Sympathetic control of heart rate in nNOS knockout mice

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Choate JK, Murphy SM, Feldman R, Anderson CR. Sympathetic control of heart rate in nNOS knockout mice. Am J Physiol Heart Circ Physiol 294: H354–H361, 2008. First published October 19, 2007; doi:10.1152/ajpheart.00898.2007.—Inhibition of neuronal nitric oxide synthase (nNOS) in cardiac postganglionic sympathetic neurons leads to enhanced cardiac sympathetic responsiveness in normal animals, as well as in animal models of cardiovascular diseases. We used isolated atria from mice with selective genetic disruption of nNOS (nNOS−/−) and their wild-type littermates (WT) to investigate whether sympathetic heart rate (HR) responses were dependent on nNOS. Immunohistochemistry was initially used to determine the presence of nNOS in sympathetic [tyrosine hydroxylase (TH) immunoreactive] nerve terminals in the mouse sinoatrial node (SAN). After this, the effects of postganglionic sympathetic nerve stimulation (1–10 Hz) and bath-applied norepinephrine (NE; 10−8−10−4 mol/l) on HR were examined in atria from nNOS−/− and WT mice. In the SAN region of WT mice, TH and nNOS immunoreactivity was virtually never colocalized in nerve fibers. nNOS−/− atria showed significantly reduced HR responses to sympathetic nerve activation and NE (P < 0.05). Similarly, the positive chronotropic response to the adenylate cyclase activator forskolin (10−7−10−5 mol/l) was attenuated in nNOS−/− atria (P < 0.05). Constitutive NOS inhibition with l-nitroarginine (0.1 mmol/l) did not affect the sympathetic HR responses in nNOS−/− and WT atria. The paucity of nNOS in the sympathetic innervation of the mouse SAN, in addition to the attenuated HR responses to neuronal and applied NE, indicates that presynaptic sympathetic neuronal NO does not modulate neuronal NE release and SAN pacemaking in this species. It appears that genetic deletion of nNOS results in the inhibition of adrenergic-adenylate cyclase signaling within SAN myocytes.

neural nitric oxide synthase; autonomic nervous system; sinoatrial node

CARDIAC SYMPATHETIC NERVE activation causes the release of the neurotransmitter norepinephrine (NE), which then interacts with sinoatrial node (SAN) β-adrenoceptors. This leads to the activation of intracellular signaling pathways that modulate ion channel function, resulting in an increase in heart rate (HR). Putatively selective pharmacological inhibition of neuronal nitric oxide synthase (nNOS or NOS1) enhances the positive chronotropic response to sympathetic nerve activation but not to β-adrenoceptor agonists (6, 22, 27). This raises the possibility that nitric oxide (NO), synthesized via nNOS in cardiac sympathetic nerves during nerve activation, inhibits sympathetic NE release and thus the postsynaptic cardiac response. Support for this idea comes from a report (26) that nNOS is colocalized in many nerve fibers in the rat atrium, which also contain tyrosine hydroxylase (TH), an enzyme involved in NE synthesis. A recent study (30) has also used an adenoviral vector with a promoter selective for noradrenergic (TH immuno-reactive) neurons to drive nNOS coupled to enhanced green fluorescence protein gene expression. In this model, overexpression of nNOS by sympathetic neurons reduced NE overflow from rat atria with field stimulation. Furthermore, in the isolated rabbit heart, the overflow of NE with sympathetic nerve activation is enhanced by constitutive NOS inhibition (26). However, NO synthesized via nNOS may also modulate postsynaptic cardiac responses. Constitutive NOS inhibition enhances the effects of ansae subclaviae stimulation and infused isoprenaline on spontaneous sinus cycle length in the anesthetized dog (10). Conversely, nNOS-derived NO could lead to enhanced cardiac responses to sympathetic activation; NO donors inhibit the reuptake of NE by cultured sympathetic neurons (18, 19). If this phenomenon also occurs in vivo, the increased levels of NE in the neuroeffector junction would enhance cardiac responses to sympathetic nerve activation.

The inconsistencies between studies investigating the role of nNOS on cardiac sympathetic function may result from the lack of specificity of pharmacological NOS inhibitors for the nNOS isoform. NOS inhibitors may also have additional actions in vivo on vascular resistance and baroreflex activation. Therefore, the aims of this study were twofold. First, to establish if nNOS was localized in postganglionic sympathetic nerve fibers innervating the mouse SAN. Second, to determine if the sympathetic control of HR was enhanced in isolated atria from nNOS−/− mice compared with atria from their wild-type (WT or nNOS+/+) littermates.

MATERIALS AND METHODS

Animals. Experimental animals were male and 3–4 mo old. The treatment of all animals was in accordance with the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th ed., 2004) and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All experimental protocols were approved by the Monash University Department of Physiology Animal Ethics Committee. Mice homozygous for targeted disruption of the nNOS gene (B6.129-NOS1tm1ph, nNOS−/−; Ref. 14) were purchased from Jackson Laboratories (Bar Harbor, ME), and a colony was established by backcrossing the nNOS−/− onto a C57BL/6 background for at least six generations. Animals were genotyped from tail clippings taken at day 20 (see Ref. 7 for genotyping methods). Age-matched littermate mice homozygous for the nNOS gene (nNOS+/+) were used as controls.

Localization of nNOS and TH immunoreactivity in WT and nNOS−/− atria. Animals (3 WT and 3 nNOS−/−) were anesthetized with pentobarbital sodium (60 mg/kg ip) and perfused with 0.9% saline followed by 2% formaldehyde in 0.1 mol/l sodium phosphate buffer (pH 7.4). Since we were interested in whether nNOS was found in sympathetic nerves that modulate HR, we fixed the SAN region of...
the mouse right atrium; the region between the inferior and superior vena cava, the interatrial septum, and the right atrium was dissected free and cryoprotected overnight in 20% sucrose in 0.1 mol/l sodium PBS (pH 7.4). Tissue was then embedded in Optimal Cutting Temperature embedding compound (Sakura Finetechanical, Tokyo, Japan) and frozen in isopentane cooled with liquid nitrogen. Sections were cut 12-μm thick on a cryostat and collected serially on gelatinized slides.

Sections were incubated over night in droplets of primary antibodies, diluted in 0.01 mol/l PBS containing 0.3% Triton X-100 and 0.1% sodium azide. Immunofluorescent single- and double-labeling was performed, using antibodies specific for TH and nNOS. Primary antibodies were as follows: nNOS (raised in sheep, 1:4,000 dilution, gift of P. Emerson; Ref. 32), nNOS (raised in rabbit, 1:500 dilution, gift of B. S. Masters; Ref. 33), and TH (raised in rabbit, 1:200 dilution, Eugene Tech, NJ). Sections were then washed three times, for 5 min each wash, in PBS, before droplets of secondary antibodies (detailed below) were applied for 1 h. After this incubation, the sections were washed again in PBS before being mounted under coverslips in Dako Fluorescent Mounting Medium (Dako Australia, Botany, New South Wales). When we performed double-labeling immunofluorescence, the primary antibodies were raised in different species. The secondary antibodies used were donkey anti-rabbit immunoglobulin G (IgG) conjugated to Texas red (Jackson Immunoresearch, West Grove, PA) at 1:150 to detect rabbit anti-nNOS and rabbit anti-TH, and donkey anti-sheep IgG conjugated to fluorescein isothiocyanate (Jackson) at 1:100 to detect sheep anti-nNOS. A different fluorophore was used to detect each of the primary antibodies in the double-labeling combinations.

Isolated mouse atrial preparation with intact sympathetic postganglionic innervation. WT mice (n = 58; body wt = 25.1 ± 1.4 g) and nNOS−/− mice (n = 57; body wt = 24.2 ± 1.5 g) were killed by cervical dislocation. The thorax and mediastinum were removed from the body and placed in mouse physiological saline solution [contain- ing (in mmol/l) 118 NaCl, 4.7 KCl, 1.2 MgSO4, 0.5 Na2EDTA, 1.2 NaH2PO4, 25 NaHCO3, 11 glucose, and 1.75 CaCl2; pH 7.4] gassed with carbogen (95% O2-5% CO2) at room temperature (~22°C). The atria, together with the right stellate ganglion and its neuronal con- nections to the SAN, were dissected free and placed into an organ bath (3 ml vol) that was maintained at 37 ± 0.1°C. For further details on this dissection and the experimental setup, see Choa and Feldman (8). The spontaneously beating preparation was left (45–90 min) to equilibrate until the HR altered by ≤10 beats/min over 20 min. After an initial measurement of HR, atropine (1 μmol/l) was added to the organ bath to eliminate any potential effects of muscarinic receptor activation due to the spontaneous activity of parasympathetic nerves. The stellate ganglion was attached to a pair of platinum electrodes connected to an isolated stimulator. The ganglia were stimulated at 10 V, 1-ms pulse width, at 1, 3, 5, and 10 Hz (random order) for 30 s at 2- to 3-min intervals. Experiments involving bath-applied drugs were done on isolated double atiral preparations.

The effects of the following were examined on HR in nNOS−/− and WT atria: 1) right stellate ganglion stimulation (three times each at 1, 3, 5 and 10 Hz); 2) the cumulative addition of bath-applied NE (10−8–10−4 mol/l); 3) the β1-adrenoceptor antagonist atenolol (1 μmol/l) on the HR responses to sympathetic nerve stimulation (SNS; 5 Hz) and NE (1 μmol/l); 4) the inhibitor of constitutive NOS isoforms, l-nitroarginine (l-NA, 100 μmol/l) and then l-arginine (1 mmol/l; both drugs equilibrated for 20 min) on the HR responses to SNS (1, 3, 5, and 10 Hz); 5) the NO donor sodium nitroprusside (SNP, 10 μmol/l) on the HR responses to SNS (1, 3, 5, and 10 Hz); 6) the cumulative addition of bath-applied forskolin (10−7–10−5 mol/l); and 7) an inhibitor of the hyperpolarization-activated Ic current Cs+ (1 mM) on the HR responses to NE (1 μmol/l).

Data analysis and statistics. All of the HR responses were calculated as both actual changes in HR (ΔHR) and as percent increases from the baseline HR. For all preparations, three HR responses to SNS (or bath-applied NE) were obtained at each stimulation frequency (or NE dose). The magnitude of these responses was calculated as the difference between the baseline HR (averaged over 3 s immediately before SNS or the addition of NE) and the maximum response to SNS (or NE, averaged over 3 s). The three responses at each stimulation frequency (or NE dose) were averaged.

SSPS software (version 12) was used to perform either two-factor or repeated measures ANOVA (with the Student-Newman-Keuls test applied for post hoc analysis when a significance level of P < 0.05 was obtained) or unpaired/paired Student’s t-tests, where appropriate. For all statistical tests, P < 0.05 was accepted as being statistically significant. Since the statistical analysis of the data as both ΔHR and percent increase in HR produced the same results, all of the data in the results are mean Δ or actual changes in HR (means ± SE).

RESULTS

nNOS and TH immunoreactivity in the mouse SAN region. The presence of nNOS and TH immunoreactivity was first examined in nerve fibers in the myocardium, the cardiac ganglia, and the coronary vasculature. In both WT and nNOS−/− mice, TH-immunoreactive nerve fibers, presumably sympathetic axons, were numerous throughout the right atrial myocardium (see Fig. 1A). As expected, no nNOS immunoreactivity was observed in tissue from nNOS−/− mice. In WT atria, nNOS-immunoreactive nerve fibers were present but uncommon. While most TH-immunoreactive fibers did not show colocalized nNOS immunoreactivity, a few fibers were immunoreactive for both TH and nNOS (Fig. 1, B and C). TH-immunoreactive nerve fibers were rarely seen within intrinsic cardiac ganglia, and none of these contained colocalized nNOS immunoreactivity. Neuronal NOS immunoreactivity was also not observed in TH-immunoreactive nerve fibers associated with the adventitia of the coronary vessels, but note that nerves present in these locations do not necessarily innervate the coronary vasculature; they may be fibers in passage to other parts of the myocardium.

The presence of nNOS and TH immunoreactivity was also examined in the nerve cell bodies that were associated with the atria. We estimated that of all the nerve cell bodies within the cardiac ganglia <5% were TH immunoreactive. Of these, only a few also expressed nNOS immunoreactivity (Fig. 1, D and E). A number of small, intensely TH immunoreactive, cells were observed in small clusters, or as isolated cells, in the myocardium (see Fig. 1F). These cells lacked axons and are likely to be catecholamine-synthesizing, small intensely fluorescent cells (i.e., SIF cells; see Ref. 29). A subset of these cells was immunoreactive for nNOS.

HR responses to SNS and bath-applied NE in nNOS−/− and WT atria. The rate of spontaneous beating was higher in atria from nNOS−/− mice (446 ± 13 beats/min; n = 57) than in atria from WT mice (372 ± 7 beats/min; n = 58; unpaired t-test, P = 0.011). However, the HR responses to right stellate ganglion stimulation were significantly smaller in atria from WT mice (n = 11) than in atria from WT mice (n = 8) at stimulation frequencies of 3, 5, and 10 Hz (ANOVA, P = 0.022; Fig. 2, A and B). In a separate series of atrial prepara- tions (n = 12 WT, n = 10 nNOS−/−), the HR responses to the cumulative addition of bath-applied NE (10−8–10−4 mol/l) were also significantly attenuated in nNOS−/− atria (ANOVA, P = 0.017; Fig. 2C).

To determine if the increase in HR after right stellate ganglion stimulation was due to the activation of β1-adreno-
ceptors, the effects of the β1-adrenoceptor antagonist atenolol (1 μmol/l) were established on the HR responses to sympathetic activation (5 Hz). Atenolol attenuated the sympathetically mediated HR increases in both the WT and nNOS−/− atria. In WT atria (n = 4), the increase in HR evoked by 5-Hz stellate ganglion stimulation under control conditions was 105 ± 12 beats/min, while after atenolol treatment the change in HR was only 14 ± 3 beats/min (paired t-test, P = 0.002). In nNOS−/− atria (n = 4), 5-Hz stellate ganglion stimulation caused an increase in HR of 58 ± 6 beats/min in control conditions, while after atenolol the increase was only 5 ± 2 beats/min (paired t-test, P = 0.004). Similarly, atenolol attenuated the HR responses to bath-applied NE (1 μmol/l) in atria from eight WT (ΔHR: control = 233 ± 21 beats/min and atenolol = 30 ± 11 beats/min; paired t-test, P = 0.003) and 8 nNOS−/− mice (ΔHR: control = 121 ± 17 beats/min and atenolol = 22 ± 12 beats/min; paired t-test, P = 0.004). Baseline HR was decreased in WT and nNOS−/− atria in the presence of atenolol (1 μmol/l; same atria as for the NE/atenolol protocol); percent decrease in HR with atenolol was −23 ± 4% for WT and −17 ± 3% for nNOS−/− atria (unpaired t-test; P = 0.270).

Effects of constitutive NOS inhibition and increased NO availability on sympathetic HR responses. Equilibration of atrial preparations from WT (n = 6) and nNOS−/− (n = 6) mice with L-NA (100 μmol/l) and then with L-arginine (1 mmol/l) did not alter the sympathetic HR responses (1–10 Hz, see responses at 5 Hz in Fig. 3; ANOVA, WT: P = 0.201 and nNOS−/−: P = 0.532). In contrast, the NO donor SNP (10 μmol/l) significantly reduced the magnitude of the sympathetic HR responses at stimulation frequencies of 1–10 Hz in atria from both WT (n = 7; ANOVA, P = 0.025) and nNOS−/− (n = 5; ANOVA, P = 0.025) mice (see Fig. 4). This reduction in the response was not due to a time-dependent run-down; time controls in a previous study (8) showed no change in the amplitude of the HR response to SNS.

Effects of forskolin on nNOS−/− and WT atrial beating rate. To determine if the attenuated sympathetic HR responses in the nNOS−/− atria resulted from an effect downstream of β1-adrenoceptor/G protein activation, the HR responses to the cumulative addition of the adenylate cyclase activator forskolin (10−7–10−5 mol/l) were examined in nNOS−/− (n = 7) and WT (n = 7) atria. As shown in Fig. 5, the positive chronotropic responses to forskolin were smaller in nNOS−/− atria only at higher doses of forskolin (10−5–10−3 mol/l).

Effect of Cs+ on the NE HR responses. To ascertain if the attenuated sympathetic HR responses in the nNOS−/− atria resulted from reduced activation of Ii, the effect of the Ii inhibitor Cs+ (1 mM) was examined on the HR responses to NE (1 μM) in nNOS−/− (n = 6) and WT (n = 6) atria. Cs+ decreased basal HR to a similar extent in WT (−45 ± 12 beats/min or %decrease of 11.0 ± 2.5%) and nNOS−/− (−41 ± 8 beats/min or %decrease of 9.2 ± 1.6%) atria. In

Fig. 1. Immunohistochemical labeling for tyrosine hydroxylase (TH) and neuronal (n) nitric oxide synthase (NOS) in the right atrium of the wild-type (WT) mouse. Scale bars in A–F = 50 μm: bar in A applies also to B; bar in D applies also to E. A: TH-immunoreactive nerve fibers in the myocardium. B and C: double-labeling immunofluorescence for TH (B) and NOS (C) reveals axons IR for both TH and NOS (indicated by arrows) and nerve fibers immunoreactive for TH alone (*). D and E: double-labeling immunofluorescence shows 2 intrinsic cardiac ganglion cells immunoreactive for TH (*) and another neuron IR for NOS (arrows). F: TH labeling shows a cluster of small intensely fluorescent (SIF) cells (*) and a less intensely IR ganglion cell.
addition, Cs+ reduced the magnitude of the NE HR response, with a larger effect in the WT atria [NE HR response in the presence of Cs+ (expressed as a % decrease from the control NE HR response); WT = -33 ± 5% and nNOS−/− = -16 ± 5% (unpaired t-test, P = 0.028)].

DISCUSSION

This study demonstrated that disruption of nNOS gene expression significantly attenuated the HR responses to SNS (neuronally released NE) and bath-applied NE in isolated mouse atria. Taken together with the paucity of nNOS in cardiac postganglionic sympathetic nerve fibers (i.e., the rare instances of colocalization of TH and nNOS immunoreactivity), these observed differences between nNOS−/− and WT atria appear to depend on processes occurring in effector cells, the cardiac myocytes, rather than in the sympathetic axons innervating the myocytes. These processes were not dependent on constitutive NOS expression, with no effect of L-NA on sympathetic HR responses in either WT or nNOS−/− atria; they involved β1-adrenoceptor activation, as responses to SNS and exogenous NE were similarly attenuated by atenolol in WT atria.
or nNOS\(^{-/-}\) atria; and they were mimicked by adenylate cyclase activation (forskolin). These results support the idea that genetic deletion of nNOS in the mouse results in the inhibition of normal adrenergic-adenylate cyclase signaling within SAN myocytes.

Is nNOS found in the mouse SAN? We found negligible colocalization of nNOS and TH immunoreactivity in nerve fibers and nerve cell bodies in the WT right atrial intercaval region (containing the SAN). It is unlikely that the tiny numbers of nNOS immunoreactive sympathetic nerves would result in NO playing a significant physiological role in the cardiac sympathetic innervation of the mouse right atrium. There is limited immunohistochemical evidence for nNOS in cardiac sympathetic postganglionic nerve terminals. Schwarz et al. (26) reported colocalization of nNOS with TH immunoreactivity in numerous nerve fibers in the rat atria, but Jew et al. (16) reported that the nNOS and TH immunoreactivity were found in separate populations of nerves innervating the rat heart mitral valve.

It is possible that nNOS is present within murine cardiac myocytes but that our immunohistochemical approach was not sensitive enough to detect this nNOS. Electron microscopic studies with immunogold labeling have been used to identify nNOS immunoreactivity on the sarcoplasmic reticulum (SR) in sections of mouse myocardium (3), as well as in isolated mouse and rabbit ventricular SR membrane vesicles (31). Furthermore, coimmunoprecipitation of proteins has shown that nNOS is specifically colocalized with ryanodine receptors and the Ca\(^{2+}\) ATPase on the mouse cardiac SR (1, 3). nNOS immunoreactivity has also been reported in the sarcolemma of atrial myocytes from the cardiac conducting system (rat and mouse) but not in normal cardiac myocytes, supporting the idea that mouse SAN myocytes contain nNOS (21, 24).

Why do nNOS\(^{-/-}\) atria have attenuated adrenergic HR responses? Since nNOS\(^{-/-}\) atria had an attenuated sympathetic HR response (compared with WT atria), we expected pharmacological NOS inhibition to attenuate the WT sympathetic HR response. Contrary to this expectation, L-NA had no effect on this WT response. Danson et al. (9) also found that L-NA or genetic disruption of endothelial NOS (eNOS\(^{-/-}\)) did not alter the increase in HR with isoprenaline in C57BL/6 (WT) atria, supporting the idea that adrenergic HR responses in the mouse atria do not involve constitutive NOS activation. In contrast, our previous work (22, 27) in the anesthetized rabbit and isolated guinea-pig atria found that pharmacological nNOS inhibition enhanced sympathetic HR responses but not HR responses to \(\beta\)-agonists. It is possible that there are differences in the cardiac expression and localization of nNOS in mammalian species. In the guinea-pig, rat, and rabbit heart, NO synthesized via nNOS appears to inhibit the presynaptic release of NE from cardiac sympathetic nerve terminals, leading to an attenuated sympathetic HR response (22, 26, 27). In the rat atria, nNOS is colocalized with TH immunoreactivity in numerous nerve fibers; in addition, L-NA increases NE overflow during cardiac SNS in isolated rat hearts (26). Furthermore,
recent studies (20, 30) in rat atria used an adenoviral vector with a promoter selective for TH-immunoreactive neurons to drive nNOS coupled to enhanced green fluorescent protein gene expression. In this model, nNOS overexpression attenuated NE overflow with field stimulation, an effect that was reversed with l-NA (30). We found little immunohistochemical evidence of nNOS in sympathetic nerves in the mouse SAN. In addition, the sympathetic and HE HR responses were both attenuated in nNOS−/− atria and l-NA did not alter the WT or nNOS−/− sympathetic HR responses. These results support the idea that the attenuated nNOS−/− sympathetic response results from a postsynaptic NOS-independent pathway.

The sympathetic control of HR has been indirectly examined in anesthetized nNOS−/− and WT mice. The reflex increase in HR after the drop in blood pressure with injection of SNP was significantly attenuated in nNOS−/− relative to WT mice (5). In addition to its vasodilator effect to lower blood pressure, SNP elevates HR via direct activation of the hyperpolarization-activated pacemaker current (Ih) and attenuates sympathetic HR responses (13). Since nNOS deletion is the only differing variable between the WT and nNOS−/− mice, it is possible that Ih the NO donor SNP attenuates sympathetic HR responses (as seen in our WT and nNOS−/− atrial preparations, see Fig. 4) and 2) nNOS−/− mice may have attenuated sympathetic HR responses to vasodilation in vivo (relative to WT mice).

Increasing NO availability via SNP reduced WT and nNOS−/− sympathetic HR responses, an effect previously reported for guinea-pig atria (6). Specifically, increasing atrial nNOS expression and activity via injection of an adenovirus encoding green fluorescent protein or nNOS into rat right atrial tissue also leads to attenuated NE HR responses in hypertensive rats (11). The effect of myocardial nNOS overexpression on HR has not been examined in mice, but elevated murine myocardial eNOS expression and activity do not alter the NE HR response in isolated hearts (2). If the attenuated sympathetic HR response in nNOS−/− atria resulted from a lack of endogenous synthesis of NO via nNOS, NO donors would be expected to enhance this response. However, NO donors do not mimic the spatial and temporal endogenous production of NO via nNOS and they mimic pathways that might involve the activation of all NOS isoenzymes. In addition, NO donors or atrial nNOS overexpression may result in high, potentially pathophysiological, concentrations of NO in the preparation. Since l-NA did not alter sympathetic HR responses in WT atria, it is possible that the attenuated sympathetic HR response with SNP (nNOS−/− and WT atria) or genetic nNOS inactivation result from different intracellular pathways. SNP may produce its effect via NO (acting pre- and/or postsynaptically), whereas the WT and nNOS−/− sympathetic HR responses may involve a postsynaptic NOS-independent pathway.

To understand how SNP or genetic inactivation of nNOS results in attenuated sympathetic HR responses in the mouse atria, it is important to understand how β-adrenoceptor activation modulates the ionic mechanisms that contribute to SAN pacemaking. In isolated guinea-pig and rabbit SAN pacemaker cells, I1 and the L- and T-type calcium currents are modulated via β-adrenoceptor activation and elevated intracellular cAMP levels to increase HR (15). In the mouse atria, we found that pharmacological inhibition of the L-type calcium current (ICa,L; with nifedipine) or I1 (with Cs+) attenuated sympathetic HR responses (8). Heaton et al. (11) reported that atrial nNOS overexpression reduces the NE-stimulated cAMP concentration, HR, and ICa,L (recorded in hypertensive SAN cells). These results support the idea that the attenuated nNOS−/− sympathetic response results from a postsynaptic NOS-independent pathway.

We did not uncover the mechanism(s) underlying the elevated baseline HR in the nNOS−/− atria. It was still present after pharmacological inhibition of autonomic receptors, with l-NA, and after I1 inhibition. Previous studies (7, 17) suggest that it may result from reduced basal parasympathetic tone in the nNOS−/− heart, but nNOS inhibition did not alter the WT baseline HR. Experiments with specific pharmacological inhi-
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It appears that the genetic deletion of nNOS results in the nNOS inhibition in WT atria failed to alter sympathetic HR increases in HR. However, we cannot eliminate the possibility that the elevated baseline HR in the nNOS−/− atria contributed to their attenuated adrenergic HR responses.

There are some limitations to using an isolated atrial preparation. It does not mimic a normally functioning heart (e.g., there is no active coronary perfusion or atrial filling) and extrinsic neuronal reflex pathways are not involved. Choate and Feldman (8) have previously shown that guanethidine virtually abolishes the sympathetic HR response in this unique isolated atrial preparation, supporting the idea that it is due to NE release from postganglionic sympathetic nerve terminals. Therefore, this murine preparation enabled us to investigate the effects of postganglionic sympathetic activation on HR, without the influence of anesthetics, extrinsic neuronal reflexes, or circulating hormones.

In summary, we found no immunohistochemical evidence to support the idea that NO, synthesized via nNOS in sympathetic TH-immunoreactive nerves, plays a physiological role in modulating NE release from cardiac sympathetic nerve terminals in the mouse SAN. The increases in HR with sympathetic nerve activation and bath-applied NE and adenylate cyclase activators were attenuated in nNOS−/− mice. Therefore, this murine preparation will help to uncover the mechanisms(s) that contribute to their attenuated adrenergic HR responses.

GRANTS

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REFERENCES

