Neuronal NOS gene transfer promotes cardiac vagal neurotransmission and gain of function

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INTRODUCTION

Nitric oxide (NO) is believed to play a fundamental role in the regulation of cardiac cholinergic function. Neuronal nitric oxide synthase (NOS-1) co-localises with choline acetyltransferase in the intra-cardiac ganglia (1). Pharmacological evidence suggests that NO generated from NOS-1 directly enhances the negative chronotropic effect of cholinergic stimulation (eg: 2 & 4), by activating the guanylate cyclase / cGMP pathway to facilitate release of ACh (5).

An indirect action via endothelial NOS-3 M_2 receptor coupled inhibition of ICaL in pacemaking cells is also suggested (3), although this is controversial (7). Importantly, the vagal heart rate (HR) response to pharmacological modulators is not mimicked by the ACh analogue carbachol (CCh), suggesting that the functional role of the pathway is site-specific (4 & 6).

Hypothesis:

We tested the hypothesis that NOS-1 gene transfer to the right atrium would enhance vagal neurotransmission and bradycardia, but would have no effect on the heart rate response to CCh.

METHODS

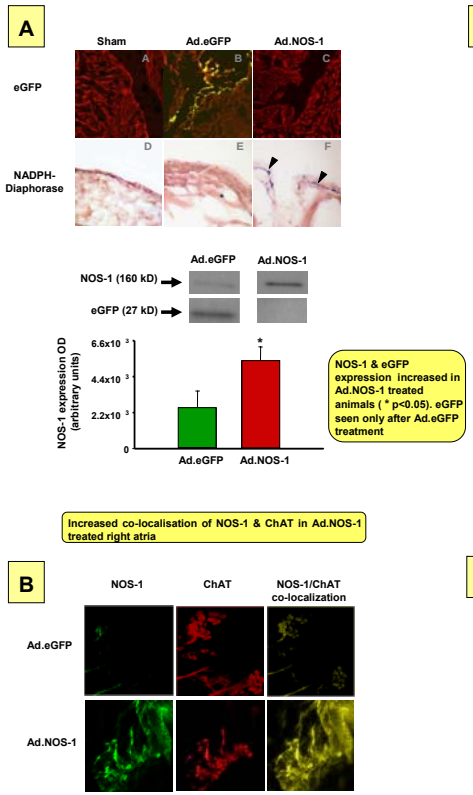
Gene transfer to the right atrium of the guinea pig

Percutaneous gene transfer to the right atrium was performed in male guinea pigs, under halothane anaesthesia. A suspension of replication-deficient adenovirus encoding either NOS-1 (Ad.NOS-1) or eGFP (Ad.eGFP; control virus) was used. Animals received a right atrial injection of $5x10^9$ – $5x10^{10}$ virus particles, or vehicle alone (sham).

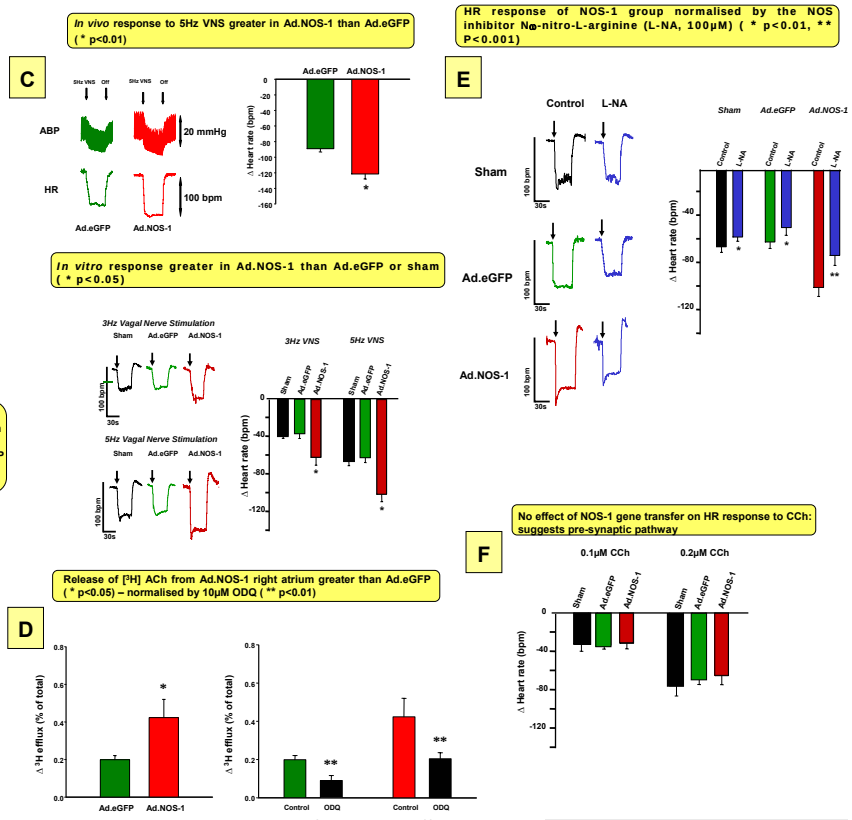
Phenotyping of transfected animals

Following an incubation period of ~5 days, HR responses to 3 and 5Hz VNS were measured, both in anaesthetised animals and *in vitro*, using a double atrial preparation with intact right vagus nerve. Isolated atrial preparations were also used to assess the HR response to CCh., and to measure release of radiolabelled ACh in response to field stimulation (10Hz). NOS-1 and eGFP protein expression was measured using a combination of Western blotting and NADPH-diaphorase / fluorescence microscopy, respectively.

Results: Molecular phenotype following gene transfer



Results: Physiological phenotyping of transfected animals



Conclusions

- Gene transfer of NOS-1 and eGFP is associated with increases in protein expression in the guinea pig right atrium. Confocal imaging shows increased NOS-1 expression in intra-cardiac ganglia.
- The heart rate response to vagal nerve stimulation is enhanced in Ad.NOS-1 treated animals, both *in vivo* and *in vitro*. This is associated with enhanced release of ACh.
- Heart rate responses to CCh are unaffected by NOS-1 gene transfer. This suggests that the dominant action of NO is site-specific to the pre-synaptic terminal.
- Gene transfer of NOS-1 to the right atrium may therefore provide a novel method for promoting cardiac vagal gain of function.

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