Effect of in vivo gene transfer of nNOS in the PVN on renal nerve discharge in rats

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Li, Yi-Fan, Shyamal K. Roy, Keith M. Channon, Irving H. Zucker, and Kaushik P. Patel. Effect of in vivo gene transfer of nNOS in the PVN on renal nerve discharge in rats. Am J Physiol Heart Circ Physiol 282: H594–H601, 2002; 10.1152/ajpheart.00503.2001.—The paraventricular nucleus (PVN) of the hypothalamus is known to be involved in the control of sympathetic outflow. Nitric oxide (NO) has been shown to have a sympathoinhibitory effect in the PVN. The goal of the present study was to examine the influence of overexpression of neuronal NO synthase (nNOS) within the PVN on renal sympathetic nerve discharge (RSND). Adenovirus vectors encoding either nNOS (Ad.nNOS) or β-galactosidase (Ad.β-Gal) were transfected into the PVN in vivo. Initially, the dose of adenovirus needed for infection was determined from in vitro infection of cultured fibroblasts. In Ad.nNOS-treated rats, the local expression of nNOS within the PVN was confirmed by histochemistry for NADPH-diaphorase-positive neurons. There was a robust increase in staining of NADPH-diaphorase-positive cells in the PVN on the side injected with Ad.nNOS. The staining peaked at 3 days after injection of the virus. In α-chloralose- and urethane-anesthetized rats, microinjection of Nω-monomethyl-L-arginine (l-NMMA), a NO antagonist, into the PVN produced a dose-dependent increase in RSND, blood pressure, and heart rate. There was a potentiation of the increase in RSND, blood pressure, and heart rate due to l-NMMA in Ad.nNOS-injected rats compared with Ad.β-Gal-injected rats. These results suggest that the endogenous NO-mediated effect in the PVN of Ad.nNOS-treated rats is more effective in suppressing RSND compared with Ad.β-Gal-treated rats. These observations support the contention that an overexpression of nNOS within the PVN may be responsible for increased suppression of sympathetic outflow. This technique may be useful in pathological conditions known to have increased sympathetic outflow, such as hypertension or heart failure.

paraventricular nucleus; renal sympathetic nerve activity; neuronal nitric oxide synthase gene transfer

The gaseous molecule nitric oxide (NO) plays an important role in cardiovascular homeostasis. It plays this role by its action on both the central and peripheral autonomic nervous systems as well as by having direct effects on vascular smooth muscle. NO synthase (NOS) activity has been demonstrated in central and peripheral sites throughout the autonomic nervous system, including the receptors and effectors of the baroreflex pathway. Localization of neuronal populations that possess neuronal NOS (nNOS) has been achieved by histochemical staining using NADPH-diaphorase and immunohistochemistry (1, 5). This close proximity of the production of NO within central sites that are involved in cardiovascular regulation have led to the belief that NO may be involved in the regulation of autonomic outflow.

There have been relatively few studies (8, 19, 20) that have examined specific sites within the forebrain involved in mediating the effect of NO on sympathetic nervous outflow. NOS is densely localized in the paraventricular nucleus (PVN) of the hypothalamus (1, 9, 14, 17). The PVN is also known to be a site of integration for autonomic and endocrine-mediated cardiovascular responses (15, 16). PVN neurons project to several areas within the central nervous system that are known to be important in regulating cardiovascular function. These regions include the nucleus tractus solitarius (NTS) and the vagal complex present in the dorsomedial medulla, the rostral ventral medulla, and the intermediolateral cell column of the thoracolumbar spinal cord, the site of sympathetic preganglionic motor neurons. The discrete presence of NOS-positive neurons in the PVN of the hypothalamus suggests that NO may serve as a physiological regulator of the sympathetic nervous system. Perfusion of the PVN with NO-containing cerebrospinal fluid or microinjection of sodium nitroprusside (SNP) into the PVN has been shown to elicit a significant reduction in arterial blood pressure (8). Taken together, these studies led us to speculate that endogenous NO mechanisms in the PVN may be responsible for regulating renal sympathetic nerve discharge (RSND) and thus arterial blood pressure.

We observed that microinjection of an inhibitor of NOS, Nω-monomethyl-L-arginine (l-NMMA), increased RSND, arterial blood pressure, and heart rate (19). These
data indicate that the endogenous NO system within the PVN is involved in mediating sympathetic outflow. We considered that the increase of blood pressure was, at least partially, mediated by an increase of sympathetic outflow because microinjection of L-NMMA also led to a concurrent increase in efferent renal sympathetic outflow (19). Specificity of NOS inhibitors is further substantiated with the observation that administration of L-arginine reversed the increases in RSND, blood pressure, and heart rate produced by NOS inhibition. In addition, subsequent administration of NOS inhibition failed to produce the increase in RSND, blood pressure, and heart rate observed before administration of L-arginine. These results indicate that endogenous NO mechanisms within the PVN contribute to regulation of changes in RSND. In further support of this hypothesis, we observed that the microinjection of SNP, a NO donor, elicited a reduction of RSND, arterial blood pressure, and heart rate. The reduction of blood pressure was, at least partially, mediated by a reduction of sympathetic outflow because microinjection of SNP also led to a concurrent reduction in efferent renal sympathetic outflow (19). These effects of SNP on arterial blood pressure and sympathetic nerve discharge were most likely mediated by NO released by SNP (8, 19).

Adenoviral vectors provide a tool for localized over-expression of a transferred gene in a target tissue. Gene transfer of nNOS using an adenoviral vector (Ad.nNOS) generates high levels of recombinant nNOS protein and augments agonist-stimulated NO production in cultured vascular smooth muscle and endothelial cells and in vascular tissues in vivo (2, 3). In the present study, we use Ad.nNOS for gene transfer within the PVN to test if the endogenous NO production can be altered within the PVN and if this change in expression of nNOS contributes to the regulation of sympathetic outflow in a functional manner in rats.

METHODS

All procedures utilized in this study were approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee, and the experiments were conducted according to the APS “Guiding Principles for Research Involving Animals and Human Beings.” Male Sprague-Dawley rats weighing 210–280 g were obtained from Sasco Breeding Laboratories (Omaha, NE). Each rat was caged individually in an environment with ambient temperature maintained at 22°C and humidity at 30–40%. Laboratory chow (Purina) and tap water were available ad libitum.

Expression of nNOS in fibroblast cells in culture and measurement of NO production. Ad.nNOS and adenoviral vectors encoding β-galactosidase (Ad.β-Gal) were constructed, isolated, and purified by double cesium chloride density centrifugation as previously described (2, 3). Ad.nNOS contains a rat nNOS cDNA under the control of the cytomegalovirus immediate/early promoter and expresses functional nNOS protein in human vascular smooth muscle cells and human umbilical vein endothelial cells (2). The construct also expresses functional nNOS protein when infused in carotid arteries of rabbits (3). To determine the optimal dosage of virus that would not cause cytotoxicity but would cause increased nNOS protein expression in cells or neurons, 5 × 10⁶ cells/well of 293P3 fibroblast cells were cultured in 1 ml of Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum in rat tail collagen-coated chamber slides (Lab-Tek, Fisher Scientific) under 5% CO₂ in air. After 24 h of seeding, cells were infected with increasing plaque-forming units (pfu) per cell of either Ad.nNOS or Ad.β-gal for 1 h at 37°C in serum-free medium. Fresh medium was replaced after the specified period, and cultures were continued for an additional 72 h. Medium from Ad.nNOS-infected cultures was saved for NO analysis using the chemiluminescence technique (NO analyzer, model 280 NOA, Sievers Instruments). Cells were fixed with 4% freshly prepared paraformaldehyde in PBS (pH 7.4) for NADPH-diaphorase staining. Cells infected with Ad.β-Gal were fixed in 2% formaldehyde-2% glutaraldehyde and incubated at room temperature for 15 min with a X-Gal reaction solution [final concentration: 50 mM Tris-HCl (pH 8.0) containing 15 mM NaCl, 1 mM MgCl₂, 2.5 mM potassium ferrous cyanide, 2.5 mM potassium ferric cyanide, and 0.5 mg/ml X-Gal]. Stained cells were dehydrated and mounted permanently for light microscopy.

NADPH-diaphorase histochemistry. Adenovirus infection was done essentially as described for the PVN (NADPH-diaphorase histochemistry).

Adenovirus injections in the PVN. On the day of the PVN injections, each rat was anesthetized with pentobarbital sodium (40 mg/kg). The rat was then placed in a stereotaxic apparatus (Davis Kopf Instruments; Tujanga, CA), and a small burr hole was placed on the skull. The coordinates for the right PVN were determined from the Paxinos and Watson Rat Atlas (11), which were 1.8 mm posterior, 0.4 mm lateral to the bregma, and 7.8 mm ventral to the dura. A cannula (outer diameter, 0.5 mm; inner diameter, 0.1 mm) connected to a microsyringe (0.5 µl, model 7000.5, Hamilton microsyringe) was advanced into the right PVN with a manipulator (Narishige Z-1). A 200-nl solution (final concentration, 1 × 10⁸ pfu/ml) of Ad.nNOS or Ad.β-gal was injected into the PVN. Initial experiments with Ad.nNOS concentrations of 1 × 10⁸–1 × 10¹² pfu/ml demonstrated destruction of neurons at the site of injection (neurons appeared fragmented upon light microscopic evaluation after diaphorase staining). With a concentration of 1 × 10⁸ pfu/ml, no damage to the neurons within the PVN was observed from light microscopic evaluation. After the injection, the wound was sutured, and analgesics (1 ml/kg sc Nubain-Stadol) were administered on each of the next 2 days.

NADPH-diaphorase histochemistry. One, three, and six days after Ad.nNOS infection, the brains were stained for NADPH-diaphorase activity (21). This stain demonstrates the presence of functional NOS protein (5). Briefly, the rats were deeply anesthetized with pentobarbital sodium (70 mg/kg ip) and perfused transcardially with 150 ml of heparinized saline as a vascular rinse, followed by 300 ml of freshly prepared 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The brain was removed from each rat, postfixed at 4°C for 4 h in 4% paraformaldehyde solution, and then placed in 20% sucrose for 24 h. The brain was blocked in the coronal plane and sectioned at 30 µm thickness in a cryostat. The sections were collected in 0.1 M phosphate (pH 7.4) containing 0.3% Triton X-100, 0.1 mg/ml nitroblue tetrazolium, and 1.0 mg/ml β-NADPH. The sections in nitroblue tetrazolium solution were then placed in an oven at 37°C for 60 min. After the reaction, the sections were rinsed in phosphate buffer (pH 7.4) and mounted onto chrome-alum-coated slides. The slides were air dried overnight, rinsed in distilled water, and then dried again. Coverslips were then mounted directly with Permount. The intensity of staining was assessed using Kodak 1D Image Analysis software. Our initial studies demonstrated that there was no obvious increase in NADPH staining after 1 or 6 days of virus...
injection. Thus we focused our attention to data obtained from rats 3 days after infection.

Western blot analysis of nNOS in the PVN. Three days after Ad.nNOS infection, the brains were sectioned, and the PVN was punched and analyzed for nNOS protein by immuno-blotting (12). Briefly, the rats were deeply anesthetized with pentobarbital sodium (70 mg/kg ip), and the brains were removed and immediately frozen on dry ice. The brains were blocked in the coronal plane and sectioned at 300 μm thickness in a cryostat. The PVN was punched according to the method of Palkovits and Brownstein (10) from the virus-injected side and the contralateral control side. The punches were homogenized in ice-cold TRI reagent (MRC) using a sonicator (GraLab 545) to extract RNA. The protein left in the organic phase was extracted according to the MRC published protocol. Protein content in the SDS-supernatant was determined using a bicinchoninic acid protein assay kit (Pierce; Rockford, IL). Because of the higher sensitivity of the antibodies, 5 μg total protein is sufficient to generate a clear signal. Therefore, 6 μg protein was mixed with SDS-PAGE buffer containing 5% mercaptoethanol and heated at 100°C for 5 min. Protein was fractionated in a 7.5% polyacrylamide gel along with molecular weight standards, transferred to an Immobilon membrane, and subjected to a Western immuno-blotting protocol (12). The membrane was probed with monoclonal anti-nNOS antibody (Transduction Labs; Lexington, KY) and peroxidase-conjugated goat anti-mouse IgG, and the signal was detected using enhanced chemiluminescence substrate (Pierce) and Renaissance X-ray film (NEN-DuPont). The film was digitized using a Kodak digital camera, and the net intensity was determined using Kodak 1D Image Analysis software.

Recording of efferent RSND. On the day of the experiment, the rat was anesthetized with urethane (0.75 g/kg ip) and α-chloralose (70 mg/kg ip). The left femoral vein was cannulated with polyethylene (PE) tubing (PE-50 filled with saline) for drug administration if necessary, and the left femoral artery was cannulated and connected to a computer-based data acquisition system (MacLab) via a pressure transducer (Gould P231D) for recording of arterial blood pressure and heart rate.

The rat was then placed in a stereotaxic apparatus (Davis Kopf Instruments), and a small burr hole was placed on the skull. The coordinates for the right PVN were determined from the Paxinos and Watson Rat Atlas (11), which were 1.8 mm posterior, 0.4 mm lateral to the bregma, and 7.8 mm ventral to the dura. A cannula (outer diameter, 0.5 mm; inner diameter, 0.1 mm) connected to a microsyringe (0.5 μL, model 7000.5, Hamilton microsyringe) was advanced into the right PVN with a manipulator (Narishige Z-1).

The left kidney was exposed through a left retroperitoneal flank incision. A branch of the renal nerve was isolated from the adipose and connective tissues. The distal end of the nerve was ligated, and the nerve was placed on a thin bipolar steel electrode. The nerve-electrode junction was insulated electrically from the surrounding tissues with mineral oil. The electrical signal from the electrode was linked via a high-impedance probe (H15P) to a Grass P511 band-pass amplifier (gain, 10–50 × 1,000) with high- and low-frequency cutoffs of 1,000 and 100 Hz. The output from the Grass amplifier was directed to a Grass integrator, which rectifies the signal and integrates the raw nerve discharge. The output of the Grass integrator was displayed as an integrated voltage that is proportional to the renal nerve discharge. The average rectified signal [resistor-capacitor circuit (RC) filtered; time constant, 0.5 s] was then recorded and stored for later analysis in a computer-based data acquisition system (MacLab). Efferent RSND that had stabilized (over 30–40 min) at the beginning of the experiment was defined as the basal nerve discharge. All renal nerve activity recordings were corrected by subtraction of background noise, defined as the signal remaining after administration of hexamethonium (20 mg/kg iv) or postmortem. The response of renal nerve discharge to the administration of drugs into the PVN during the experiment was subsequently expressed as the percent change from the basal value.

Microinjections. Experiments were performed to examine if the endogenous NO-mediated inhibitory effects in the PVN on renal sympathetic outflow are enhanced in rats with gene transfer of nNOS in the PVN. In five Ad.β-Gal-injected control rats and five Ad.nNOS-injected experimental rats, an inhibitor of NOS, L-NMMA, was consecutively injected (50, 100, and 200 pmol in 50, 100 and 200 nl, respectively, using 1 mM solution of L-NMMA over 2 min) into the PVN in intervals of 20–30 min. The vehicle solution was artificial cerebrospinal fluid (pH 7.4) for all compounds. The responses in mean arterial blood pressure, heart rate, and RSND over the 20–30 min were recorded after each dose of L-NMMA.

Brain histology for identification of site of injection. At the end of the experiments, monastral blue dye was injected into the brain for histological verification of injection. After the rat was euthanized, the brain was removed and fixed in 4% formaldehyde for at least 24 h. The brain was then frozen, and serial transverse sections (30 μm) were cut using a cryostat (IEC, model CT, International-Harris Cryostat) at −20°C. The sections were thaw mounted on microscope slides and stained with 1% aqueous neutral red staining procedures. Presence of the blue dye within the PVN was verified microscopically.

Data analysis. Responses of RSND to the various doses of drugs were expressed as the percent change over the basal value. Responses of arterial blood pressure and heart rate to drugs were expressed as the difference between the basal value and the value after each dose of drug. The data were subjected to one-way repeated-measures ANOVA, followed by comparison for individual differences using the Newman-Keuls test (18). Blood pressure, heart rate, and NADPH-diaphorase staining intensities were compared between groups using the unpaired t-test. P < 0.05 was considered to indicate statistical significance. All data are presented as means ± SE.

RESULTS

Adenovirus-mediated gene transfer of nNOS to cultured fibroblasts. No staining was observed for cells cultured without any viral particles, suggesting that 293P3 fibroblast cells do not express either nNOS or β-galactosidase (Fig. 1). However, distinct β-galactosidase or diaphorase staining was visible for cells infected with the either viral construct. That the nNOS-infected cells expressed functional nNOS was evident from the linear increase in NO accumulation in the medium after an increasing concentration of virus exposure (Fig. 1). However, viral concentration over 50 pfu/cell appeared to affect cell morphology and their ability to stay attached on the culture surface (data not shown). Therefore, 50 pfu/cell were found to be the optimum concentration for fibroblast cells, and the dosage was used to estimate the optimum dosage for in vivo injection.
Adenovirus-mediated gene transfer of nNOS within the PVN: NADPH-diaphorase histochemistry. We evaluated the efficacy of Ad.nNOS gene transfer in the PVN by comparing the NADPH-diaphorase staining of the PVN infected with Ad.nNOS with the contralateral PVN in the same rat. An example of the differences in staining of the infected versus uninfected PVN is shown in Fig. 2, A–C. There was a significant increase in the number of diaphorase-positive cells as well as the intensity of NADPH-diaphorase staining in the Ad.nNOS-infected PVN compared with the contralateral uninfected PVN (Fig. 2D). Similarly, Ad.β-Gal demonstrated infection of cells in the ipsilateral injected PVN and no labeled cells in the contralateral PVN (Fig. 2E).

Western blot analysis of nNOS in the PVN. To confirm the results of histochemistry, we evaluated the efficacy of Ad.nNOS gene transfer in the PVN by comparing the nNOS protein levels of the PVN infected with Ad.nNOS with the contralateral PVN in the same rat. An example of the differences in levels of nNOS protein of the infected versus uninfected PVN is shown in Fig. 3A. There was a significant increase in the intensity of the bands of nNOS in the Ad.nNOS-infected PVN compared with the contralateral uninfected PVN (Fig. 3B).

RSND responses to adenovirus-mediated gene transfer of nNOS within the PVN. There were no significant differences in mean arterial pressure, heart rate, or RSND in the Ad.nNOS group compared with the Ad.β-Gal group (Table 1). Typically, microinjection of l-NMMA produced a gradual increase in RSND, blood pressure, and heart rate that peaked between 6–12 min from the start of the injection, followed by a gradual decrease back to the preinjection level (within 20–30 min). Microinjections of 50, 100, and 200 pmol l-NMMA elicited significant increases in efferent RSND, arterial blood pressure, and heart rate, reaching 49 ± 7%, 14 ± 2 mmHg, and 22 ± 4 beats/min, respectively, at the highest dose in Ad.β-Gal-injected rats (Fig. 4). The increase in RSND, arterial blood pressure, and heart rate to l-NMMA in rats injected with Ad.nNOS were significantly potentiated (84 ± 13%, 21 ± 3 mmHg, and 37 ± 5 beats/min at the highest dose) compared with Ad.β-Gal-injected rats. These data indicate that blockade of endogenous NO synthesis in rats with Ad.nNOS transfection is more
effective in raising RSND, blood pressure, and heart rate.

The responses in RSND and blood pressure to micro-injection of L-NMMA into the PVN in noninjected (virus) normal rats (49 ± 11%, 17 ± 5 mmHg, and 24 ± 6 beats/min, respectively, at the highest dose, n = 6) were similar to those from rats injected with Ad.β-Gal.

DISCUSSION

The results of the present study demonstrate for the first time the efficacy of adenoviral gene transfer of NOS into cells of the PVN of rats. We show that Ad.nNOS infects cells in the PVN and leads to a functional effect on renal sympathetic nerve activity mediated by the PVN. Our results provide a novel approach to restore neuronal levels of NOS, thus providing a potentially important candidate gene for cardiovascular gene therapy in diseases states, such as heart failure and hypertension lacking central nNOS (7, 21).

We used adenovirus vectors encoding the β-galactosidase gene or the nNOS gene to transfect fibroblasts to determine the dose of virus to use in the PVN. The successful gene transfer was confirmed by several methods. First, β-galactosidase protein expression was confirmed by histochemical staining of β-galactosidase in fibroblasts. Second, nNOS protein expression/activity was confirmed by NADPH-diaphorase staining of fibroblasts. Third, NO production by the fibroblasts was measured by monitoring the nitrite or nitrate (NOx) levels in the medium from fibroblasts infected with Ad.nNOS. There was increasing production of NOx with increasing concentration of the virus exposure. Taken together, this evidence indicates that 50 pfu/cell of Ad.nNOS is the optimal concentration for infecting fibroblasts that can be used for the optimal dose when injecting into the PVN.

Subsequently, we injected this dose of Ad.nNOS into the PVN of rats. Our initial results of time course (1, 3, and 6 days) demonstrated that there was lack of dramatic changes in NADPH-diaphorase in the PVN of rats infected for 1 day. However, there was a robust increase in NADPH-diaphorase-positive cells, both in
The number and intensity of staining in the PVN of Ad.nNOS-infected rats compared with the contralateral uninfected PVN in the same rat, 3 days after viral injection. These data demonstrate a successful transfer of the nNOS gene into cells in the PVN. Consistent with this observation, we also observed successful gene transfer of the β-galactosidase gene into cells in the PVN when Ad.β-Gal was injected. The intensity and number of NADPH-positive cells in the PVN of Ad.nNOS-injected rats was decreased in rats examined 6 days after the infection. Taken together, these data indicate that Ad.nNOS at a dose of 50 pfu/cell in the PVN is efficient in transferring the nNOS gene into the cells in the PVN 3 days after infection. However, the transient expression of nNOS protein after Ad.nNOS injection limits this mode of gene transfer to acute studies. On the contrary, adenovirus-associated viruses, such as human parvovirus and retrovirus vectors, have been successfully used for gene therapy (for a review, see Ref. 6) because of the weaker immune response and long-term effects. A variety of experimental manipulations, including the use of recombinant virus particles and improvement of intracellular traf-

![Graph A](image1.png)

**Fig. 3.** Western blot analysis of nNOS in the PVN of virus-infected and contralateral control PVN of rats. A: nNOS protein in the PVN of 3 rats infected with Ad.nNOS (V) and the contralateral control (C) PVN. B: summarized intensity data in 3 rats.

![Graph B](image2.png)

**Fig. 4.** The change in renal sympathetic nerve discharge (RSND; A), change in arterial blood pressure (B), and change in heart rate (C) to the microinjection of NG'-monomethyl-L-arginine (L-NMMA) into the PVN in Ad.β-Gal-treated rats (n = 5) and Ad.nNOS-treated rats (n = 5). Values represent means ± SE. *P < 0.05 vs. control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>RSND, μV·s</th>
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<tbody>
<tr>
<td>Ad.β-Gal (n = 5)</td>
<td>91 ± 3</td>
<td>333 ± 18</td>
<td>5.1 ± 0.8</td>
</tr>
<tr>
<td>Ad.nNOS (n = 5)</td>
<td>86 ± 3</td>
<td>306 ± 19</td>
<td>4.6 ± 0.6</td>
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Values represent means ± SE; n = no. of rats. RSND, renal sympathetic nerve discharge; Ad.β-Gal and Ad.nNOS, adenovirus-mediated transfection of the β-galactosidase and neuronal nitric oxide synthase genes, respectively. There were no statistically significant differences between the groups.
ficking, have been used to improve the efficacy of adenovirus-associated viruses in long-term gene therapy (6). Therefore, further experiments need to be done using improved adenovirus-associated virus-nNOS constructs for maintaining the long-term effect of nNOS in the PVN; hence, the beneficial effect of NO in the PVN in chronic disease conditions such as hypertension and heart failure.

It could be possible that inflammation and cytotoxicity caused by adenovirus infection might have affected the present findings (4). Adenoviral infection can cause a dose-dependent acute inflammatory injury (4). To avoid this, we conducted our studies at a relatively low infectious titer (1 × 10⁸ pfu/ml), a dose that we have shown yields maximal transgene expression without apparent neuronal injury or vascular injury (3). Furthermore, Ad.β-Gal gene transfer did not alter basal arterial blood pressure or heart rate, and the responses in RSND responses to L-NMMA were larger in rats infected with Ad.nNOS, L-NMMA into the PVN elicited significant increases in arterial blood pressure and heart rate. However, overall, the two studies are in general agreement that overexpression of NO within the central nervous system decreases overall sympathetic tone (13).

In summary, we demonstrated a technique of nNOS gene transfer into the PVN of rats. We demonstrated that Ad.nNOS into the PVN generates a high level of NO in cells in the PVN and that the expressed nNOS is functional in inhibiting renal sympathetic nerve activity by the PVN. Ad.nNOS injection in the PVN provides a novel approach to replacing NO activity in the PVN of disease conditions known to have depleted levels of NO, such as heart failure and hypertension.

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