Gene Transfer of Neuronal Nitric Oxide Synthase into Intracardiac Ganglia Reverses Vagal Impairment in Hypertensive Rats

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Abstract—Hypertension is associated with reduced cardiac vagal activity and decreased atrial guanylate cyclase and cGMP levels. Neuronal production of NO facilitates cardiac parasympathetic transmission, although oxidative stress caused by hypertension may disrupt this pathway. We tested the hypothesis that peripheral vagal responsiveness is attenuated in the spontaneously hypertensive rat (SHR) because of impaired NO-cGMP signaling and that gene transfer of neuronal NO synthase (nNOS) into cholinergic intracardiac ganglia can restore neural function. Cardiac vagal heart rate responses in the isolated SHR atrial/right vagus preparation were significantly attenuated compared with age-matched normotensive Wistar–Kyoto rats. [3H] acetylcholine release was also significantly lower in the SHR. The NO donor, sodium nitroprusside, augmented vagal responses to nerve stimulation and [3H] acetylcholine release in the Wistar–Kyoto rat, whereas the soluble guanylate cyclase inhibitor 1H-(1,2,4)oxadiazolo(4,3-a)quinoxaline-1-one attenuated [3H] acetylcholine release in Wistar–Kyoto atria. No effects of sodium nitroprusside or 1H-(1,2,4)oxadiazolo(4,3-a)quinoxaline-1-one were seen in the SHR during nerve stimulation. In contrast, SHR atria were hyperresponsive to carbachol-induced bradycardia, with elevated production of atrial cGMP. After gene transfer of adenoviral nNOS into the right atrium, vagal responsiveness in vivo was significantly increased in the SHR compared with transfection with adenoviral enhanced green fluorescent protein. Atrial nNOS activity was increased after gene transfer of adenoviral nNOS, as was expression of α1-soluble guanylate cyclase in both groups compared with adenoviral enhanced green fluorescent protein. In conclusion, a significant component of cardiac vagal dysfunction in hypertension is attributed to an impairment of the postganglionic presynaptic NO-cGMP pathway and that overexpression of nNOS can reverse this neural phenotype. (Hypertension. 2007;49:380-388.)

Key Words: NO • gene transfer • autonomic nervous system • acetylcholine • heart rate • hypertension • guanylate cyclase

Cardiac autonomic imbalance has been implicated in the etiology of hypertension and is characterized by sympathethetic overactivity that is coupled with depressed baroreflex-mediated cardiac vagal responsiveness.1,2 Reduced cardiac vagal tone has been demonstrated in hypertensive patients3,4 and in hypertensive animal models.5,6 Moreover, similar autonomic responses are observed in normotensive subjects with a family history of hypertension,7 suggesting that parasympathetic dysfunction may be an early feature of the pathophysiology of this disease. This is clinically significant, because impaired cardiac vagal function is a powerful independent predictor of mortality.8,9 In addition, vagal activation exerts strong antiarrhythmic effects during coronary artery occlusion in exercising dogs10 and may improve survival in rats after myocardial infarction.11

The mechanisms responsible for cardiac vagal impairment in hypertension remain elusive. However, it is now widely established that reduced bioavailability of NO, associated with increased oxidative stress, is characteristic of the hypertensive phenotype.12,13 Moreover, downregulation of soluble guanylate cyclase (sGC), the key mediator of cGMP-dependent effects of NO, is observed in the aorta and atria of the spontaneously hypertensive rat (SHR).14,15 Under normal conditions, NO derived from neuronal NO synthase (nNOS) facilitates vagal neurotransmission and bradycardia, via a cGMP-dependent pathway.16 Emerging evidence shows that adenoviral gene transfer of nNOS to cardiac vagal neurons within the cervical vagus and right atrium in normotensive animals results in a rapid enhancement of parasympathetic function.17,18 nNOS gene transfer also normalizes hyperre-
sponsiveness to β-adrenergic stimulation in the sino-atrial node of the SHR.\textsuperscript{15} We tested the hypothesis that impaired NO-cGMP signaling in hypertension attenuates peripheral vagal responsiveness in the SHR. From this we further hypothesized that overexpression of nNOS in postganglionic cholinergic neurons would restore peripheral parasympathetic function.

**Methods**

**Animal Care**
The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (National Institutes of Health Publication No. 85-23, revised 1996) and the Animals (Scientific Procedures) Act 1986 (United Kingdom) and was performed under British Home Office license requirements (PPL 30/2130). Age-matched (16 to 24 weeks old) male SHR and Wistar–Kyoto (WKY) rats were housed under standard laboratory conditions.

**Isolated Rat Sino-Atrial Node/Right Vagus Nerve Preparation: Assessment of Vagal Function**

**Dissection**
Animals were killed by exsanguination under deep halothane anesthesia (4% in 100% O\textsubscript{2}). The heart was exposed and the ventricles removed, allowing the atria to be back perfused with 10 mL of heparinized (1000 U/mL) Tyrode’s solution. The thorax and mediastinum were rapidly removed and placed in oxygenated (95% O\textsubscript{2} and 5% CO\textsubscript{2}) Tyrode’s solution at room temperature in a Perspex dissecting dish with a Sylgard base. The atria and right vagus were carefully separated from surrounding tissues, and the proximal end of the right vagus was tied off.

**Experimental Preparation**
Sutures (Ethicon, 5/0 Mersilk) were placed at the lateral edges of both atria, and the preparation was transferred to a preheated (37±0.1°C) water-jacketed organ bath containing 100 mL of continuously oxygenated Tyrode’s solution. The atria were vertically mounted with the suture in the left atrium connected to a stainless steel hook and the suture in the right atrium attached to an isometric force transducer (Harvard Apparatus, model 60-2997) connected to an amplifier. Heart rate was triggered from contraction and recorded in real time (Biopac MP100 with Acqknowledge software).

**Protocols**
Preparations were equilibrated in Tyrode’s solution for 60 to 90 minutes at 37°C until a stable baseline heart rate was achieved. The right vagus was placed through a pair of custom-built platinum ring electrodes and stimulated at 3, 5, 7, and 10 Hz (15 V, 1-ms pulse duration; order of stimulations randomized) for 25 seconds, with an interval of ≥1 minute between successive stimulations. In some experiments, vagal stimulation was repeated after application of the NO donor sodium nitroprusside ([SNP] 20 μmol/L; 10-minute incubation; Sigma). In addition, muscarinic responsiveness of atrial preparations was assessed using cumulative concentration–response curves to carbachol (0.1 to 0.5 μmol/L; Sigma; 2-minute incubation at each concentration).

**Measurement of Acetylcholine Release**
**Experimental Preparation**
Animals were killed and the right atria removed as described above. The preparation was then transferred to a preheated (37±0.2°C), continuously oxygenated, water-jacketed organ bath containing 4 mL of Tyrode’s solution where the atrium was pinned flat between 2 parallel silver stimulating electrodes 10 mm apart. Our methodology was similar to that described previously.\textsuperscript{16} After a 45-minute equilibration period (where the Tyrode’s solution was replaced every 15 minutes), the atrium was stimulated at 5 Hz (15 V, 1-ms pulse duration) for 1 minute and then again after another minute to stimulate acetylcholine (ACh) turnover. The preparation was then incubated for 30 minutes with [H\textsuperscript{3}]choline chloride (10 μCi, Amersham United Kingdom) during which the atrium was stimulated at 5 Hz for 10 seconds every 30 seconds to incorporate the radiolabeled choline into parasympathetic transmitter stores. Tyrode’s solution containing 50 μmol/L of hemicholinium-3 (Sigma) was used after the incubation period to reduce reuptake of radioactively labeled transmitter. Excess [H\textsuperscript{3}]choline was washed from the preparation by superfusing for 60 minutes at a rate of 3 mL/min with Tyrode’s solution.

**Protocol**
After the wash period, superfusion was stopped and the bath solution replaced every 3 minutes with a 0.5-mL sample being taken on every change of solution. This sample was added to 4.5 mL of scintillation fluid (Ecocint A, National Diagnostics) and the amount of radioactivity in each sample (disintegrations per minute) measured using a liquid scintillation counter (Tri-carb 2800TR, Packard). After 16 and 94 minutes, the atrium was stimulated at 5 Hz for 1 minute, and after 34 and 112 minutes, it was stimulated again at 10 Hz for 1 minute (Figure 2A). In some experiments the sGC inhibitor 1H,1,2-oxadiazolo(4,3-a)quinazoline-1-one ([ODQ] 10 μmol/L; Sigma) was introduced to the solution after the first (control) 5-Hz stimulation and allowed to incubate for 45 minutes before the second 5-Hz stimulation was performed, whereas in additional experiments, SNP (20 μmol/L; Sigma) was added and allowed to incubate for 15 minutes before the second stimulation. At the end of the experiment, the atrium was immersed overnight in Tyrode’s solution containing 4 μmol/L of papain (Sigma) and the radioactivity contained in the extract determined. [H\textsuperscript{3}]outflow was expressed as a percentage of the total radioactivity in the atrium at the end of the experiment and that released after superfusion.

**Measurement of Right Atrial cGMP Concentration**

**Experimental Preparation**
Isolated, perfused, beating atria were prepared by methods described previously.\textsuperscript{19} In brief, the animal was killed and the right atrium removed as described above. A cannula containing 2 narrow bore catheters (4.5 mm OD) was inserted into the atrium and secured by ligatures. The outer tip of the atrial cannula was open to allow for outflow. The cannulated atrium was transferred to a preheated (36.5±0.2°C), continuously oxygenated, water-jacketed organ chamber and immediately perfused with oxygenated Tyrode’s solution by means of a peristaltic pump (0.5 mL/min).

**Protocols**
The atria were allowed to stabilize for 60 minutes. Once the stabilization period was completed, [H\textsuperscript{3}]inulin (5 μCi, Amersham United Kingdom) was introduced to the perfusional fluid 20 minutes before the start of the sample collection to measure translocation of extracellular fluid. The perfusate was collected at 2-minute intervals at 4°C for analysis. Collections were performed during perfusion with Tyrode’s solution containing carbachol (0.3 μmol/L) for 10 minutes after a 20-minute control collection period and again after 10 minutes of washout with Tyrode’s solution.

**Measurement of Extracellular Fluid Translocation**
The radioactivity of [H\textsuperscript{3}]inulin in atrial perfusate samples was measured with a liquid scintillation counter, and the amount of extracellular fluid translocated through the atrial wall was calculated, as described elsewhere.\textsuperscript{20}

**Radioimmunoassay of cGMP Concentration**
For measurement of cGMP concentration in the atrial perfusate, 500 μL of the perfusate was treated with trichloroacetic acid to a final concentration of 6% for 15 minutes at room temperature and centrifuged at 4°C. The supernatant (200 μL) was extracted with water-saturated ether 3 times and then dried using a SpeedVac concentrator (Savant). The dried samples were reconstituted, and a [125I]cGMP radioimmunoassay kit (Amersham United Kingdom) was used to measure the amount of cGMP after the bound form was
separated from the free form by magnetic separation. The amount of cGMP efflux was expressed as picomoles of cGMP per minute per gram of atrial tissue. The molar concentration of cGMP in the interstitial space fluid\(^1\) was calculated as cGMP efflux concentration (nanomoles per liter) = cGMP (in picomoles per minute per gram)/extracellular fluid translocated (in microliters per minute per gram) × 1000.

Right Atrial nNOS Gene Transfer and In Vivo Assessment of Cardiac Vagal Responsiveness

Gene Transfer Procedure
SHRs underwent gene transfer via percutaneous injection into the right atrium using methods similar to those described previously in the guinea pig.\(^2\) Animals were anesthetized with halothane (3% to 4% for induction and 2% to 3% for maintenance, in 100% \(\text{O}_2\)) and injected with \(5\times10^6\) particles of replication-deficient adenoviral vector encoding nNOS (Ad.nNOS) or enhanced green fluorescent protein (Ad.eGFP) control vector in sterile PBS (300 \(\mu\)L injectate). The injection was performed using a 26-gauge needle, placed through the third intercostal space on the right side of the animal and directed toward the left axilla. Localization of the tip of the needle within the right atrial chamber was confirmed before injection by flashback of blood into the syringe, and the injection was performed during withdrawal of the needle from the atrial cavity. Phenotyping of transfected animals was performed \(\approx\) 5 days after injection.

Anesthesia and Surgery
Surgical anesthesia was induced and maintained using halothane as described above, and a tracheostomy was performed after injection by flashback of blood into the syringe, and the injection was performed during withdrawal of the needle from the atrial cavity. Phenotyping of transfected animals was performed \(\approx\) 5 days after injection.

Intensive Care
Body temperature was monitored using a rectal thermocouple, and heating lamps placed above and below the animal were used to maintain body temperature within the range 37°C to 38°C. Arterial blood samples were regularly taken into preheparinized capillary tubes and used to measure blood gases and pH (ABL505, Radiometer Copenhagen); alteration of ventilatory parameters and/or infusion of 4.2% sodium bicarbonate solution (in 0.9% NaCl) was used to maintain blood gases and pH within acceptable limits (Pa\(_O_2\) >100 mm Hg; Pa\(_CO_2\) 35 to 45 mm Hg [pH 7.4±0.02]).

Experimental Protocol
Animals were bilaterally vagotomized, and the distal end of the right vagus was placed over a pair of hooked platinum stimulating electrodes. Vagal nerve stimulation was performed for 30 seconds at 3, 5, 7, and 10 Hz (15 V, 3-ms pulse duration; order of stimulations randomized), with an interval of \(\approx\) 1 minute between successive stimulations. Rats were euthanized using an intravenous overdose of sodium pentobarbitone (Sagatal; Rhône Mérieux Ltd) on completion of the experimental protocol.

Measurement of sGC and nNOS Protein Expression and nNOS Activity
Western blotting for sGC and nNOS in right atria was performed using standard techniques as described previously.\(^1\) Animals were bilaterally vagotomized, and the distal end of the right vagus was placed over a pair of hooked platinum stimulating electrodes. Vagal nerve stimulation was performed for 30 seconds at 3, 5, 7, and 10 Hz (15 V, 3-ms pulse duration; order of stimulations randomized), with an interval of \(\approx\) 1 minute between successive stimulations. Rats were euthanized using an intravenous overdose of sodium pentobarbitone (Sagatal; Rhône Mérieux Ltd) on completion of the experimental protocol.
of Tris (pH 7.5), 5 mmol/L of CaCl₂, 1 mmol/L of MgCl₂, 14 μmol/L of tetrahydrobiopterin, 10 μg/mL of calmodulin, 4 μmol/L of flavin adenine dinucleotide, 4 μmol/L of flavin adenine mononucleotide, 1 mmol/L of reduced nicotinamide adenine dinucleotide phosphate, and 1 μL of 1 mCi/mL [³H]-l-arginine. The activities of the nNOS isoforms were measured using a specific endothelial NO synthase inhibitor (L-N⁵-(1-iminoethyl) ornithine, Dihydrochloride, Calbiochem Ltd) added to the assay buffer at a concentration of 10 μg per assay. After 30 minutes of incubation at 37°C, the reactions were stopped with 20 mmol/L of sodium acetate (pH 5.5), 0.2 mmol/L of EGTA, 1 mmol/L of l-citrulline, and 2 mmol/L of EDTA and poured over Dowex AG-50W-X8 columns (Bio-Rad). [³H]-l-citrulline was eluted with 2 mL of deionized water, and radioactivity was quantified by liquid scintillation counting. The results are expressed as femtomoles of citrulline per milligram of protein per minute.

**Solutions and Drugs**

Rat Tyrode’s solution contained (in mmol/L): NaCl 120, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, CaCl₂ 2, and glucose 11. The solution was constantly aerated (95% O₂ and 5% CO₂) to maintain pH at 7.4. All of the solutions were prepared fresh on the day of use using deionized water obtained from an Elga water purification system. Experiments using SNP were performed in a darkened room because of the light sensitivity of this drug.

**Statistical Analysis**

Data are presented as mean±SEM. Differences in the data were assessed using the t test or Mann–Whitney rank sum test as appropriate (SigmaStat, Systat Software Inc). Statistical significance was accepted at P<0.05.

**Results**

**Rate Response to Vagal Nerve Stimulation**

The heart rate response to right vagal stimulation was significantly reduced in the SHR (n=9) relative to the WKY (n=7) rats at 3, 5, and 7 Hz (P<0.05, unpaired t test; Figure 1A and 1B). No significant difference in responsiveness was observed at 10 Hz, although a trend toward impaired responsiveness in the SHR was present (P=0.07; see Figure 1).

**Measurement of Right Atrial ACh Release**

Release of [³H]ACh in response to right atrial field stimulation was significantly impaired at both 5 and 10 Hz in the SHR (n=6) compared with the WKY rats (n=7; 5 Hz: P<0.01; 10 Hz: P<0.05; unpaired t test; see Figure 2). S1 and S2 represent the first and second field stimulation, respectively. There was no difference between S1 and S2 at 5 or 10 Hz in either hypertensive or normotensive animals.

**Pharmacological Manipulation of NO-cGMP Pathway**

**Effect of NO Donor**

Administration of 20 μmol/L of SNP significantly enhanced the release of [³H]ACh in response to 5-Hz field stimulation in the WKY rats (n=8; P<0.05, paired t test; see Figure 3A and 3C), whereas there was no effect in the SHRs (n=6; Figure 3B and 3C). This translated functionally where SNP significantly enhanced the rate responsiveness to vagal stimulation in the isolated double atrial preparation in the WKY rats (n=7; P<0.05, paired t test; Figure 3D). However, no response was seen in the SHRs (n=6) despite a similar increase in basal heart rate in the 2 strains (WKY: +49±7 [n=8] versus SHR: +43±6 [n=6] bpm) because of the well-established action of NO on the pacemaker itself.

**Effect of sGC Inhibitor**

Incubation with ODQ significantly decreased the release of [³H]ACh in response to 5-Hz field stimulation in the WKY rats (n=7; P<0.01, paired t test; see Figure 4). However, the release of [³H]ACh from SHR atria (n=7) was unaffected by ODQ.

**Stimulation of Muscarinic Receptors**

A comparison of cumulative concentration–response curves to carbacol revealed a significant increase in responsiveness in SHR atria at 0.1 and 0.5 μmol/L (P<0.05, unpaired t test; see Figure 5A for representative raw data traces and Figure 5B for grouped data).
carbachol (0.3 μmol/L) also increased cGMP efflux concentration in the SHR (n = 6), which was 21.5% higher than WKY responses (Figure 6; n = 5; P < 0.05, unpaired t test). In addition, atrial endothelial NO synthase activity was significantly greater in the SHR (SHR: 12.70 ± 0.09 fmol/mg per minute versus WKY: 13.52 ± 0.30 fmol/mg per minute), suggesting postsynaptic cellular remodeling of the receptor-coupled NO-cGMP pathway.

**nNOS Activity and Guanylate Cyclase Protein Levels After nNOS Gene Transfer**

We evaluated the efficacy of Ad.nNOS gene transfer by measuring nNOS activity together with nNOS and α₁sGC protein expression in the atria of transfected WKY and SHR atria. Western blot analysis confirmed that there was a significant increase in both groups of rats in the intensity of the bands of nNOS (Figure 7A and 7B) and sGC (Figure 8) after Ad.nNOS compared with Ad.eGFP-treated rats. nNOS activity in Ad.nNOS-transfected rats was significantly increased in both groups compared with Ad.eGFP (WKY: +23.3 ± 8.4%, P < 0.05, unpaired t test; SHR: +15.7 ± 3.2%, P < 0.05, unpaired t test; Figure 7C).

**Effects of nNOS Gene Transfer on Parasympathetic Function**

A comparison of mean arterial pressures in Ad.eGFP- and Ad.nNOS-transfected SHRs showed no effect of nNOS gene transfer on blood pressure (126 ± 2 mm Hg Ad.nNOS versus 116 ± 6 mm Hg Ad.eGFP; n = 6 for both groups). However, transfection with Ad.nNOS significantly increased vagal responsiveness in the SHR at all of the frequencies tested (eg, 3 Hz: −40 ± 3 bpm [Ad.nNOS, n = 5] versus −19 ± 4 [Ad.eGFP, n = 7]; P = 0.001, unpaired t test; Figure 9A).

Rate responses of SHR atria to carbachol were unaffected by nNOS gene transfer at all of the concentrations tested (eg, 0.1 μmol/L: −29 ± 2 bpm [Ad.eGFP, n = 22] versus −28 ± 4 [Ad.nNOS, n = 8]; Figure 9B) suggesting that parasympathetic gain of function was occurring via facilitated presynaptic transmission. When in vivo SHR results were viewed quantitatively with the response of the WKY, it can be seen from Figure 10 that nNOS gene transfer enhanced vagal responsiveness in both groups of rats compared with the eGFP-treated controls. Moreover, nNOS-treated SHRs demonstrated a vagal response similar to that of eGFP-transfected WKYs at 3 and 5 Hz with an augmented response at 7 and 10 Hz.

**Discussion**

The main findings of the current study are as follows: (1) at the level of the cardiac postganglionic neuron, bradycardia and acetylcholine release in response to vagal nerve stimulation are impaired in the SHRs compared with the normotensive WKY rats; (2) downregulation of the atrial NO-coupled guanylate cyclase pathway contributes to the parasympathetic phenotype in the SHR; and (3) upregulation of this pathway by gene transfer of adenoviral nNOS in the SHR restores peripheral cardiac vagal function in vivo.

**Defective Peripheral Cardiac Vagal Function in the Hypertensive Rat**

Increasing evidence supports the concept of sympathetic hyperactivity and parasympathetic underactivity as a significant component in the etiology of not only early and borderline hypertension but also in the maintenance of sustained essential hypertension.24–26 Furthermore, this is accompanied by a reduction in sensitivity of the parasympathetic component of the arterial baroreflex.27 The data presented here give direct evidence that vagally induced bradycardia and radiolabeled acetylcholine release are significantly impaired in the hypertensive rat at the level of the postganglionic cholinergic synapse. This demonstrates that a signif-
icant component of parasympathetic dysfunction occurs peripherally within efferent cardiac vagal neurons of the adult SHR. This is interesting in light of previous studies performed in young hypertensive rats in which others failed to observe an attenuation of cardiac vagal responsiveness.28 Our results also show that activation of postsynaptic muscarinic receptors in right atrial tissue with carbachol results in enhanced bradycardia and production of cGMP in the SHR, implicating hyperreactivity of the cholinergic system to a bath-applied agonist in this model. This may be related to the observation that atrial endothelial NO synthase activity is significantly higher in the SHR and, therefore, could lead to more NO-cGMP-dependent inhibition of pacemaker currents, like the inward calcium current ICaL.15 This could represent a postsynaptic compensatory mechanism resulting from the dysfunctional signaling at the prejunctional level.

Defective NO-cGMP Signaling in the Hypertensive Rat

Previous studies have shown that a presynaptic NO-cGMP pathway can modulate cardiac cholinergic transmission in adult guinea pig,16,29 rabbit,30 and nNOS knockout mice.31 Upregulation of nNOS expression within cardiac vagal neurons is essential for the increase in cardiac parasympathetic function observed as a result of aerobic exercise training.32,33 However, downregulation of sGC is observed in the aorta14 and right atrium15 of the SHR, where reduced atrial levels of cGMP are also observed.15

Attention has focused on the potential role of nNOS in the pathophysiology of hypertension based on its putative actions within the autonomic nervous system.14 It is well established...
that NO modulates activity within the central autonomic nuclei and that there may be defective nNOS-mediated signaling within the dorsal brain stem in hypertension. Our results show that an aspect of vagal impairment in the SHR resides within the peripheral nervous system at the level of the NO–sGC–cGMP pathway. Neither ODQ or SNP affected ACh release in the SHR, whereas ODQ could inhibit and SNP facilitate transmission in the WKY rat in a similar fashion to the normotensive guinea pig.

Effect of nNOS Gene Transfer on Parasympathetic Function in the SHR

To determine whether our in vitro data were significant in the intact animals, heart rate responsiveness to vagal nerve stimulation was measured in anesthetized SHRs after adenoviral gene transfer of either nNOS or eGFP. nNOS-transfected animals displayed enhanced vagally mediated bradycardia at all of the frequencies of stimulation tested in comparison to eGFP-transfected animals. This result is consistent with those data published previously in the normotensive guinea pig. We have reported recently that a similar gene transfer technique with nNOS increased nNOS protein expression and the production of right atrial tissue levels of cGMP in both the SHR and WKY rats. Consistent with this observation, we observed here that nNOS activity and the α1 subunit of sGC protein expression in WKY atria were significantly increased after Ad.nNOS, indicating that gene transfer upregulated NO bioavailability that then translated into vagal gain of function in both strains of rats.

The ability of nNOS gene overexpression to reverse peripheral vagal impairment in the hypertensive rat is somewhat surprising in light of our in vitro data from experiments...
using SNP. Administration of the NO donor SNP produced a prejunctional enhancement of cholinergic neurotransmission in right atrial preparations from the normotensive WKY rat. However, this response was absent in the atrial preparations from the SHR. It is possible that acute pharmacological administration of NO donors at a relatively low concentration (20 μmol/L) may not fully mimic the action of NO generated intracellularly by the nNOS transgene given its labile and highly reactive properties. In particular, a relatively high local concentration of NO may be required to increase the bioavailability of NO under conditions of increased oxidative stress that have been demonstrated previously in hypertension, because in the presence of the superoxide anion, NO will undergo rapid conversion to peroxynitrite. Indeed, evidence from experiments using synaptosomes isolated from the electric organ of Torpedo suggests that peroxynitrite may inhibit choline acetyltransferase and, therefore, reduce synthesis of ACh. It is, therefore, highly likely that nNOS gene transfer provided a more potent NO signal than the NO donor and that gene transfer also upregulated sGC in the SHR in a similar manner to our observations in WKY tissue.

Perspectives
The overall pathophysiological significance of these observations remains to be established. The vagus is nature’s cardiac calcium channel antagonist, and our data suggest that strategies designed to upregulate NO bioavailability or the downstream sites of sGC-cGMP (with NO synthase gene transfer) may be therapeutically beneficial in targeting the impaired parasympathetic phenotype associated with hypertension. Emerging evidence suggests that stimulating sGC inhibits cardiovascular remodeling in a model of hypertension. However, NO can have diverse effects within the nervous system where the spatial localization of the enzyme seems to be important for conferring specificity of action. This has been illustrated recently where NO has been shown to differentially modulate neurotransmission to premotor cardiac vagal neurons in the nucleus ambiguous. Therapeutically the brain is clearly a difficult structure to target with precision gene delivery. A realistic challenge would be to design a gene delivery system that specifically upregulates the NO synthase pathway in cardiac cholinergic ganglia and show that this strategy is effective in restoring neural control. Importantly, can it be demonstrated that this approach has an impact on the etiology of the disease itself.

In conclusion, we have established that a significant component of cardiac vagal impairment in the SHR resides at the end organ level because of abnormal NO-cGMP signaling in intracardiac ganglia. In a proof-of-principle study, nNOS gene transfer into these ganglia restores the neural phenotype by facilitating the release of ACh and improving cardiac parasympathetic function in vivo.

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Disclosures
None.

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