VAGAL MODULATION OF HEART RATE IN THE nNOS KNOCKOUT MOUSE *IN VITRO*

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INTRODUCTION

The role of nitric oxide (NO) synthesized from neuronal NO-synthase (nNOS) in the vagal modulation of heart rate (HR) is controversial. Pharmacological inhibitors of nNOS have been reported to significantly attenuate[1] or to have little effect on the decrease in heart rate with vagal nerve stimulation (VNS) [2,3]. This effect may depend on the expression of NOS and bioavailability of NO[4].

AIMS of the study

(1) To identify nNOS within the SA nodal innervation of the mouse heart.
(2) To compare the heart rate responses to vagal stimulation in isolated right vagus/double atria preparations from wild-type homozygous (WT, nNOS+/+), heterozygous (nNOS+/-), and nNOS knockout (nNOS-/-) mice.
(3) To assess the role of upstream and downstream NO-cGMP pathways in cholinergic modulation of HR.

METHODS

Animals

All animals were 3-4 month old males. WT, nNOS(+/-) and nNOS(-/-) genotypes were all confirmed in tissue samples taken from tail clippings.

Anatomy

The right atrium and slices of the hypothalamus (40µm) were processed for immunohistochemistry. Immunoreactivity was revealed by the chromogenic substrate diaminobenzidine with hydrogen peroxide.

Physiology and Pharmacology

A double atrial/right vagal preparation was dissected free, placed into an organ bath containing mouse physiological saline bubbled with carbogen (95% O_2, 5% CO_2) and connected to an isometric force transducer. Heart rate was triggered from contraction. The change in heart rate with vagal stimulation for 30s or bath-applied CCh (10^{-8} - 10^{-4}M) was measured. Drugs were added to the organ bath after control protocols were completed.

RESULTS

Anatomy - nNOS in right atrium & hypothalamus

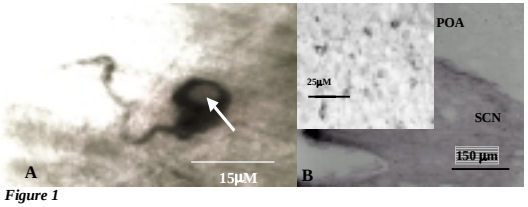

Figure 1
(A) Light photomicrograph showing a nNOS positive neurone in the SA node region taken from the isolated WT right atrium (n=3). Arrow marks the unstained nucleus.
(B) Positive and negative control staining for nNOS in the WT hypothalamus (n=6). Immunoreactivity is present in the suprachiasmatic nucleus (SCN), but not the adjacent preoptic area (POA). Inset shows the SCN at higher magnification.
No nNOS immunoreactivity was found in the right atrium or hypothalamus of nNOS-/- mice (n=3).

Physiology - In-vitro vagal HR responses

* There were no differences in ventricular : body weight ratios

* Baseline HR was elevated in nNOS-/- compared to WT atria **(Table 1)**.

* There was no difference in the magnitude of the HR response to vagal stimulation **(Figure 2)**.

* The rate of decline in HR (TT50%) with vagal stimulation was significantly slower in nNOS-/- than WT mice **(Figure 2 & Table 1)**.

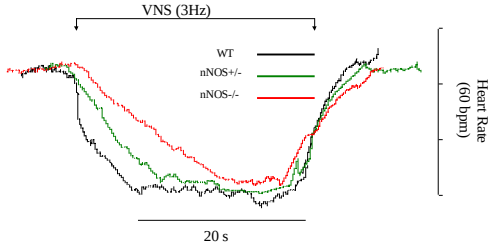

Figure 2 Typical raw data traces for WT, nNOS+/- and nNOS-/-

* The baseline HR, vagal HR responses and TT50% in nNOS+/- were not significantly different from those in either WT or nNOS-/- . The mean values for nNOS+/- preparations fell in-between those for WT and nNOS-/- **(Table 1)**.

Table 1 Baseline HR, TT50% (3 & 5Hz) in WT, nNOS-/- and nNOS+/-.

	Baseline HR (bpm)	3Hz TT50% (s)	5Hz TT50% (s)
WT (n=56)	322 ± 6	5.93 ± 0.28	4.79 ± 0.28
nNOS -/- (n=39)	360 ± 7*	7.30 ± 0.38*	5.74 ± 0.23*
nNOS +/- (n=15)	338 ± 8	6.66 ± 0.18	5.56 ± 0.33

(Mean ± S.E.M.) * p < 0.05 ***nNOS-/-*** vs. ***WT***, unpaired t-test

* There were no statistical differences in the IC_{50} concentrations of bath-applied carbamylcholine (CCh) for heart rate responses in WT and nNOS-/- **(Table 2).**

Table 2 IC_{50} values for CCh HR Response (10^{-8}-10^{-4}M) in WT vs. nNOS-/-

	IC_{50} for CCh HR Response (10^{-x}M)
WT (n=12)	6.37 ± 0.39
nNOS -/- (n=16)	6.00 ± 0.37

Pharmacology - effects of NOS inhibition

N-Monomethyl L-Arginine (L-NMMA) (100µM) significantly slowed the rate of the decrease in heart rate with vagal stimulation (3 & 5Hz) in WT (n=8), but not in nNOS-/- (n=8) atria. This effect was reversed with L-arginine (1mM).

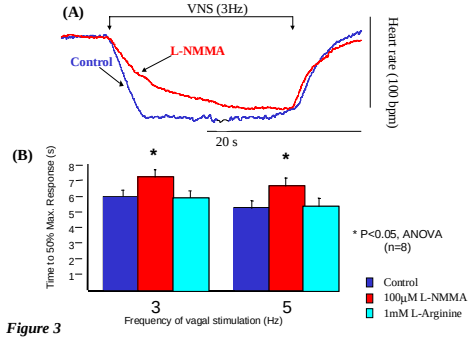

Figure 3
(A) Typical raw data trace from a WT preparation (VNS at 3Hz)
(B) Averaged data from 8 WT preparations

The nNOS inhibitors N5-(1-Imino-3-butenyl)-L-ornithine (L-VNIO) or 1-(2-trifluoromethylphenyl) imidazole (TRIM) (100µM) significantly attenuated the heart rate response to vagal nerve stimulation at 3Hz in WT but not in nNOS-/- (n=8) or nNOS+/- (n=9). This was reversed with L-arginine (1mM).

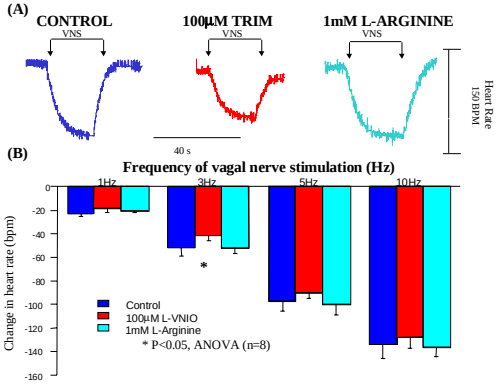

Figure 4
(A) TRIM: Typical raw data trace from a WT preparation (VNS at 5Hz)
(B) L-VNIO: Averaged data from 8 WT preparations (VNS at 1,3,5 and 10Hz)

Pharmacology - effects of a NO donor and a guanylate cyclase inhibitor

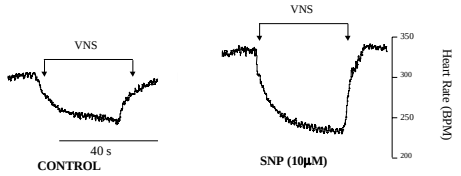

Figure 5
Heart rate trace from WT preparation during vagal stimulation at 3Hz before and after the addition of 10µM sodium nitroprusside (SNP). Identical effects were obtained in WT (n=10), nNOS(-/-) (n=7) and nNOS(+/-) (n=15) preparations, showing downstream NO signalling was intact.

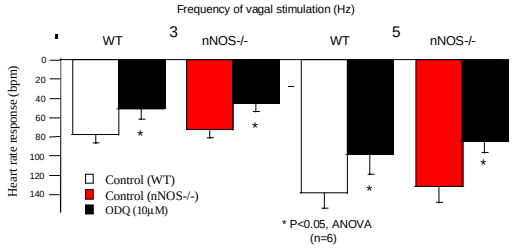

Figure 6
Graph showing effect of inhibition of soluble guanylate cyclase with 10µM 1H-(1,2,4)-oxadiazolo-(4,3-a)-quinoxalin-1-one (ODQ) on the HR response to vagal stimulation at 3 & 5Hz. There is no difference in the effect of ODQ in WT (n=6) and nNOS(-/-) preparations (n=6).

CONCLUSION

The findings of this study suggest that nNOS present in the parasympathetic nerves supplying the atria produces NO that facilitates presynaptic cholinergic neurotransmission, and contributes to the decrease in heart rate in response to vagal nerve stimulation.

References

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[2] Sears C.E., Choate J.K., and Paterson D.J. 1998. *J. Aut. Nerv. Syst.* **73**, 63-73.
[3] Sears C.E., Choate J.K., and Paterson D.J. 1998. *J. Appl Physiol.* **84**(5):1596-603.
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