# NO-cGMP PATHWAY FACILITATES VAGAL NEUROTRANSMISSION AND BRADYCARDIA

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## 1. Introduction

Neuronal nitric oxide synthase (nNOS) has been immunohistochemically located in parasympathetic ganglion around the pacemaker of the heart[1]. We have shown that inhibition of nNOS or guanylyl cyclase (GC) reduces the heart rate (HR) response to vagal nerve stimulation (VNS) via a presynaptic pathway in the adult guinea pig *in-vitro*[2]. However, the mechanism by which this occurs is unknown. NO has been implicated in increasing the release of acetylcholine in the rat forebrain[3] and the cAMP system has been shown to augment acetylcholine release in isolated atria[4].

We therefore tested the following hypotheses:

**1.** Does NO act presynaptically via cGMP to augment the exocytotic release of acetylcholine during nerve stimulation?

**2.** Can this be mimicked by stimulation of particulate guanylyl cyclase with natriuretic peptides?

**3.** Is this achieved by inhibition of phosphodiesterase (PDE) 3 to increase cAMP - protein kinase A (PKA) dependent phosphorylation of pre-synaptic calcium channels?

## 2. Methods

**Guinea-pig double atrial/right vagus nerve preparation**

The atria and right vagus nerve were dissected from adult (550-750g) female guinea pigs and transferred to a preheated (37±0.2ºC) 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.

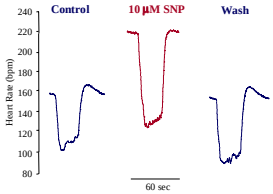
**Measuring right atrial $^3$H-acetylcholine release**

Guinea pig right atrial preparations were placed in a 2ml organ bath at 37±0.5°C and loaded with $^3$H-choline by repeated (10s every 30s) field stimulation (10Hz, 20V, 1ms pulse duration) for 30mins. Excess radioactivity was washed from the preparation by 30 mins perfusion at 2ml/min. The organ bath Tyrode's solution was then replaced every 3 minutes and its radioactive content measured with a liquid scintillation counter. After 28 mins and again after 43 mins the preparation was stimulated for 1 minute at 10Hz and the change in efflux of radioactivity measured. Remaining $^3$H in the atria was measured at the end of the experiment following incubation with papain (4units/mL) and results are expressed as an efflux of total atrial $^3$H content.

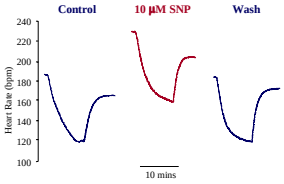
## 3. Results

### 3a. NO-cGMP pathway augments acetylcholine release

Vagal nerve stimulation

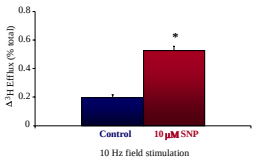



Carbamylcholine

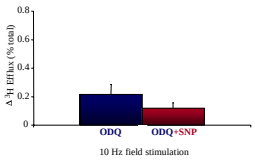



• Sodium nitroprusside (SNP, 10 µM or 100 µM, n=6) significantly ($p<0.05$) increased the HR response to VNS at 5 Hz (and 3 Hz - not shown), but did not increase the HR response to 100 nM carbamylcholine (CCh, n=8). This suggests that SNP acts pre-synaptically to increase vagal neurotransmission.

NO and $^3$H-ACh release



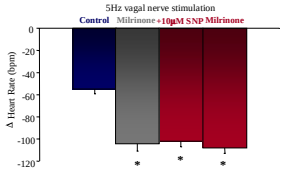

GC inhibition



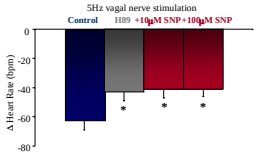

• SNP (10 µM, n=4) significantly increased the evoked release of $^3$H-acetylcholine (ACh) to field stimulation. However SNP had no effect on the release of $^3$H-ACh (n=4) or the HR response to VNS (n=5) in the presence of the guanylyl cyclase inhibitor ODQ (10 µM). (*$p<0.05$)

### 3b. PDE3 pathway increases PKA phosphorylation $I_{CaN}$

PDE3 inhibition

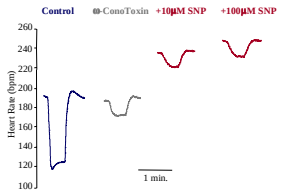



PKA inhibition

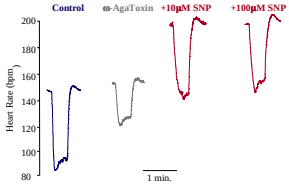



• SNP had no effect on the HR response to VNS at 5 Hz (n=7) or the release of $^3$H-acetylcholine (n=4) in the presence of the phosphodiesterase (PDE) 3 inhibitor milrinone (1µM). The protein kinase A (PKA) inhibitor H-89 (0.5µM, n=5), but not the PKG inhibitor KT5823 (1 µM, n=6), abolished the increase in the HR response to VNS with SNP at 5 Hz. Similar results were observed at 3 Hz VNS - data not shown. (*$p<0.05$)

$I_{CaN}$ inhibition



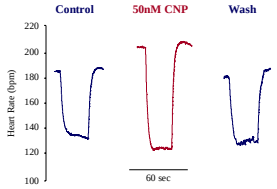

$I_{CaP}$ inhibition



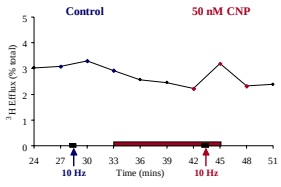

• Inhibition of either N-type (with 100 nM ω-conotoxin, n=6) or P-type (with 50 nM ω-agatoxin, n=5) calcium channels significantly reduced the HR response to VNS at 5 Hz (and 7 and 9 Hz not shown). However SNP did not increase the HR response to VNS in the presence of the N-type calcium channel blocker ω-conotoxin.

### 3c. Other substances that raise cGMP

CNP and vagal stimulation




CNP and $^3$H-ACh release

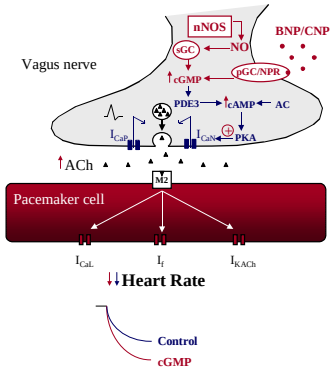



• Like NO, C-type natriuretic peptide (CNP, 50 nM, n=6) and BNP (not shown) significantly increased the HR response to VNS at 5 Hz . CNP also increases the release of $^3$H-ACh (n=6). In further experiments (not shown) the effect of CNP on the HR response to VNS was abolished by the particulate guanylyl cyclase coupled natriuretic peptide receptor antagonist HS-142-1 (100 µg/ml, n=5), the PDE 3 inhibitor milrinone (1 µM, n=7), the PKA inhibitor H-89 (0.5 µM, n=6) and the N-type calcium channel blocker ω-conotoxin (100 nM, n=6).

## 4. Summary

•SNP releases NO that acts pre-synaptically via a soluble guanylyl cyclase dependent pathway to facilitate vagal release of acetylcholine. This effect can be mimicked by stimulation of particulate guanylyl cyclase with the natriuretic peptides BNP and CNP.

•This is achieved via GMP dependent inhibition of phosphodiesterase 3 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 phosphorylates N-type calcium channels to facilitate the exocytotic release of acetylcholine[5].




References

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