PRE-SYNAPTIC NO-cGMP PATHWAY AND VAGAL CONTROL OF HEART RATE

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Introduction

Neuronal nitric oxide synthase (nNOS) has been immunohistochemically located in parasympathetic ganglion around the pacemaker of the heart [1]. Inhibition of nNOS in the adult guinea pig *in-vivo* dramatically reduces the heart rate (HR) response to vagal nerve stimulation (VNS)[2]. However, this is not seen in the young guinea pig *in-vitro* or rabbit *in-vivo*[3] although high concentrations of exogenous NO or cGMP are still able to modulate the vagal bradycardia [4]. It is also unclear how NO from pre-synaptic nNOS modulates the HR response to VNS.

We tested the following hypotheses:

1. inhibition of endogenous nNOS or guanylyl cyclase (GC) only reduces the HR response to VNS in adult but not young guinea pigs and this is dependent on the levels of nNOS protein.
2. NO from nNOS acts on a pre-synaptic GC dependent pathway to increase vagal neurotransmission.

Methods

Guinea-pig double atrial/right vagus nerve preparation

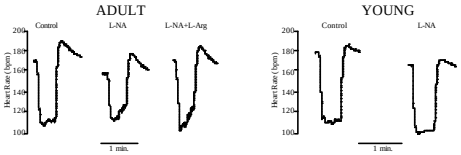
The atria and right vagus nerve were dissected from young (150-250g) and adult (550-750g) female guinea pigs and transferred to a preheated (37±0.2°C) organ bath containing oxygenated Tyrode's solution. Following an equilibration period (60–90 mins), the vagus nerve was stimulated at 1, 3, and 5Hz (10-15V,1ms pulse duration) for 30 seconds before and after pharmacological interventions.

Western Blot

Protein was extracted from freshly dissected right atria. 200µg was separated on 7.5 % SDS-polyacrylamide and transferred to a MSI PVDF membrane. Membranes were incubated for 12 hours at 4°C with primary antibody and again with the secondary HRP-conjugated antibody. Antibody-bound proteins were detected using luminol-based chemiluminescent and autoradiography.

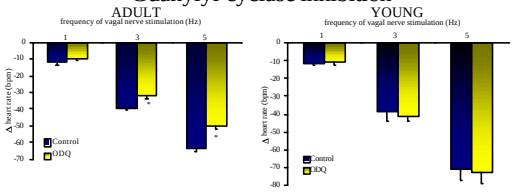
1. Pre-synaptic NO-cGMP: *a developmental role*

NOS inhibition



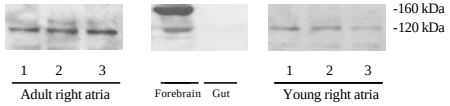
- The non-specific NOS inhibitor 100 µM Nω-Nitro-L-Arginine (L-NA, n=5) and the specific nNOS inhibitor 100 µM vinyl N5 -(1-imino-3-butenyl)-L-ornithine (L-VNIO, n=6) significantly reduced the HR response to VNS at 5 Hz and this effect was reversed by L-arginine. However, L-NA had no effect on the HR response to VNS in young guinea pigs (n=7).

Guanylyl cyclase inhibition



- Inhibiting GC with 10µM 1H-(1,2,4) oxadiazolo (4,3-a) quinoxalin-1-one (ODQ) significantly (* $p<0.05$) reduced the HR response to VNS at 5 and 3 Hz in adult (n=7) but not young (n=7) guinea pigs.

nNOS protein levels

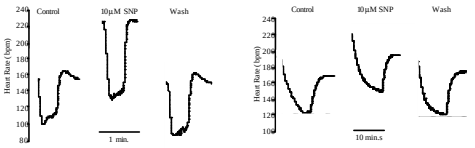


- Western blot analysis showed significantly less 120 kDa nNOS protein in right atria from young compared to adult guinea pigs (young, 146±32 OD, n=3, adult, 620±111 OD, n=3). 140 kDa eNOS protein was found at similar levels in both adult and young guinea pigs.

2. Pre-synaptic NO-cGMP: *an intracellular pathway*

Vagal nerve stimulation

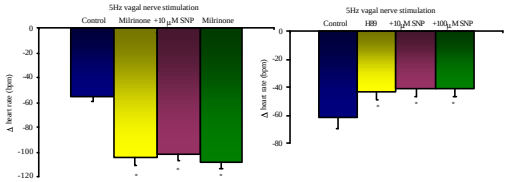
Carbamylcholine



- 10 µM (n=6) or 100 µM (n=7) sodium nitroprusside (SNP) increased the HR response to VNS at 5 Hz, but did not increase the HR response to 100 nM carbamylcholine (CCh, n=8). Neither L-NA (n=6), L-VNIO (n=5) nor ODQ (n=6) had any effect on the HR response to cumulative dose (50, 100, 150, 200nM) of CCh. This suggests that NO from nNOS acts on pre-syantpic GC to increase vagal neurotransmission.

PDE3 inhibition

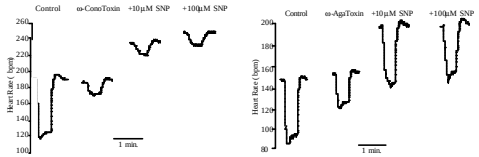
PKA inhibition



- SNP still increased the HR response to VNS at 5 Hz in the presence of the protein kinase G inhibitor 1 µM KT5823 (n=6) but not in the presence of the phosphodiesterase (PDE) 3 inhibitor 1µM milrinone (n=7). The protein kinase A (PKA) inhibitor 0.5µM H-89 also abolished the increase in the HR response to VNS with SNP (n=5). (*$p<0.05$)

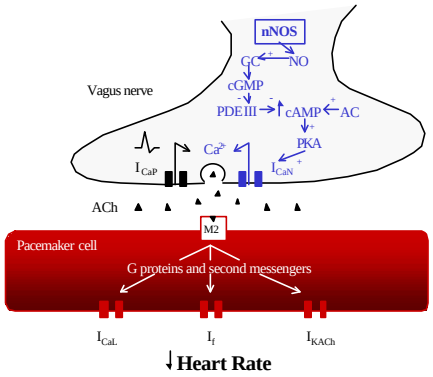
I_{CaN} inhibition

I_{CaP} inhibition



- Inhibition of either N-type (with 100 nM ω-Cono Toxin, n=6) or P-type (with 50 nM ω-Aga Toxin, n=5) calcium channels reduced the HR response to VNS at 5 Hz. However SNP did not increase the HR response to VNS in the presence of the N-type calcium channel blocker ω-Cono Toxin.

Summary



Conclusions

•These results are consistent with the hypothesis that NO from nNOS acts on pre-synaptic GC to facilitate the HR response to vagal nerve stimulation in adult guinea pigs. This effect is not seen in young guinea pigs where right atrial nNOS levels are significantly lower.

•Pre-synaptic NO may modulate the HR response to vagal nerve stimulation via cGMP acting on phosphodiesterase III to raise levels of cAMP and increase the activity of protein kinase A. Although both N and P-type calcium channels are involved in vagal neurotransmission, protein kinase A may phosphorylate N-type calcium channels to increase the influx of calcium and vesicular acetylcholine release on vagal stimulation.

References

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4. Sears, C.E., Choate J.K., & Paterson D.J. (1999). *J. Appl. Physiol.* **86**, 510-6.

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