Remodeling of the Cardiac Pacemaker L-Type Calcium Current and Its β-Adrenergic Responsiveness in Hypertension After Neuronal NO Synthase Gene Transfer

Daniel A. Heaton, Ming Lei, Dan Li, Simon Golding, Tom A. Dawson, Ravi M. Mohan, David J. Paterson

Abstract—Hypertension is associated with abnormal neurohumoral activation. We tested the hypothesis that β-adrenergic hyperresponsiveness in the sinoatrial node (SAN) of the spontaneously hypertensive rat occurs at the level of the L-type calcium current because of altered cyclic nucleotide-dependent signaling. Furthermore, we hypothesized that NO, a modulator of cGMP and cAMP, would normalize the β-adrenergic phenotype in the hypertensive rat. Chronotropic responsiveness to norepinephrine (NE), together with production of cAMP and cGMP, was assessed in isolated atrial preparations from age-matched hypertensive and normotensive rats. Right atrial/SAN pacemaking tissue was injected with adenovirus encoding enhanced green fluorescent protein (control vector) or neuronal NO synthase (nNOS). In addition, L-type calcium current was measured in cells isolated from the SAN of transfected animals. Basal levels of cGMP were lower in hypertensive rat atria. These atria were hyperresponsive to NE at all of the concentrations tested, with elevated production of cAMP. This was accompanied by increased basal and norepinephrine-stimulated L-type calcium current. Using enhanced green fluorescent protein, we observed transgene expression within both tissue sections and isolated pacemaking cells. Adenoviral nNOS increased right atrial nNOS protein expression and cGMP content. NE-stimulated cAMP concentration and L-type calcium current were also attenuated by adenoviral nNOS, along with the chronotropic responsiveness to NE in hypertensive rat atria. Decreased calcium current after cardiac nNOS gene transfer contributes to the normalization of β-adrenergic hyperresponsiveness in the SAN from hypertensive rats by modulating cyclic nucleotide signaling. (Hypertension. 2006;48:443-452.)

Key Words: sinoatrial node ■ norepinephrine ■ nitric oxide synthase

Many cardiovascular diseases (eg, heart failure, hypertension, and postmyocardial infarction) are also diseases of the autonomic nervous system. Neurohumoral activation and high levels of circulating catecholamines are negative prognostic indicators for sudden cardiac death and strong independent predictors of mortality.1-4 In hypertension, cardiac sympathetic hyperactivity5-7 and decreased cardiac parasympathetic tone8,9 result in tachycardia and contribute to the pathophysiological phenotype. However, a significant component of the neural impairment may also occur postjuncturally at the level of the β-adrenoceptor, because the arrhythmic action of isoprenaline is enhanced in papillary muscles from the spontaneously hypertensive rat (SHR).10 This correlates with increased basal and isoprenaline-stimulated L-type calcium current (ICaL) in ventricular myocytes from the SHR compared with the normotensive Wistar Kyoto rat (WKY).11 Whether similar events occur in supraventricular tissue has not been established.

It is now widely recognized that reduced bioavailability of NO, secondary to oxidative stress, is important in the pathophysiology of hypertension.12,13 Moreover, downregulation of soluble guanylate cyclase (sGC), a key precursor of cGMP-dependent effects of NO, is observed in the aorta of the SHR.14 Under normal conditions, NO is thought to attenuate myocardial responsiveness to β-adrenergic stimulation15 via activation of cGMP-stimulated phosphodiesterase II and subsequent inhibition of ICaL.16,17 Ventricular myocytes from the neuronal NO synthase (nNOS) knockout mouse show an enhanced inotropic response to β-adrenergic stimulation18 and an associated increase in calcium current density, suggesting that nNOS-derived NO plays a role in the negative feedback modulation of calcium entry.19 This is significant, because nNOS has been localized to the sarcoplasmic reticulum (SR) in ventricular myocardium of rabbits, mice, and humans.20 Data regarding the precise role of nNOS in modulating calcium currents in the...
sinoatrial node (SAN) remain limited. However, evidence link-
ing SR calcium release to the generation of pacemaker rate21–23
and the positive chronotropic action of β-agonists24,25 suggests
that nNOS-mediated regulation of ICaL could modulate the
chronotropic response of the heart to β-adrenergic stimulation.
Moreover, recent work has reported high levels of cAMP–
protein kinase A (PKA)-mediated phosphorylation of calcium
channels in SAN cells.26 This further underscores the important
role that cyclic nucleotides and calcium may play in the
β-adrenergic control of pacemaking rate.

We tested the hypothesis that impaired NO–cyclic nucle-
otide signaling caused by hypertension removes a “brake” on
the β-adrenergic signaling cascade, resulting in hyperrespons-
siveness to norepinephrine (NE) via cAMP-mediated activa-
tion of ICaL. From this we further hypothesized that gene
transfer of nNOS to the pacemaker region would enhance
local expression of nNOS protein and production of cGMP,
thus leading to the normalization of heart rate responsiveness
to NE via attenuation of cAMP-mediated activation of ICaL.

Methods
An extended methods and results section can be found in the online
data supplement available at http://hyper.ahajournals.org.

Animals
Age-matched (20- to 24-week-old) male SHRs and WKYs were
purchased from Harlan UK (Bicester, Oxon, United Kingdom). All
of the animal procedures were performed in accordance with national
and institutional guidelines.

Gene Transfer
Replication-deficient adenoviral vectors encoding recombinant enhanced
green fluorescent protein (eGFP) or nNOS under control of the
cytomegalovirus promoter were generated as described previously.27,28
Targeted percutaneous gene transfer to the right atrium was performed
under halothane (Fluothane, Concord Pharmaceuticals Ltd) or isoflurane
(Iscare, Animalcare Ltd) anesthesia (4% for induction and 2% to 3%
for maintenance in 100% O2), using a technique similar to that described
previously for the guinea pig.29 Animals received an injection of 5 × 109
particles of adeno-nNOS (Ad.LnNOS) or adeno-eGFP (Ad.eGFP) in 300
µL of PBS. Molecular and physiological phenotyping was performed
5 days after injection.

Molecular Phenotyping
Western blotting for nNOS in right atria was performed using
standard techniques as described previously.29 In addition, fluores-
cence microscopy was used to assess expression of eGFP in tissue
sections (stained with hematoxylin/eosin) and isolated cells using a
Leica DC Camera mounted on a Leica DMIRB inverted microscope
in conjunction with Leica TWAIN software. Expression of native
nNOS, endothelial NO synthase (eNOS) monoclonal mouse anti-
sera, Transduction Laboratories), and the α1 and β subunits of sGC
(polyclonal rabbit antisera, Cayman Chemical) was compared in
right atria and aorta from untreated SHRs and WKYs. Tetrahydro-
biopterin (BH4) and total biopterin (BT) were measured by high
performance liquid chromatography as described previously.30

Physiological Phenotyping
Isolated Atrial Preparation
Chronic responsiveness to NE was assessed using an isolated
double-atrial preparation; this allowed us to study the peripheral
β-adrenergic phenotype in the absence of central autonomic input
and circulating neurohumoral factors. Details may be found in the
online data supplement.

Perfused Beating Atrial Preparation
Isolated, perfused, beating atria were prepared using methods de-
scribed previously with minor modifications. Details may be
found in the online data supplement.

Measurement of Extracellular Fluid Translocation
The amount of [1H]inulin in samples of atrial perfusate was
measured with a liquid scintillation counter (Tri-carb 2800TR, Packard),
and the amount of extracellular fluid (ECF) translocated through the atrial
wall was calculated as follows:

ECF translocated (microliters per minute per gram of atrial wet
weight) = total radioactivity in the perfusate (counts per minute per
minute) × 1000/radioactivity in the organ bath solution (counts per
minute per microliter) × atrial wet weight (milligrams).

Radioimmunoassay of cAMP and cGMP
The amount of cAMP and cGMP present in samples of perfusate was
determined using a 125I-cAMP and 125I-cGMP radioimmunoassay kit
(Amersham UK) after the bound form was separated from the free
form by magnetic separation. The amount of cAMP (or cGMP) efflux
was expressed as picomoles of cAMP (or cGMP) per minute
per gram of atrial tissue. The molar concentration of cAMP (or
cGMP) in the interstitial space fluid was calculated as:

cAMP (or cGMP) efflux concentration (nanomoles) = cAMP (or
cGMP) (picomoles per minute per gram) × 1000/ECF translocated
(microliters per minute per gram).

Figure 1. A, Representative raw data traces and I-V curves (nor-
malized to cell capacitance, Cm) for ICaL recorded from isolated
SAN cells from SHR and WKY under basal conditions and after
2-minute exposure to 2 µmol/L of NE. B, Data showing peak
ICaL normalized to cell capacitance in the WKY (n = 5) and SHR
(n = 5). Both basal and NE-stimulated ICaL are significantly
increased in the SHR relative to the WKY (P < 0.05, unpaired t
test).
Measurement of Tissue cGMP Levels

For cGMP measurements, atria were snap-frozen in liquid nitrogen. The tissue was minced in 800 μL of ice-cold trichloroacetic acid (6%) and homogenized at 4°C. The homogenates were centrifuged at 1000 g for 10 minutes at 4°C, and the supernatant was extracted with water-saturated ether 3 times and then dried using a SpeedVac concentrator (Savant). The dried samples were resuspended, and a 125I-cGMP radioimmunoassay kit (Amersham UK) was used to measure the amount of cGMP after the bound form was separated from the free form by magnetic separation. The pellet was treated with 500 μL of NaOH (1 N) and used for protein determination. The results are expressed in picomoles per milligram of protein.

Single Cell Experiments

Individual SAN cells were isolated from right atria of the SHR and WKY based on methods described previously36 with some modifications (see Methods in the online supplement).

Cells were placed in a 400-μL superfusion chamber on the stage of an inverted microscope; the base of the chamber was coated with poly-L-lysine to aid adherence of viable cells. Patch clamping was performed using the amphotericin-perforated patch technique to minimize intracellular dialysis (amphotericin, Sigma, 200 μg/mL added to the pipette solution). During current measurements, cells were superfused with Tyrode solution at a rate of ~1 mL/min at 37°C. An Axopatch-200B patch clamp amplifier was used for voltage clamping, and I_{Ca,L} was obtained by voltage clamp steps of 200-ms duration from a −50 mV holding potential to test potentials between −40 and +50 mV. This protocol was designed to minimize contamination of our recordings by the transient-type calcium current (I_{Ca,T}), because I_{Ca,T} is almost completely inactivated at −40 mV.37,38 Furthermore, some experiments were performed in the presence of 20 μmol/L of tetrodotoxin (Calbiochem) to eliminate possible contamination by the fast-type sodium channel; application of tetrodotoxin did not affect our recordings of I_{Ca,L}. Peak currents (normalized to cell capacitance) were measured before and after stimulation with 2.0 μmol/L of NE (a concentration chosen to near-maximally activate I_{Ca,L} based on our concentration-response curves to NE in isolated atria).

Solutions

Rat Ringer’s solution contained (in mM): NaCl, 120; KCl, 4.7; KH₂PO₄, 1.2; MgSO₄, 1.2; glucose, 11; NaHCO₃, 25; and CaCl₂, 1.8. The solution was constantly bubbled with 95% O₂/5% CO₂ to maintain pH at 7.4.

Calcium-free Tyrode solution contained (in mM): NaCl, 130; KCl, 5.4; MgCl₂, 3.5; NaH₂PO₄, 0.4; glucose, 10; taurine, 20; and HEPES, 5 titrated to pH 7.4 with NaOH and bubbled with 100% O₂. Tyrode solution used during Ca²⁺ current recordings contained 1.8 mmol/L of CaCl₂.

KB solution contained (in mM): KCl, 25; EGTA, 0.5; MgCl₂, 3; L-glutamic acid, 80; taurine, 20; KH₂PO₄, 10; glucose, 10; and HEPES, 10 titrated to pH 7.4 with KOH.
The pipette solution used for recordings of ICaL contained (in mM): KCl, 140; MgSO4, 1.8; HEPES, 5; and EGTA, 0.1 titrated to pH 7.3 with KOH.

**Data Analysis**
All of the data passed normality and equal variance tests. Analysis was performed using the paired or unpaired Student t test as appropriate, and significance was accepted at P<0.05. Results are presented as mean±SE.

**Results**

**Phenotype of the SHR**

**Molecular**
Hearts from the SHR were significantly hypertrophied compared with the WKY (ventricular weight: body weight ratios of 3.2×10⁻³±8.5×10⁻³ [SHR, n=7] versus 2.5×10⁻³±3.2×10⁻³ [WKY, n=6]; P<0.001, unpaired t test). We compared the expression of native nNOS, eNOS, and sGC (α1 and β1 subunits) proteins in right atria from untreated SHRs and WKYS. Expression of nNOS, eNOS, and the β1 subunit of sGC was not different between the 2 strains (supplemental Table I, available at http://hyper.ahajournals.org). However, expression of the α1 subunit in both aorta and atria was significantly lower in the SHR (P<0.05, see supplemental Figure I, available at http://hyper.ahajournals.org). To assess whether NOS might be uncoupled in the SHR, we also compared levels of BH4 and BT in atrial tissue from SHRs and WKYS. Our data indicate no difference in levels of either BH4 or bioperin between the 2 groups (supplemental Table I).

**Basal and Adrenergically Stimulated ICaL**
SAN cells from the SHR (n=5) showed increased peak amplitude of both basal (22.9±5.4 versus 9.6±1.9 pico Amp/pico Faraday [pA/pF]; P<0.05) and NE-stimulated ICaL (48.0±9.6 versus 20.9±4.7 pA/pF; P<0.05) relative to the WKY (n=5; Figure 1). This suggests that the increase in calcium current density noted previously in the ventricle is accompanied by a similar increase within the cardiac pacemaker.

**Heart Rate Responsiveness to NE**
Basal heart rate was not significantly different between atrial preparations from Ad.eGFP-transfected SHRs (234±6 bpm; n=20) and WKYS (243±4 bpm; n=7). However, a comparison of dose-response curves to NE indicated that SHR atria were hyperresponsive to β-adrenergic stimulation at all of the concentrations tested (eg, 0.1 μmol/L: 66±7 bpm versus 25±5 bpm [WKY]; P<0.01; see Figure 2).

**Right Atrial Production of cAMP and cGMP**
In perfused beating atria, basal concentrations of both cyclic nucleotides and heart rate were not different between the 2 strains. SHR atria (n=6) displayed significantly increased cAMP efflux concentration (eg, in 20 minutes: 56.2±2.4 versus 44.5±3.2 nM; P<0.05; relative to the WKY [n=5]; Figure 3A) and heart rate (eg, in 20 minutes: 270±19 versus 226±4 bpm [WKY]; P<0.05; Figure 3B) in response to NE administration. No detectable difference in cGMP efflux concentration was observed between the WKY and SHR (data not shown). However, tissue levels of cGMP were significantly lower in the SHR compared with the WKY (P<0.05; Figure 3C).

**Effect of nNOS Gene Transfer**

**Molecular**
Fluorescence microscopy detected cGFP expression in both SAN sections and isolated SAN myocytes from Ad.eGFP transfected SHRs (Figure 4A through 4G), confirming transgene expression within pacemaking tissue. Furthermore, Western blotting showed increased nNOS expression in Ad.nNOS-transfected atria (Figure 4E) compared with atria transfected with Ad.eGFP; this confirms our previous results in the guinea pig where the same percutaneous injection method was used.

**Basal and Adrenergically Stimulated ICaL**
Basal ICaL in cells from the SHR was unaffected by nNOS gene transfer (see Figure 5A for representative examples). However, the NE-induced increase in ICaL was significantly attenuated (Figure 5B; 35.7±6.5% [n=6] versus +122.4±20.2% [n=5]; P<0.01). Furthermore, nNOS gene transfer abolished the difference in NE-stimulated ICaL between the WKY and SHR groups. This indicates that increased nNOS expression blunts β-adrenergic hyperresponsiveness via an autocrine mechanism involving attenuation of NE-stimulated ICaL.

**Heart Rate Responsiveness to NE**
Right atrial transfection with Ad.nNOS (n=8) significantly attenuated chronotropic responsiveness of SHR atria to NE.
relative to the Ad.eGFP group (n=20; eg, 0.1 μmol/L: 22±8 versus 66±7 bpm; \( P<0.001 \)) and normalized heart rate responsiveness of the SHR relative to the WKY (Figure 6A). This effect was partially reversed by NOS inhibition at NE concentrations ≥0.5 μmol/L (supplemental Table II), suggesting that NO-independent pathways may also contribute to modulation of I_{Cal} and HR after Ad.nNOS gene transfer. Interestingly, only the response to 0.1 μmol/L of NE was significantly attenuated after nNOS gene transfer in the WKY (Figure 6B), indicating that NO may play only a relatively minor role in the normotensive animal. Basal heart rate was unaffected by nNOS gene transfer in both SHR (248±4 bpm of [Ad.nNOS] versus 234±6 bpm [Ad.eGFP]) and WKY (249±4 bpm [Ad.nNOS; n=11] versus 243±4 [Ad.eGFP; n=7]) atria.

Right Atrial Production of cAMP and cGMP
Basal cAMP levels were significantly greater than cGMP levels. However, basal concentrations of both cyclic nucleotides and heart rate were unaffected by right atrial transfection with Ad.nNOS in both the SHR and WKY. Transfection with Ad.nNOS significantly attenuated cAMP efflux concentration and heart rate in both WKY (n=5) and SHR (n=7) atria during perfusion with NE (0.3 μmol/L; WKY, n=5; SHR, n=7; see Figure 7). cGMP efflux concentration was unaffected by Ad.nNOS gene transfer (data are not shown) in either group and probably reflects the lack of sensitivity of the technique. In contrast, the tissue levels of cGMP were significantly increased after transfection with Ad.nNOS compared with Ad.eGFP in both strains (n=6 in each group; \( P<0.05 \)). However, tissue levels of cGMP were still signifi-
cantly less in the SHR compared with the WKY after Ad.nNOS gene transfer (Figure 8).

Discussion
The novel findings presented here are threefold. First, the SHR displays hyperresponsiveness to β-adrenergic stimulation at the level of the SAN (in the absence of central autonomic input), suggesting that a significant component of the hypertensive adrenergic phenotype resides at the end-organ level. Second, a significant component of this hyperresponsiveness results from an elevated density of ICaL within the SAN, because we have shown increased ICaL under both basal and NE-stimulated conditions in isolated cells from the SHR. This was also accompanied by elevated production of atrial cAMP and reduced atrial tissue levels of cGMP. Third, nNOS gene transfer normalizes β-adrenergic responsiveness of the SHR by decreasing the NE-induced increase in cAMP-dependent modulation of ICaL.

NO Signaling in the Right Atrium of the SHR
We compared expression of native nNOS, eNOS, and sGC in the SHR and WKY in an attempt to determine whether hypertension is associated with a disruption of NO–cGMP-dependent signaling pathways within the right atrium. Western blotting indicated no difference in right atrial expression of nNOS or eNOS between the SHR and WKY. However, greater oxidative stress in hypertension and a reduction in the bioavailability of NO (by reaction with O₂⁻ to form ONOO⁻), may occur even in the absence of changes in NO synthase protein expression. Furthermore, “uncoupling” of the enzyme, leading to a switch from production of NO to O₂⁻, may occur if cofactors such as BH₄ are depleted. However, we observed that levels of BH₄ and BT were normal in atria from the SHR indicating that uncoupling of nNOS is unlikely. In contrast, decreased expression of the ₁ subunit of sGC in the right atrium of the SHR suggests that downstream signaling via NO–cGMP-dependent pathways is disrupted in hypertension, as first shown in the aorta.

Calcium Currents and Heart Rate Responsiveness to NE in the SHR
The measurements of ICaL presented here are, to our knowledge, the first recordings in SAN myocytes isolated from the SHR. Cell isolation from the SHR SAN produced only a very low yield of cells that were often fragile and difficult to patch.
For these reasons, we were unable to make longer-term current recordings to test the effect of NO synthase inhibition. Nevertheless, we consistently observed a greater ICaL in the SHR under basal conditions and during acute exposure to NE. Tissue cGMP was also significantly less in the SHR, and levels of cAMP were significantly elevated during ampere-stimulated ICaL and cAMP concentration in the SHR. We could detect no change in cGMP efflux concentration between strains or within strains after gene transfer of Ad.nNOS or Ad.eGFP. The low basal levels of cGMP relative to cAMP probably reflect the poor sensitivity of our measurement system. Therefore, the possibility exists that a nonmeasurable change did occur to increase phosphodiesterase (PDE)2-mediated breakdown of cAMP, or, alternatively, nNOS gene transfer interfered in some other way with β-receptor–coupled adenylate cyclase signaling. In contrast, we observed that tissue levels of cGMP were significantly less in the SHR compared with the WKY and that gene transfer of nNOS increases tissue levels of cGMP under basal conditions in both strains. No changes in basal ICaL and cAMP concentration were observed after transfection with Ad.nNOS. This concurs with results in isolated atria showing no difference in basal heart rate between Ad.eGFP- and Ad.nNOS-treated preparations and suggests that NO-mediated regulation of ICaL may be significant only during β-adrenergic stimulation. Furthermore, the lack of difference between baseline heart rate in atrial preparations from the SHR and WKY, in spite of differences in basal ICaL, suggests that the contribution of the L-type calcium current to the determination of basal pacemaking rate may be rather less important than the contribution of this current to adrenergically induced tachycardia.

Our voltage clamp protocol was designed to minimize any contamination by the ICaT. Furthermore, we suggest that this current is unlikely to be important during β-adrenergic stimulation, because its amplitude is unaffected by isoproterenol.37,38 Previous work suggests that the effect of β-agonists on the L-type current is mediated predominantly by an increase in the amplitude of the current, rather than a change in its kinetics.37 Evidence indicates that release of calcium from the SR also plays an important role in pacemaking, because pharmacological interventions that deplete the SR calcium store slow the rate of the diastolic depolarization and increase cycle length in pacemaker cells.21–23 The mechanism involved seems to involve highly localized SR calcium release “sparks,” occurring during the final third of the diastolic depolarization.41 Adrenergic stimulation increases SR Ca2+ load,24,42 and increases the frequency of calcium sparks,43 although it is not known whether NO modulates calcium sparks in pacemaking cells.

How Does NO Decrease Responsiveness of the SHR to NE?
NO synthase inhibition potentiates the tachycardia produced by sympathetically derived NE or cAMP analogs, suggesting that NO is involved in a inhibitory regulation of heart rate.44 Evidence indicates cGMP-dependent activation of PDE2,17,45 activation of protein kinase G,46 and/or cGMP-independent S-nitrosylation47 as candidate mechanisms. Data showing downregulated expression of sGC in the right atrium and only partial reversal of the effects of Ad.nNOS by L nitro arginine suggest that S-nitrosylation may be an important contributor toward nNOS-induced attenuation of ICaL in the SHR (because it is difficult to imagine that this longer-term modulation of the channel protein would be reversed by acute inhibition of nNOS). Moreover, it is possible that the effects of nNOS-derived NO may occur as a result of the reaction with superoxide (O2) to form highly reactive peroxynitrite (ONOO-), particularly in the hypertensive animal where oxidative stress is increased.40

Other Pacemaking Currents Modulated by β-Stimulation
It is important to note that other pacemaker currents, in addition to ICaL, are modulated by the β-adrenoceptor signaling cascade. For example, a role for the hyperpolarization-activated inward current (Ih) in the positive chronotropic action of β-agonists is now well established.48 However,
pharmacological evidence suggests that the normalization of \( \beta \)-adrenergic responsiveness in the SHR by nNOS gene transfer is unlikely to be mediated by actions of NO on If, because under basal conditions, NO donors induce an If-dependent tachycardia that is maintained during \( \beta \)-adrenergic stimulation.

**Perspectives**

The overall physiological significance of these observations remains to be established. In particular, does gene transfer of nNOS to pacemaking tissue suppress chronotropic hyperresponsiveness to sympathetic activation in intact hypertensive animals? Because basal chronotropic responses were unaltered, we speculate that such a strategy warrants further development. Moreover, the challenge of a gene transfer strategy will be to design a vector that targets only pacemaking cells. The precise signal transduction pathway involving nNOS cannot be ascertained from this study. We hypothesize that nNOS-derived NO may normalize \( \beta \)-adrenergically mediated activation of ICaL in the SHR by attenuating cAMP–PKA-mediated phosphorylation of the calcium channel. One potential mechanism may be via the cGMP-activated PDE2, which decreases cAMP levels. Although we do not report NOS activity levels after gene transfer, indirect evidence suggests that they were increased. Specifically, we observed that cGMP levels were significantly raised in both SHR and WKY after nNOS gene transfer, with the response in the SHR being marginally less, because it had a lower starting value because of the disease state (Figure 8). It is interesting to note that a stable analog of cAMP produces a tachycardia that is enhanced in the presence of NOS inhibition; this agrees with our findings and suggests that cAMP–PKA signaling within the SAN is subject to an inhibitory regulation by NO. Whether gene transfer of nNOS directly enhances sGC or PDE2 to affect PKA modulation of ICaL has not been established.

**Conclusions**

We have established in a proof-of-principle study that nNOS gene transfer to the SAN of the SHR decreases \( \beta \)-adrenergic hyperresponsiveness of cardiac rate in vitro by modulating cyclic nucleotide regulation of the pacemaker current ICaL.

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Disclosures

None.

References


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