NO-cGMP PATHWAY ENHANCES THE HEART RATE RESPONSE TO PERIPHERAL VAGAL NERVE STIMULATION IN EXERCISE-TRAINED MICE

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INTRODUCTION

Aerobic exercise training increases cardiac vagal tone and decreases resting heart rate. The mechanisms by which these effects are brought about are not fully understood. The nitric oxide – cyclic guanosine monophosphate (NO-cGMP) pathway is implicated in cholinergic regulation of heart rate[1], and plays a role in adaptations taking place in coronary vasculature[2], and cardiac sympathetic innervation[3] following training.

AIMS of the study

(1) To investigate whether male mice that had been selectively bred for increased wheel-running[4] had increased heart rate responses to peripheral vagal nerve stimulation or bath-applied carbamylcholine following a period of 20 weeks voluntary wheel-running.

(2) To assess the role of upstream and downstream NO-cGMP pathways in cholinergic modulation of HR following training.

METHODS

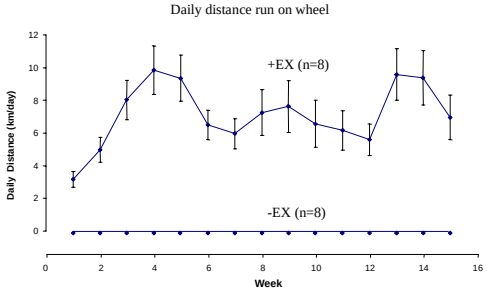
Animals & Training

Mice were selectively bred for wheel-running over 10 generations. Male mice, 8-12 weeks old were provided with wheels (+EX, n=8) and running distances were logged daily. Controls (-EX, n=8) were singly-housed in cages without wheels.

Physiology and Pharmacology

A double atrial/right vagal preparation was dissected free, placed into an organ bath containing mouse physiological saline aerated 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 ($3x10^{-8}$ – $3x10^{-7}$M) was measured. Drugs were added to the organ bath after control protocols were completed.

Running Performance



Ventricle/Body Weight Ratio

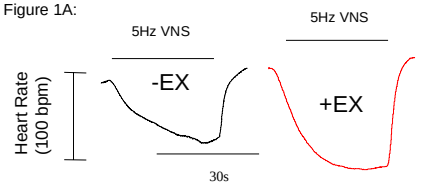
	Body Weight (g)	Ventricular Weight (mg)	Ventricle/Body Weight Ratio (mg/g)
-EX	42.89±1.62	167.18±9.34	3.97±0.34
+EX	34.33±2.05*	220.39±12.97*	6.60±0.58*

*P<0.001, –EX (n=8) vs. +EX (n=8), one-way ANOVA

RESULTS

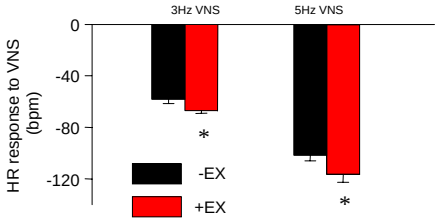
Cholinergic regulation of heart rate

Figure 1A:



Raw data traces showing decrease in heart rate during vagal nerve stimulation (VNS) at 5Hz was enhanced in atria taken from +EX mice

Figure 1B:

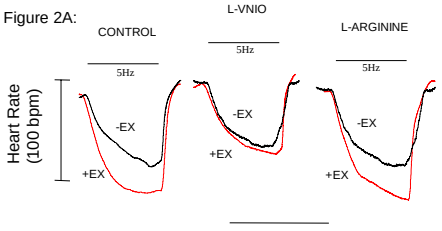


Vagal bradycardia was significantly enhanced in +EX mice during VNS at 3 and 5Hz. *P<0.05, +EX (n=8) vs. –EX (n=8), one-way ANOVA.

NO-cGMP in cholinergic regulation of heart rate

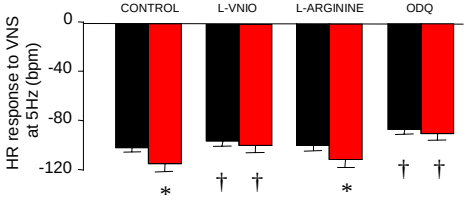
(i) Inhibition of NO-cGMP

Figure 2A:



Raw data trace showing that the nNOS inhibitor, vinyl-l-nio hydrochloride (L-VNIO, 100μM) attenuated vagal bradycardia in +EX and –EX atria and abolished the control differences between them at 5Hz VNS. This effect was reversed by 1mM L-arginine

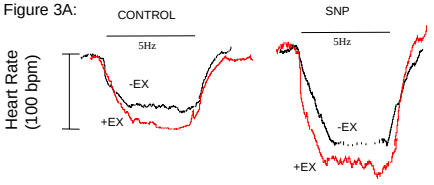
Figure 2B:



L-VNIO significantly attenuated responses to VNS in +EX and –EX atria: †P<0.01, Control (n=8) vs. L-VNIO (n=8) vs. L-arginine (n=8), one-way RM ANOVA. Also, the significantly enhanced bradycardia in +EX vs. –EX (*P<0.05, one-way ANOVA) was abolished by L-VNIO, and restored by L-arginine. The soluble guanylyl cyclase inhibitor, 1H-[1,2,4]Oxadiazolo -[4,3-a]quinoxalin-1-one (ODQ, 10μM), had the same effect as L-VNIO.

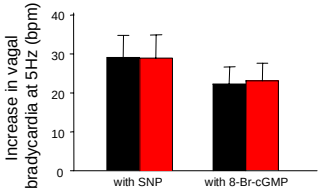
(ii) Activation of NO-cGMP

Figure 3A:



Raw data trace showing that the NO donor, sodium nitroprusside (SNP, 10μM) enhanced vagal bradycardia in +EX and –EX groups, and that the enhanced bradycardia observed in +EX atria was still present when NO-cGMP was amplified by exogenous NO.

Figure 3B:



SNP and the cGMP analogue, 8-Br-cGMP (0.5mM) enhanced vagal bradycardia to the same extent in +EX and –EX atria, suggesting that mediators downstream to NOS enzymes were unchanged after exercise.

Pre-synaptic role

Table: There was *no difference* between the HR response to carbamylcholine (CCh) in –EX (n=8) and +EX (n=8)

HR Response (bpm) vs. conc. of CCh (mol/L)	$3 x10^{-8}$	$1 x10^{-7}$	$3 x10^{-7}$
-EX (n = 8)	24 ± 6	67 ± 9	139 ± 27
+EX (n = 8)	25 ± 8	73 ± 11	145 ± 31

Western Blot Analysis

Figure 4A:

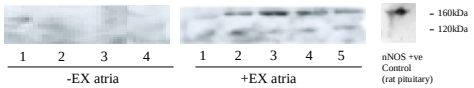
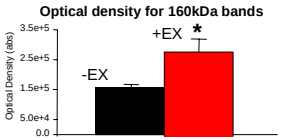
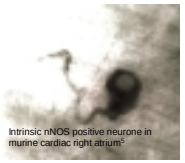


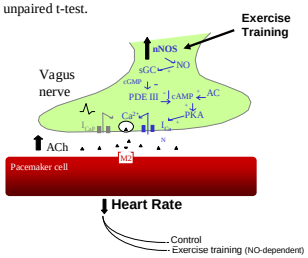
Figure 4B:



Western blot analysis showed significantly increased expression of nNOS protein in atria taken from +EX animals. * p<0.01, unpaired t-test.

CONCLUSION





Evidence presented here suggests that exercise-trained mice have an increased peripheral vagal bradycardia, and that this may be due to upregulation of neuronal NOS resulting in NO facilitating ACh release[6].

References

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[4] Koteja, P., Garland, T., Sax, J.K., Swallow, J.G. & Carter, P.A.(1999) *Anim Behav* 58(6):1307-1318

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