and the corresponding ICP response after the 1-mA neurostimulation (see Fig. 2). The protocol for these studies was as follows: rats were subjected to 2 mo of STZ diabetes before a single intracavernous injection of one of five doses of pcDNA-hSlo, vector, or vehicle alone. Age-matched control rats were run in parallel. The demographic profile and weights of all animals at the initiation of the experiment, at the time of injection, and at the termination of the cavernosometry experiment are shown in Table 1.

**Effects of gene transfer on CN-stimulated ICP response 3–4 mo postinjection.** A representative cavernosometry experiment conducted on these animals is shown in Fig. 3, and the mean values for all experiments are displayed in Fig. 4. As shown in Table 2, at 3 mo after gene transfer, there were no significant differences in either the resting ICP levels or BP among any of the treatment groups. In stark contrast, statistical analysis of the data at the 0.5-mA level of current stimulation revealed that virtually all of the gene transfer animals had significantly greater ICP-to-BP ratios than all of the untreated groups. At 10-mA neurostimulation, all of the gene transfer groups except the 10  $\mu\text{g}$  group were also significantly greater than the

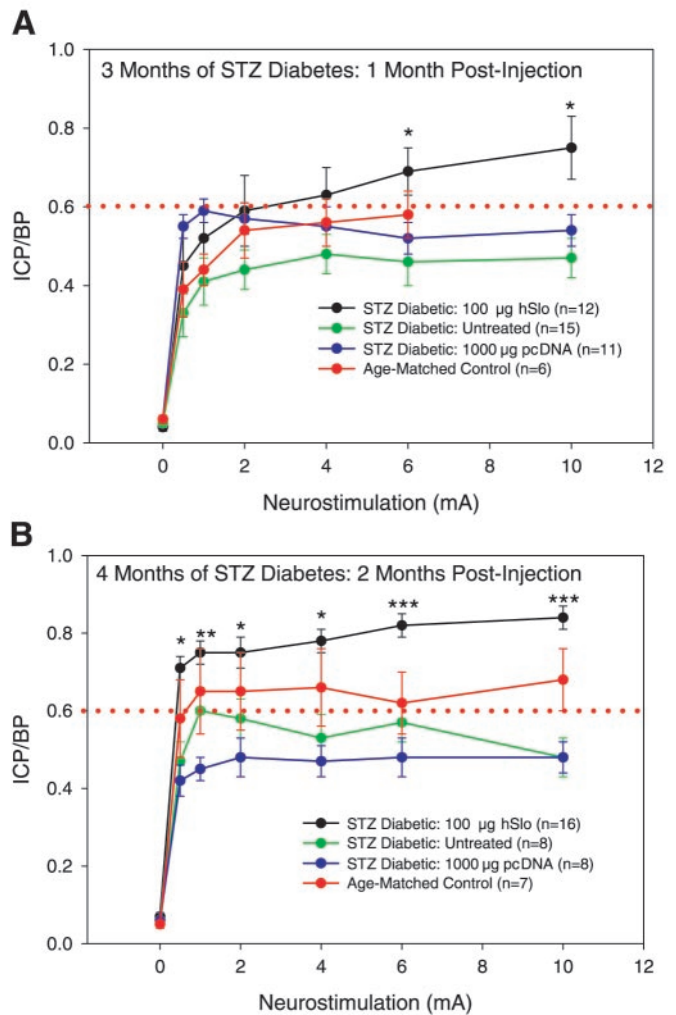


Fig. 2. Line graph summary of mean data from cavernosometry experiments performed on all rats at the 1-mo (A) and 2-mo (B) time points postinjection, that is, a total of 3 and 4 mo of STZ-diabetes. \*Significantly different from STZ untreated and STZ vector-only treated rats; \*\*significantly different from all other groups,  $P < 0.5$  in all cases.

untreated STZ-diabetic rats but similar to the age-matched control animals (Fig. 4A).

As summarized in Table 2, 4 mo after gene transfer, there were no significant differences in either the resting ICP levels or BP among any of the treatment groups. However, as illustrated in Fig. 4B, statistical analysis of the data at the 0.5-mA level of current stimulation revealed clear evidence for the presence of both dose- and duration-dependent effects of gene transfer on the CN-stimulated ICP response. More specifically, at this time point, the 10- $\mu\text{g}$  group was similar to the untreated groups as well as the age-matched control animals. As shown (Fig. 4B), there were significant differences between all other gene transfer groups (i.e., 30, 100, 300, and 1,000  $\mu\text{g}$ ) and at least one of the untreated groups. At the 10-mA level of neurostimulation, the 10- $\mu\text{g}$  group was once again similar to the untreated groups as well as the age-matched control animals. All other STZ-diabetic gene transfer groups had mean ICP-to-BP values significantly greater than at least one of the untreated STZ-diabetic groups. For example, at both the 30-

Table 2. Mean  $\pm$  SE values for resting ICP and BP for the various treatment groups after 3–6 mo of STZ-diabetes

	hSlo Treatment							
	10 $\mu$ g	30 $\mu$ g	100 $\mu$ g	300 $\mu$ g	1,000 $\mu$ g	Untreated	pcDNA (1,000 $\mu$ g)	Control
3 mo of STZ-diabetes (1 mo postinjection)								
ICP, cmH <sub>2</sub> O			5.8 $\pm$ 1.0			10.1 $\pm$ 1.2	7.5 $\pm$ 1.1	8.5 $\pm$ 2.0
BP, cmH <sub>2</sub> O			135.9 $\pm$ 5.2			152.4 $\pm$ 3.9	148.2 $\pm$ 4.8	148.2 $\pm$ 4.8
n			12			15	11	6
4 mo of STZ-diabetes (2 mo postinjection)								
ICP, cmH <sub>2</sub> O			10.7 $\pm$ 1.0			8.5 $\pm$ 1.7	7.4 $\pm$ 1.8	8.9 $\pm$ 1.4
BP, cmH <sub>2</sub> O			156.9 $\pm$ 6.5			158.0 $\pm$ 8.0	153.0 $\pm$ 6.9	163.5 $\pm$ 6.0
n			16			8	8	7
5 mo of STZ-diabetes (3 mo postinjection)								
ICP, cmH <sub>2</sub> O	9.9 $\pm$ 1.9	13.6 $\pm$ 4.9	11.0 $\pm$ 1.5	11.6 $\pm$ 3.7	10.4 $\pm$ 2.2	12.5 $\pm$ 2.6	7.5 $\pm$ 1.1	10.7 $\pm$ 0.9
BP, cmH <sub>2</sub> O	159.8 $\pm$ 2.6	150.9 $\pm$ 9.3	152.0 $\pm$ 8.3	143.1 $\pm$ 5.2	156.2 $\pm$ 9.9	156.9 $\pm$ 2.6	174.0 $\pm$ 6.3	183.4 $\pm$ 19.6
n	7	5	8	5	5	4	10	6
6 mo of STZ-diabetes (4 mo postinjection)								
ICP, cmH <sub>2</sub> O	6.1 $\pm$ 1.9	9.3 $\pm$ 1.1	11.0 $\pm$ 3.1	12.6 $\pm$ 2.8	8.4 $\pm$ 1.3	12.5 $\pm$ 2.6	7.5 $\pm$ 1.1	7.7 $\pm$ 1.1
BP, cmH <sub>2</sub> O	160.1 $\pm$ 7.8	164.1 $\pm$ 6.8	147.5 $\pm$ 12.9	173.8 $\pm$ 9.7	162.6 $\pm$ 7.4	156.9 $\pm$ 2.6	174.0 $\pm$ 6.3	162.7 $\pm$ 7.3
n	5	9	4	4	8	4	10	8

Values are means  $\pm$  SE; n, no. of rats. hSlo was injected intracavernously in a pcDNA vector (see MATERIALS AND METHODS). Control, age-matched control rats injected intracavernously with vehicle only (i.e., PBS; see MATERIALS AND METHODS).

and 1,000- $\mu$ g doses, the mean ICP-to-BP values were significantly greater than those observed for the 10- $\mu$ g gene transfer group as well as both untreated groups and the age-matched control rats.

**Expression of hSlo in corporal tissue after intracavernous injection of pcDNA-hSlo.** To further document that expression of the recombinant hSlo transcript was required for amelioration of the diabetes-related decline in erectile capacity, RT-PCR studies were conducted in a subset of experimental animals. A representative Southern blot is shown in Fig. 5A. Consistent with the physiological data in vivo, the molecular data document that recombinant hSlo expression was detected

for up to 4 mo postinjection and, furthermore, that hSlo expression was never observed in the vehicle control animals.

**Evaluation of the impact of STZ-diabetes on endogenous Slo expression.** To gain further mechanistic insight into the effects of STZ-diabetes on maxi-K channel subtype expression, we evaluated the impact of STZ-diabetes on endogenous Slo expression (i.e., rSlo). Thus we conducted a separate series of experiments on a distinct population of rats after 8 wk of STZ-diabetes (n = 3), with age-matched control rats run in parallel (n = 3). An RNase protection assay was used to determine the rSlo transcript level, with cyclophilin A used as the control “housekeeping” gene. As shown in Fig. 5B, there was little effect of STZ-diabetes on rSlo transcript levels. Consistent with this observation, densitometry analysis revealed similar transcript ratios (i.e., rSlo/cyclophilin) of  $0.33 \pm 0.1$  and  $0.37 \pm 0.01$  in STZ-diabetic and age-matched control rats, respectively.

**Histological evaluation.** To evaluate the impact of the diabetic state on corporal tissue architecture, histological and immunochemical studies were conducted. Consistent with a previous report (33), STZ-diabetes >8-wk duration was associated with a significant effector neuropathy, as illustrated by the reduced synaptophysin immunostaining seen in STZ-diabetic rat corporal tissue sections compared with corresponding studies on age-matched control rats (Fig. 6). In addition, the similarity in the hematoxylin and eosin staining among the treatment groups revealed no overt corporal myopathy or fibrosis associated with STZ-diabetes of 8- to 24-wk duration. Additionally, there was no evidence of corporal fibrosis or lymphocytic infiltrates in any of the treatment groups, consistent with the absence of a local immune response to the gene transfer protocol.

**Biodistribution of injected “naked” DNA.** In a separate group of 15 rats, a single intracavernous injection of 100  $\mu$ g pcDNA-hSlo was administered, the rats were killed at 8 different time points, and tissues were harvested from 11 different tissues (Fig. 7 and Table 3). Although no attempt was made to restrict blood flow out of the penis, the intracavernous injection

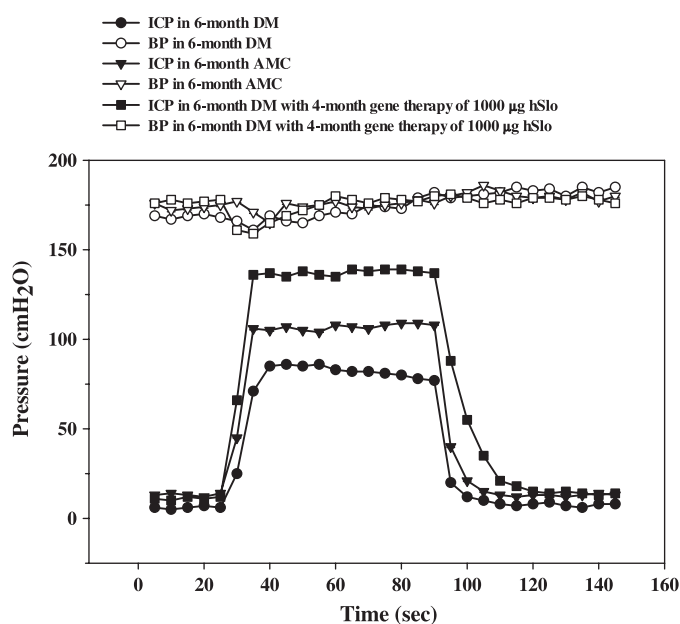
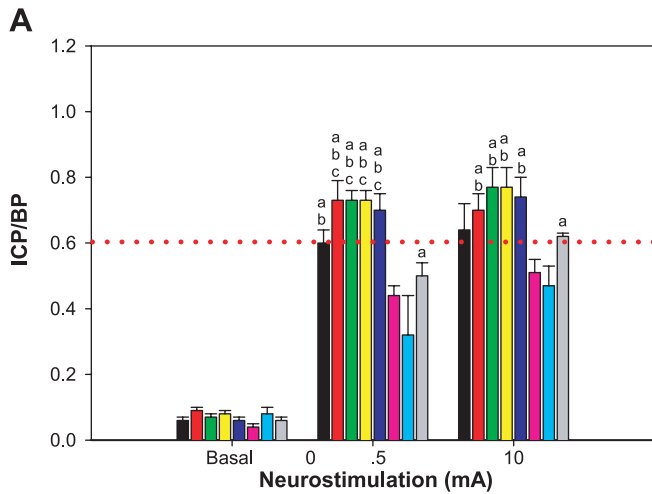


Fig. 3. Representative original records of digitized data from cavernosometry experiments performed at 10 mA current stimulation on 3 distinct rats 4 mo postinjection or after 6 mo of STZ-diabetes.

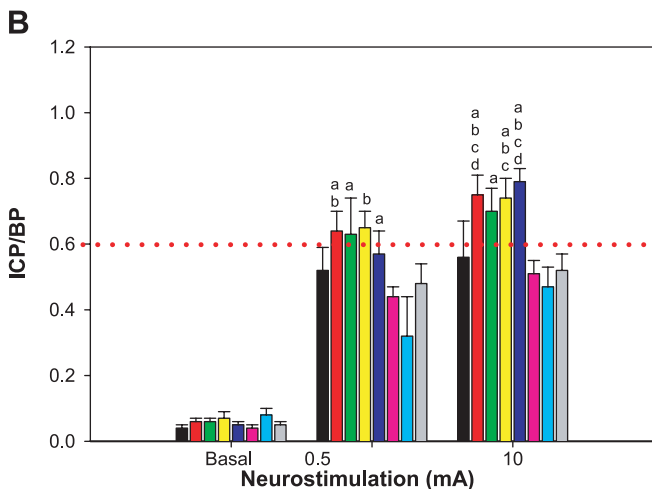
of recombinant *hSlo* resulted in a transcript expression pattern that was largely locally confined to the original tissue injection, that is, the corpora, at time points  $\geq 24$  h after injection.

*Pharmacological studies of maxi-K channel function in vitro.* To obtain mechanistic insight into recombinant maxi-K channel function after gene transfer, we conducted pharmacological studies in vitro with the selective maxi-K channel blocker IBTX on a cohort of retired breeder Sprague-Dawley rats ( $n = 7$  rats injected with 1,000  $\mu\text{g}$  *hSlo*) as well as their



■ 10  $\mu\text{g}$  hSlo/pcDNA ( $n=7$ )  
 ■ 30  $\mu\text{g}$  hSlo/pcDNA ( $n=5$ )  
 ■ 100  $\mu\text{g}$  hSlo/pcDNA ( $n=8$ )  
 ■ 300  $\mu\text{g}$  hSlo/pcDNA ( $n=5$ )  
 ■ 1000  $\mu\text{g}$  hSlo/pcDNA ( $n=5$ )  
 ■ 1000  $\mu\text{g}$  pcDNA ( $n=10$ )  
 ■ Untreated DM ( $n=4$ )  
 ■ AMC ( $n=6$ )

a: Significantly different from untreated DM group;  
 b: Significantly different from 1000  $\mu\text{g}$  pcDNA group;  
 c: Significantly different from age-matched control group;  
 d: Significantly different from 10  $\mu\text{g}$  hSlo/pcDNA group;  
 e: Significantly different from 100  $\mu\text{g}$  hSlo/pcDNA group;  
 f: Significantly different from 1000  $\mu\text{g}$  hSlo/pcDNA group;  
 One Way ANOVA with Fisher LSD.



■ 10  $\mu\text{g}$  hSlo/pcDNA ( $n=5$ )  
 ■ 30  $\mu\text{g}$  hSlo/pcDNA ( $n=9$ )  
 ■ 100  $\mu\text{g}$  hSlo/pcDNA ( $n=4$ )  
 ■ 300  $\mu\text{g}$  hSlo/pcDNA ( $n=4$ )  
 ■ 1000  $\mu\text{g}$  hSlo/pcDNA ( $n=8$ )  
 ■ 1000  $\mu\text{g}$  pcDNA ( $n=10$ )  
 ■ Untreated DM ( $n=4$ )  
 ■ AMC ( $n=8$ )

a: Significantly different from untreated DM group;  
 b: Significantly different from 1000  $\mu\text{g}$  pcDNA group;  
 c: Significantly different from age-matched control group;  
 d: Significantly different from 10  $\mu\text{g}$  hSlo/pcDNA group;  
 e: Significantly different from 100  $\mu\text{g}$  hSlo/pcDNA group;  
 f: Significantly different from 1000  $\mu\text{g}$  hSlo/pcDNA group;  
 One Way ANOVA with Fisher LSD.

Fig. 4. Bar graph summary of mean data from cavernosometry experiments performed on all rats at the 3-mo (A) and 4-mo (B) time points postinjection, that is, a total of 5 and 6 mo of STZ-diabetes. AMC, age-matched control rats; LSD, least-significant difference test.

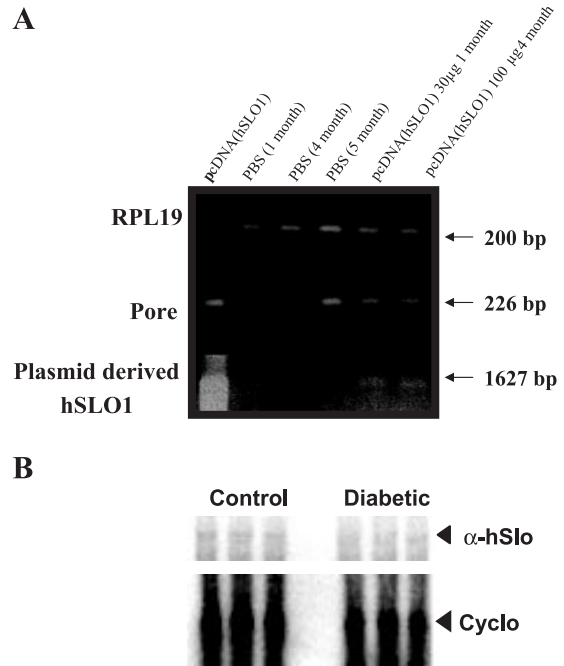


Fig. 5. Expression of *hSlo* (A) and *rSlo* (B) in flash-frozen corporal tissue. A: vector-derived *hSlo* is only found in the animals in which it was injected and then for up to 4 mo postinjection. B: there was little or no change in the level of *rSlo* transcript after 8 wk of STZ-diabetes. RPL19, ribosomal protein large subunit 19 (see MATERIALS AND METHODS).

corresponding age-matched controls ( $n = 5$  rats injected with vehicle only). A summary of the mean data is graphically depicted in Fig. 8. As illustrated in Fig. 8A, *hSlo* gene transfer revealed the presence of a statistically significant IBTX-sensitive portion of the SNP-induced relaxation response. However, in the age-matched control rats, there was no detectable effect of IBTX on the SNP-induced relaxation response (Fig. 8B).

Of note, the magnitude of the IBTX-sensitive portion of the relaxation response (between  $\sim 65\%$  relaxation and  $\sim 50\%$  relaxation) seen in the gene transfer rats was remarkably similar to the fractional increase in the ICP-to-BP ratio observed after gene transfer (see Figs. 1–4) (32). That is, compromised erectile capacity consisted of ICP-to-BP ratios of  $\leq 0.5$  and the absence of an erectile response to CN stimulation. *hSlo* gene transfer restored the ICP-to-BP ratio to  $\geq 0.6$  and resulted in the appearance of rigid erections (i.e.,  $\sim 0.5$  to  $0.65$ ). Thus the presence of an IBTX-sensitive portion of the SNP-induced relaxation response in *hSlo* gene transfer rats in vitro correlates with the enhanced ICP response observed in vivo after *hSlo* gene transfer.

DISCUSSION

The decline in erectile capacity observed in this animal model is associated with a reproducible autonomic neuropathy and a decline in erectile capacity (7, 10, 33) (i.e., a decrease in the CN-stimulated ICP-to-BP value). As in the corresponding clinical condition, diabetes-related erectile dysfunction in the STZ-treated rat is presumably related to heightened contractility and/or an impaired relaxation of corporal smooth muscle. It is important to note that the inability of the corporal smooth muscle to relax promotes penile flaccidity and thus erectile dysfunction. Given the proximal importance of hyperpolariz-

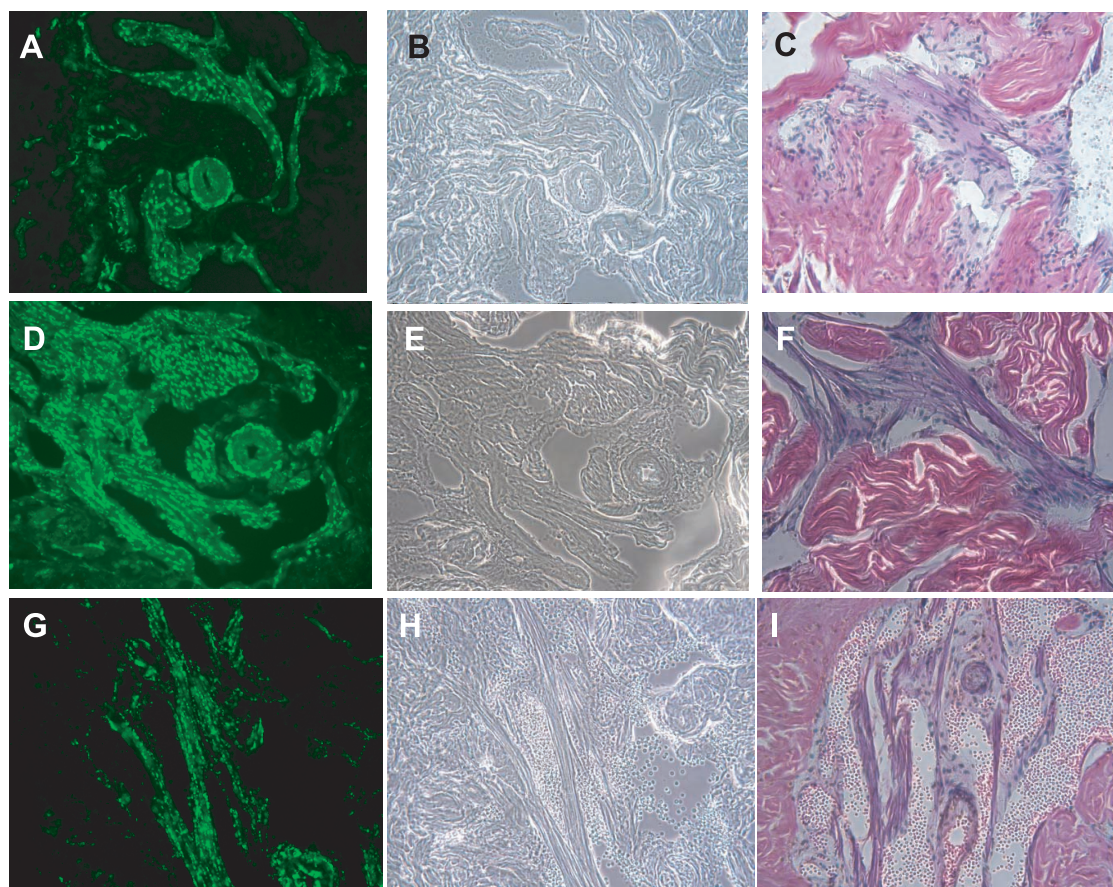


Fig. 6. Corporal tissue histology and synaptophysin immunostaining. *A–I*: photomicrographs of synaptophysin immunostaining (*A*, *D*, and *G*); phase-contrast images of the same sections (*B*, *E*, and *H*); and hematoxylin and eosin-stained images of different sections (*C*, *F*, and *I*) of tissue from the same rat corpora. *A–C* are from a 3-mo STZ-diabetic rat 1 mo after the injection of 1,000 µg pcDNA-*hSlo*. *D–F* are from an AMC rat. *G–I* are from a 6-mo STZ-diabetic rat 4 mo after the injection of 1,000 µg pcDNA-*hSlo*.

Fig. 7. Incorporation and expression of pcDNA-*hSlo* plasmid and *hSlo* recombinant transcript. For these experiments, rats were given a single intracorporal injection of 100 µg pcDNA-*hSlo*, and no attempt was made to restrict blood from the cavernous spaces. Corporal tissue was then collected and frozen at various time points after injection. *A* and *B*: distribution of the pcDNA-*hSlo* plasmid (PCR) and *hSlo* transcript (RT-PCR) in corporal tissue from the same rat 1 h after injection. Note that, whereas PCR assays documented the presence of the plasmid in numerous tissues (*A*), RT-PCR studies (*B*) documented the expression of recombinant transcript in a smaller number of tissues (see MATERIALS AND METHODS for primer details, etc.). The results of many such experiments are summarized in Table 3, and especially note that at longer time points (i.e., ≥2 wk), the *hSlo* transcript can only be detected in the corpora. *C*: RT-PCR products from the human transcript are detectable only in injected rats (+, injected; –, not injected) for time points as early as 1 h and, moreover, for up to 1 mo postinjection. *D*: results of yet another blot on RT-PCR products. One single band with the expected base size (~260 bp) in each lane was identified.

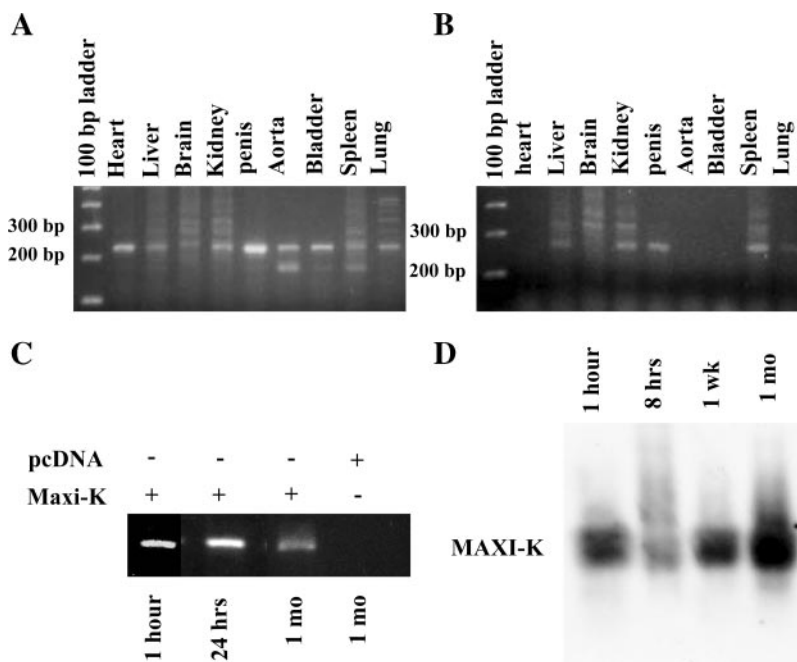


Table 3. RT-PCR: summary of tissue distribution of hSlo mRNA after a single bolus injection of the hSlo-pcDNA

	Time							
	1 h	8 h	24 h	48 h	1 wk	2 wk	1 mo	2 mo
Heart								
Liver	◆▽	▲						
Brain					▲			
Kidney	◆							
Corporal	X◆▽	▲	∩♣	◇	∅▲	√+	⊗#	
Aorta			♣					
Bladder					∅			
Spleen	◆▽	▲						
Lung	◆		♣			√		
Eye								
Testis								

For these studies, all of the indicated tissues were harvested from 15 different rats after a single intracorporal injection of 100  $\mu$ g pcDNA-hSlo (see MATERIALS AND METHODS). Note that each unique symbol reflects the detection of the recombinant transcript in a particular tissue for a particular rat.

ing K currents to corporal smooth muscle relaxation and erectile capacity, the explicit goal of these studies was to evaluate the efficacy and duration of gene transfer with a recombinant maxi-K  $\alpha$ -subunit in ameliorating the observed diabetes-related decline in erectile capacity. Although we cannot unequivocally exclude a potential impact of hSlo gene transfer on the expression, regulation, or function of other K channel subtypes (e.g., voltage-gated K channels), the data described below clearly point to an important role for the maxi-K channel in this process.

*Effects of diabetes mellitus on erectile capacity, that is, the CN-stimulated ICP response.* The CN-stimulated ICP response after 3–4 mo of STZ-diabetes was associated with a significant decrease in the mean amplitude of the ICP response to all levels of current stimulation relative to observations on age-matched control rats. Furthermore, whereas the ICP response in the age-matched control animals was relatively constant during the stimulation period (Fig. 1), the values of the mean ICP response in the STZ-diabetic rats were of lower amplitude and actually further declined during the stimulation period. These characteristics presumably reflect the presence of a functional autonomic effector neuropathy in the STZ-diabetic rat (33).

*Effects of gene transfer with 100  $\mu$ g pcDNA-hSlo on erectile capacity 1–2 mo postinjection.* The supposition that was tested in these experiments is that expression of hSlo, which mediates a hyperpolarizing current of major importance to penile erection, would increase the responsiveness of a sufficient fraction of corporal smooth muscle cells to result in a restoration of the erectile response (i.e., ICP) in the face of a diminished excitatory neural stimulus (i.e., nitric oxide) (7). As illustrated in Figs. 1 and 2, a single intracavernous injection of 100  $\mu$ g pcDNA-hSlo was associated with ICP responses that were significantly greater than those observed in both untreated and age-matched control rats. This was true at the two highest levels of neurostimulation at the 1-mo time point postinjection (Fig. 2A), without any detectable effect on resting ICP or BP (Table 2). Furthermore, at the 2-mo time point, the ICP responses in the gene transfer animals were significantly greater than those observed in all untreated animals at all levels

of neurostimulation (Fig. 2B) and sustained throughout the duration of the stimulus interval (Fig. 1).

At both time points, the mean ICP-to-BP ratio value in the gene transfer rats was  $\geq 0.6$ . The physiological significance of an ICP-to-BP ratio  $\geq 0.6$  in the rat model of penile erection has been previously noted (32) and is further supported by data summarized in Table 4. Similar measurements have been made in humans and reported as the penile-to-brachial index (PBI) (1, 8, 18, 21, 23, 25, 35). In clinical studies, a PBI value of  $< 0.6$  has been used as the cut off below which BP in the penis is considered insufficient to permit penile rigidity. Although PBI and ICP are not directly equivalent, it is of interest that

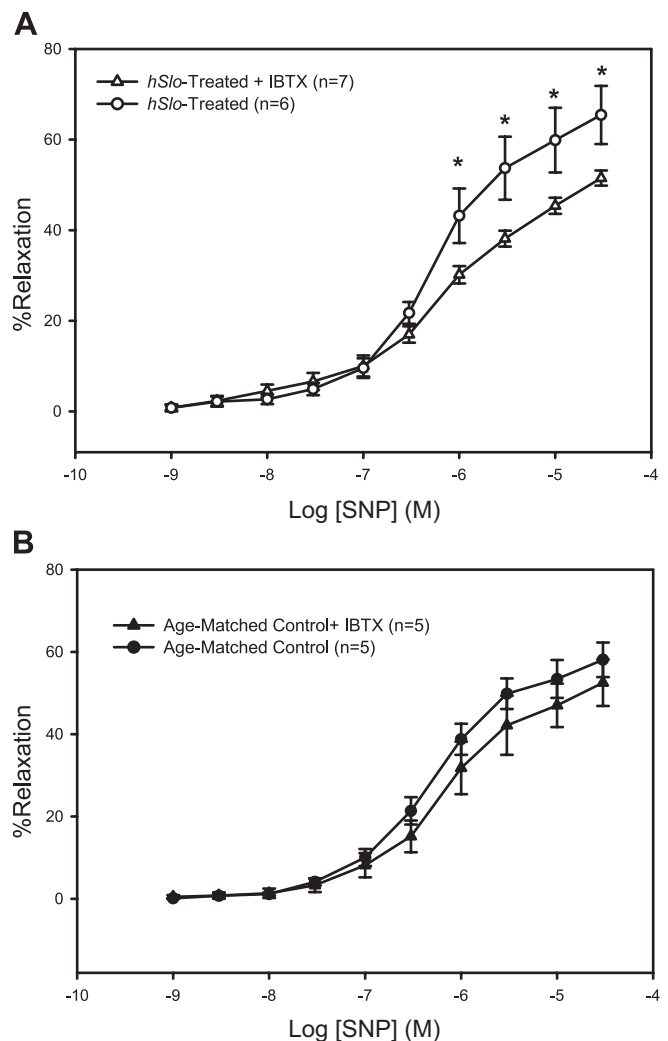


Fig. 8. Concentration-response curves (CRCs) for sodium nitroprusside (SNP)-induced relaxation of equivalently phenylephrine (PE)-precontracted corporal tissue strips derived from rats that had received a single intracorporal injection of pVAX-hSlo (1,000  $\mu$ g; A; see MATERIALS AND METHODS for description). A second set of pharmacological experiments was conducted on corporal tissue strips from AMC rats (B). Values are means  $\pm$  SE for the percent relaxation at each concentration of SNP. All strips were equivalently precontracted to near maximum with 10  $\mu$ M PE, and a cumulative CRC to SNP was constructed at 0.5 log increments (see MATERIALS AND METHODS). A single SNP CRC was performed on each corporal tissue strip in either the absence or presence of 200 nM iberiotoxin (IBTX; 15–20 min; see MATERIALS AND METHODS). \*Statistically significant difference at the  $P < 0.05$  level (Student's *t*-test for unpaired samples). In all cases, *n* refers to the number of corporal tissue strips.

Table 4. *Effects of IBTX in vivo: visual erectile scale*

	N	T	PE	FE
DM ( <i>n</i> = 5)				
Control		1	3	1
IBTX (50 $\mu$ l, $10^{-6}$ M)		1	3	1
IBTX (10 $\mu$ l, $10^{-5}$ M)	3	1	1	
IBTX (50 $\mu$ l, $10^{-5}$ M)	4	1		
AMC ( <i>n</i> = 5)				
Control				5
IBTX (50 $\mu$ l, $10^{-6}$ M)			1	4
IBTX (10 $\mu$ l, $10^{-5}$ M)		2	1	2
IBTX (50 $\mu$ l, $10^{-5}$ M)	5			

*n*, No. of rats. DM, STZ-diabetic for 2 mo; N, no visible change in penis; T, tumescence (expansion and/or elongation of penis); PE, partial erection (any movement of penis observed but no sustained lift of penis); FE, full erection (sustained lift of penis).

their values and physiological significance are qualitatively similar in humans and rats. In the present report, when the CN-stimulated ICP-to-BP value was  $\geq 0.6$ , there was a dramatic increase in the probability of a visible full erection.

*Evaluation of the effects of the dose and duration of gene transfer.* Figure 3 shows that after gene transfer, there was an increase in the mean amplitude of the nerve-stimulated ICP response and, furthermore, that the magnitude of the response was maintained throughout the duration of neurostimulation. In addition, Fig. 4 provides some evidence for the presence of a dose-dependent effect of gene transfer, because the 10- $\mu$ g dose was clearly less effective than the other doses, although the efficacy of the remaining doses was relatively similar. In fact, 4 mo after transfection, the 10- $\mu$ g group had ICP responses that were no longer distinguishable from responses observed on untreated STZ-diabetic rats, and, therefore, under these conditions, this clearly represents an apparent "threshold" for the efficacy of gene transfer in vivo. In addition, because the number of statistically significant differences in the mean ICP values between the treated and untreated STZ-diabetic rats was obviously greater 3 mo postinjection than 4 mo postinjection [particularly at submaximal levels of current stimulation (i.e., 0.5 mA)], this provides compelling evidence for the presence of a duration-dependent efficacy of gene transfer as well.

*Expression of hSlo and rSlo in STZ-diabetic rats.* As shown in Fig. 5A, the increased ICP responses observed in the STZ-diabetic rats occurred in the presence of expression of vector-derived hSlo (see MATERIALS AND METHODS for details). These data document a putative mechanistic link for the increased CN-stimulated ICP response observed in the gene transfer-treated STZ-diabetic rats. As displayed in Fig. 5B, there were no detectable changes in rSlo transcript expression levels in 8-wk STZ-diabetic rats, although the data summarized in Table 4 indicate that hSlo gene transfer may be associated with differences in maxi-K channel function (see Table 4).

*Effects of STZ-diabetes and gene transfer on corporal histology.* Consistent with previous studies, STZ-diabetes was associated with a significant decline in autonomic effector innervation (Fig. 6) (10, 33). However, there were no other striking differences in corporal histology that were associated with the presence of the diabetic state under the conditions of these studies. In addition, even at the highest injected dose of pcDNA-hSlo (i.e., 1,000  $\mu$ g), there were no obvious signs of inflammation or fibrosis for up to 4 mo after a single injection.

These observations are consistent with the supposition that using this gene transfer protocol is not associated with any detectable adverse impact on corporal tissue structure.

*Putative biodistribution of hSlo after intracavernous injection.* A preliminary study was also conducted to assess the distribution of the hSlo transcript after intracavernous injection. No attempt was made to restrict the outflow of blood from the penile vascular spaces after the intracavernous injection of genetic material. As summarized in Table 3, despite this "worst case" scenario (i.e., a tourniquet could be safely placed at the base of the human penis for up to 0.5 h to limit plasmid outflow), injection of rSlo results in transcript expression that is largely locally confined to the original tissue injection at time points  $\geq 24$  h after injection.

*Potential role of the maxi-K channel in the diabetes-related decrease in the CN-stimulated ICP-to-BP ratio.* To gain preliminary mechanistic insight into the potential role of the maxi-K channel in the observed STZ-diabetes-related decline in erectile capacity, pharmacological studies were conducted with the selective maxi-K channel blocker IBTX in vitro. IBTX was selected because it binds with high affinity to the external vestibule of the maxi-K channel  $\alpha$ -subunit, thus occluding K<sup>+</sup> flux through affected channels (9, 20). The rationale was that an IBTX-induced diminution in the hyperpolarizing ability of the maxi-K channel in corporal myocytes in response to the addition of SNP would inhibit the observed relaxation response.

As shown in Fig. 8A, the demonstration of IBTX-sensitive relaxation responses elicited using the nitric oxide donor SNP are consistent with the supposition that gene transfer results in the expression of functional hSlo channels and, moreover, that these channels participate in the modulation of corporal smooth muscle cell tone in a physiologically detectable/relevant fashion. Thus activation of the nitric oxide pathway, a critical endogenous component of the normal erectile response, results in activation of IBTX-sensitive Slo channels in rats with increased erectile capacity after hSlo gene transfer [i.e., an increased (normalized) ICP-to-BP ratio and rigid erections]. Conversely, there is no consistent IBTX-sensitive component to activation of the nitric oxide pathway in age-matched control rats with diminished erectile capacity. These data provide compelling evidence that perhaps diminished K channel-mediated hyperpolarization is an important component of age-related erectile dysfunction in this animal model and, furthermore, that hSlo gene transfer results in the expression of physiologically relevant K channels that can ameliorate this observed decline in erectile capacity.

*Perspectives.* Heightened contractility and/or impaired relaxation of corporal smooth muscle represent a major mechanism of diabetes-related alterations in erectile capacity. The fact that hSlo gene transfer in vivo was associated with IBTX-sensitive relaxation responses to SNP in corporal tissue strips in vitro is consistent with the supposition that gene transfer produces functional maxi-K channels that participate in the modulation of corporal smooth muscle cell tone. Taken together, these observations suggest a fundamental diabetes-related change in corporal myocyte maxi-K channel regulation, expression, or function that may be corrected by expression of recombinant hSlo. In this regard, other recent pharmacological studies have shown a decreased sensitivity of human corporal tissue strips obtained from impotent diabetic patients to relax-

ation with K channel modulators [i.e., pinacidil (38)]. In fact, a common theme of several other recent publications is that altered K channel expression, regulation, or function may be a contributing factor to the development of the vascular and erectile pathologies associated with diabetes (19, 24, 26, 27, 36). If disruption of normal ionic mechanisms, in the form of a putative K channelopathy (in this instance, an apparently diminished myocyte hyperpolarizing ability) is partially responsible for diabetic erectile dysfunction, then utilization of gene transfer to increase expression of a *Slo* transcript may represent an important new therapeutic avenue.

#### GRANTS

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-60037 and DK-55076 and a grant from Ion Channel Innovations.

#### REFERENCES

- Abber JC, Lue TF, Orvis BR, McClure DR, and Williams RD. Diagnostic tests for impotence: a comparison of papaverine injection with the penile-brachial index and nocturnal penile tumescence monitoring. *J Urol* 135: 923–925, 1986.
- Andersson KE. Erectile physiological and pathophysiological pathways involved in erectile dysfunction. *J Urol* 170: S6–S14, 2003.
- Archer SL. Potassium channels and erectile dysfunction. *Vasc Pharmacol* 38: 61–71, 2002.
- Bivalacqua TJ, Armstrong JS, Biggerstaff J, Abdel-Mageed AB, Kadowitz PJ, Hellstrom WJG, and Champion HC. Gene transfer of extracellular SOD to the penis reduces  $O_2^-$  and improves erectile function in aged rats. *Am J Physiol Heart Circ Physiol* 284: H1408–H1421, 2003.
- Bivalacqua TJ, Champion HC, Abdel-Mageed AB, Kadowitz PJ, and Hellstrom WJ. Gene transfer of prepro-calcitonin gene-related peptide restores erectile function in the aged rat. *Biol Reprod* 65: 1371–1377, 2001.
- Bivalacqua TJ and Hellstrom WJG. Potential application of gene therapy for the treatment of erectile dysfunction. *J Androl* 22: 183–190, 2001.
- Bivalacqua TJ, Usta M, Champion HC, Adams D, McNamara DB, Abdel-Mageed AB, Kadowitz PJ, and Hellstrom WJG. Gene transfer of endothelial nitric oxide synthase partially restores nitric oxide synthesis and erectile function in streptozotocin diabetic rats. *J Urol* 169: 1911–1917, 2003.
- Blaivas JG, O'Donnell TF, Gottlieb P, and Labib KB. Comprehensive laboratory evaluation of impotent men. *J Urol* 124: 201–204, 1980.
- Candia S, Garcia M, and Latorre R. Mode of action of iberiotoxin a potent blocker of the large conductance  $Ca^{2+}$ -activated  $K^+$  channel. *Biophys J* 63: 583–590, 1992.
- Cellek Rodrigo S, R, Lobos E, Fernandez P, Serrano J, and Moncada S. Selective nitrenergic neurodegeneration in diabetes mellitus—a nitric oxide-dependent phenomenon. *Br J Pharmacol* 128: 1804–1812, 1999.
- Chancellor MB, Yoshimura N, Pruchnic R, and Huard J. Gene therapy strategies for urological dysfunction. *Trends Mol Med* 7: 301–306, 2001.
- Chitale K, Bivalacqua TJ, Champion HC, Usta MF, Hellstrom WJG, Mills TM, and Webb RC. Adeno-associated viral gene transfer of dominant negative RhoA enhances erectile function in rats. *Biochem Biophys Res Commun* 298: 427–432, 2002.
- Christ GJ. Gene therapy for erectile dysfunction: where is it going? *Curr Opin Urol* 12: 497–501, 2002.
- Christ GJ. K channels as molecular targets for the treatment of erectile dysfunction. *J Androl* 23: S10–S19, 2002.
- Christ GJ. Frontiers in gene therapy. *Int J Impot Res* 15, Suppl 5: S33–S40, 2003.
- Christ GJ, Rehman J, Day N, Salkoff L, Valcic M, Melman A, and Geliebter J. Intracorporal injection of hSlo cDNA in rats produces physiologically relevant alterations in penile function. *Am J Physiol Heart Circ Physiol* 275: H600–H608, 1998.
- Costabile RA. Optimizing treatment for diabetes mellitus induced erectile dysfunction. *J Urol* 170: S35–S39, 2003.
- Dow JA, Gluck RW, Golimbu M, Weinberg GI, and Morales P. Multiphasic diagnostic evaluation of arteriogenic, venogenic, and sinusoidal impotency. Value of noninvasive tests compared with penile duplex ultrasonography. *Urology* 38: 402–407, 1991.
- Fan SF, Brink PR, Melman A, and Christ GJ. An analysis of the maxi- $K^+$  ( $K_{Ca}$ ) channel in cultured human corporal smooth muscle cells. *J Urol* 153: 818–825, 1995.
- Garcia ML, Gao Y, McManus OB, and Kaczorowski GL. Potassium channels: from scorpion venoms to high-resolution structure. *Toxicon* 39: 739–748, 2001.
- Goldstein I, Siroky MB, Nath RL, McMillian TN, Menzolan JO, and Krane RJ. Vasculogenic impotence: role of the pelvic steal test. *J Urol* 128: 300–306, 1982.
- Guay AT. Sexual dysfunction in the diabetic patient. *Int J Impot Res* 13: S47–S50, 2001.
- Jevitch MJ. Importance of penile arterial pulse sound examination in impotence. *J Urol* 124: 820–824, 1980.
- Karicheti V and Christ GJ. Physiological roles for  $K^+$  channels and gap junctions in urogenital smooth muscle: implications for improved understanding of urogenital function, disease and therapy. *Curr Drug Targets* 2: 1–20, 2001.
- Kim SC, Moon YT, and Oh CH. Non-visualization versus normal appearance of cavernous arteries on selective internal pudendal pharmacangiograms: comparison with Duplex scanning, cavernosal artery systolic occlusion pressure and penile brachial index. *Br J Urol* 73: 185–189, 1994.
- Korovkina VP and England SK. Detection and implications of potassium channel alterations. *Vasc Pharmacol* 38: 3–12, 2002.
- Lawson K and Dunne MJ. Peripheral channelopathies as target for potassium channel openers. *Expert Opin Investigat Drugs* 10: 1345–1259, 2001.
- Magee TR, Ferrini M, Garban HJ, Vernet D, Mitopni K, Rajfer J, and Gonzalez-Cadavid NF. Gene therapy of erectile dysfunction in the rat with penile neuronal nitric oxide synthase. *Biol Reprod* 67: 20–28, 2002.
- Meera P, Wallner M, and Toro L. A neuronal  $\beta$  subunit (KCNMB4) makes the large conductance, voltage- and  $Ca^{2+}$ -activated  $K^+$  channel resistant to charybdotoxin and iberiotoxin. *Proc Natl Acad Sci USA* 97: 5562–5567, 2000.
- Melman A and Christ GJ. Integrative erectile biology: the effects of age and disease on gap junctions and ion channels and their potential value to the treatment of erectile dysfunction. *Urol Clin North Am* 28: 217–231, 2001.
- Melman A and Gingell JC. The epidemiology and pathophysiology of erectile dysfunction. *J Urol* 165: 5–11, 1999.
- Melman A, Zhao W, Davies KP, Bakal R, and Christ GJ. The successful long-term treatment of age-related erectile dysfunction with hSlo cDNA in rats in vivo. *J Urol* 170: 285–290, 2003.
- Rehman J, Chenven E, Brink PR, Grine B, Walcott B, Melman A, and Christ GJ. Diminished neurogenic- but not pharmacological-induced intracavernous pressure responses in the 3-mo STZ-diabetic rat. *Am J Physiol Heart Circ Physiol* 272: H1960–H1971, 1997.
- Schenk G, Melman A, and Christ GJ. Gene therapy: future therapy for erectile dysfunction. *Curr Urol Rep* 2: 480–487, 2001.
- Schwartz AN, Lowe MA, Ireton R, Berger Richardson RE, ML, and Graney DO. A comparison of penile brachial index and angiography: evaluation of corpora cavernosa arterial inflow. *J Urol* 143: 510–513, 1990.
- Sobey CG. Potassium channel function in vascular disease. *Arterioscler Thromb Vasc Biol* 21: 28–38, 2001.
- Tirney S, Mattes CE, Yoshimura N, Yokoyama T, Ozawa H, Tzeng E, Birder LA, Kanai AJ, Huard J, de Groat WC, and Chancellor MB. Nitric oxide synthase gene therapy for erectile dysfunction: comparison of plasmid adenovirus, and adenovirus-transduced myoblast vectors. *Mol Urol* 5: 37–43, 2001.
- Venkateswarlu K, Giraldo J, Zhao Wang W, HZ, Melman A, Spektor M, and Christ GJ. K channels and human corporal smooth muscle cell tone: diabetes and relaxation of human corpus cavernosum smooth muscle by  $K_{ATP}$  channel openers. *J Urol* 168: 355–361, 2002.