# NO-cGMP PATHWAY FACILITATES ACETYLCHOLINE RELEASE AND BRADYCARDIA DURING VAGAL NERVE STIMULATION

Neil Herring and David J. Paterson

University Laboratory of Physiology, Parks Road, Oxford, U.K. OX1 3PT

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

## Methods

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

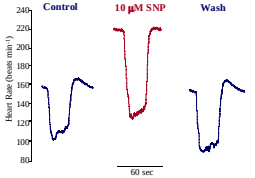
The atria and right vagus nerve were dissected from adult (550-750g) female guinea pigs and transferred to a preheated (37±0.2ºC) organ bath containing oxygenated Tyrode's solution. Following an equilibration period (60–90 mins), the vagus nerve was stimulated at 1, 3, and 5Hz (10-15V,1ms pulse duration)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

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

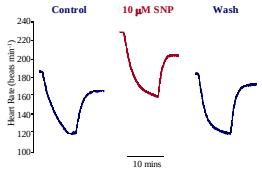
Guinea pig right atrial preparations were placed in a 2ml organ bath at 37±0.5°C and loaded with $^3$H-choline by repeated (10s every 30s) field stimulation (10Hz, 20V, 1ms pulse duration) for 30mins. Excess radioactivity was washed from the preparation by 30 mins perfusion at 2ml/min. The organ bath 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. Experiments were performed in the presence of 50µM hemicholinium 3 to prevent re-uptake of released $^3$H-acetylcholine and any remaining $^3$H in the atria was measured at the end of the experiment following incubation with papain (4units/mL). Results are expressed as an efflux of total atrial $^3$H content.

## Results 1. NO-cGMP pathway augments acetylcholine release

Vagal nerve stimulation

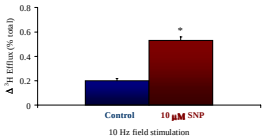



Carbamylcholine

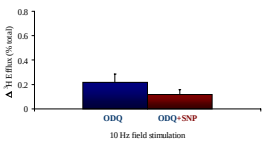



- 10 µM (n=6) or 100 µM (n=7) sodium nitroprusside (SNP) significantly ($p<0.05$) increased the HR response to VNS at 5 Hz (and 3Hz - not shown), but did not increase the HR response to 100 nM carbamylcholine (CCh, n=8). This suggests that SNP acts pre-syantpically to increase vagal neurotransmission.

NO and $^3$H-ACh release



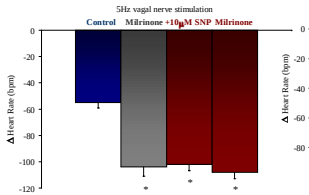

GC inhibition



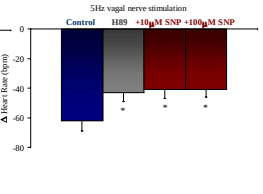

- 10 µM (n=4) SNP increased the evoked release of $^3$H-acetylcholine to field stimulation. However SNP had no effect on the release of acetylcholine (n=4) or the HR response to VNS (n=5) in the presence of the guanylyl cyclase inhibitor ODQ (10 µM).

## Results 2. cGMP-PDE3 pathway increases PKA phosphorylation of $I_{CaN}$

PDE3 inhibition

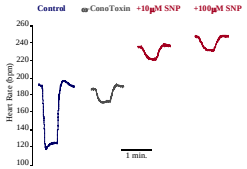



PKA inhibition

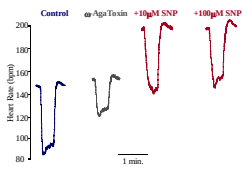



- SNP had no effect on the HR response to VNS at 5 Hz (n=7) or the release of $^3$H-acetylcholine (n=4) in the presence of the phosphodiesterase (PDE) 3 inhibitor 1µM milrinone. The protein kinase A (PKA) inhibitor 0.5µM H-89 (n=5), but not the PKG inhibitor 1 µM KT5823 (n=6), abolished the increase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

$I_{CaN}$ inhibition

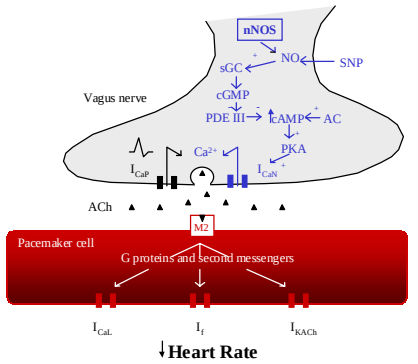



$I_{CaP}$ inhibition




- 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 (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-Cono Toxin.

## Summary




## Conclusions

•SNP releases NO that acts pre-synaptically via a guanylyl cyclase dependent pathway to facilitate vagal release of acetylcholine.

•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.

## References

1. Klimaschewski L, Kummer W, Mayer B, Couraud J.Y, Preissler U, Philippin B, & Heym C. (1992). *Circ.Res.* **71**, 1533-7.
2. Herring N, Golding S, & Paterson D.J. (2000). *J. Moll. Cell. Cardiol.* **32**, 1795-1804.
3. Prast H, & Philippu A. (1992). *Euro. J. Pharm.* **216**, 139-140.
4. Dawson J.J, Iannazzo L, & Majewski H. (1996). *J. Auton. Pharmacol.* **16**, 79-85.

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