Sympathetic neuron-specific adenoviral vector mediated nNOS gene transfer decreases neurotransmission by modulating intracellular Ca^{2+}

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Introduction

Adenoviral gene transfer of neuronal NOS (nNOS) can decrease central sympathetic outflow and facilitate cardiac cholinergic neurotransmission, indicating that NOS may confer site specific actions in relation to its target. Our previous work provided direct evidence that nNOS targeted to cardiac sympathetic neurons inhibits sympathetic neurotransmission (1). Inhibition of nNOS or soluble guanylate cyclase (sGC) enhances both noradrenaline release and the heart rate responses to peripheral cardiac sympathetic nerve stimulation *in vitro* and *in vivo* (2). A proposed model suggested that NO generated by nNOS decreased NE release via cGMP-mediated activation of phosphodiesterase2 in the sympathetic varicosities, which causes enhanced degradation of cAMP that could de-activate PKA and lead to a decrease in Ca^{2+} influx (3). To test this hypothesis, we investigate depolarizing K^+ concentration evoked Ca^{2+} influx in excitation-secretion coupling in nNOS transduced cardiac sympathetic neurons.

Methods

Noradrenergic neuron-specific adenoviral vector encoding nNOS was constructed using a synthetic promoter (PRS) with motifs for transcription factors Phox 2a/2b, found in the dopamine β-hydroxylase (DBH) promoter. Stellate sympathetic ganglia from neonatal SD rats were dissected and digested using a combination of collagenase and trypsin. An empty adenoviral vector was used as control for comparing the effect of viral transduction on the neurons. Fura2-AM based, ratio-metric measurements of intracellular free calcium $[Ca^{2+}]_i$ were obtained from cells bathed in HEPES buffered Tyrode solution. Ca^{2+} imaging was performed 48 hours post-transduction. Cell depolarization was induced by using 50mM KCl in HEPES Tyrode buffer for 20 seconds. To acquire accurate measurement of second messengers (cGMP and cAMP) in cardiac sympathetic neurons, we employed TR-FRET technology for cAMP measurement and enzyme fragment complementation (EFC) detection technology for cGMP.

Results

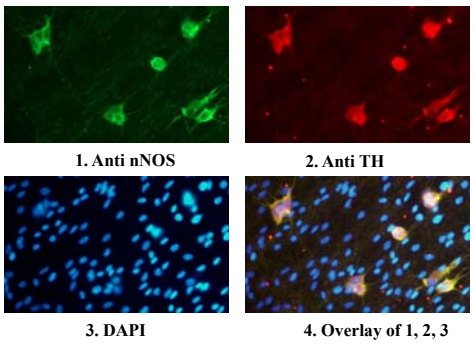


Fig 1. Transduction of the cardiac sympathetic neurons with Ad.PRS-nNOS resulted in exclusive expression of nNOS in tyrosine hydroxylase (TH) positive neurons.

nNOS positive staining (green) is only found in sympathetic neurons, identified by anti-tyrosine hydroxylase (red). An nNOS signal is not detectable in Schwann cells and fibroblasts labelled by DAPI.

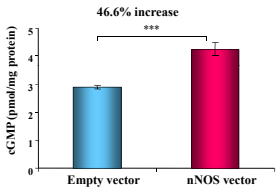


Fig 2. nNOS gene transfer into sympathetic neurons increased cGMP level

Data are from 6 independent gene transduction followed by cGMP assay.

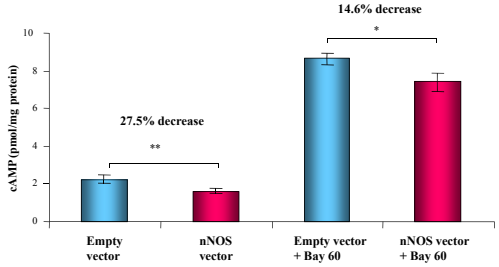


Fig 3. NOS gene transfer decreased cAMP level, PDE2 inhibitor Bay60 partially reversed this effect

Data are from 7 independent gene transduction followed by cAMP assay. PDE2 specific inhibitor Bay60 robustly increased cAMP level, but did not fully reverse cAMP inhibition.

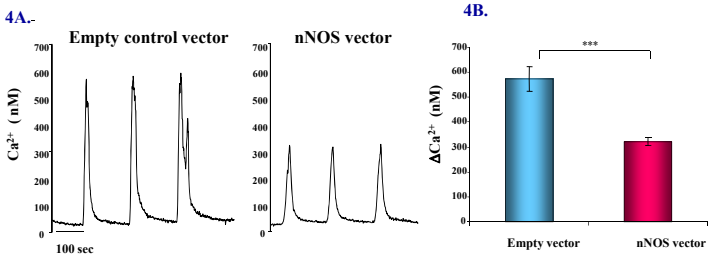


Fig 4. nNOS gene transfer reduced high K^+ evoked $[Ca^{2+}]_i$

4A shows an example recording from Ad.PRS-nNOS transduced sympathetic neurons loaded with Fura-2Am. 50 mM K^+ causes robust rises in $[Ca^{2+}]_i$. This is significantly lower than an empty vector control. **4B** shows the average $[Ca^{2+}]_i$ in sympathetic neurons treated with an nNOS vector (15 cells) and control empty vector (15 cells), the average decrease of $[Ca^{2+}]_i$ in nNOS transduced neurons is 43.8%. $p<0.001$, unpaired T test.

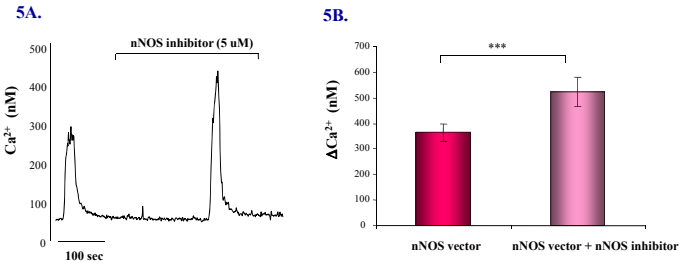
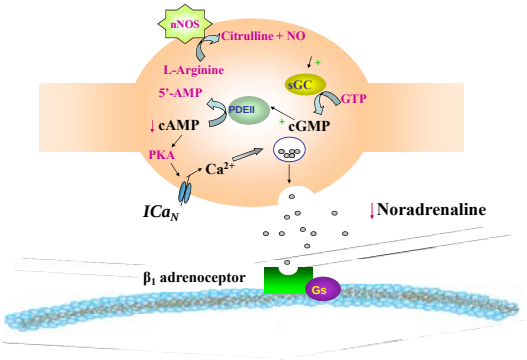


Fig 5. Effect of nNOS inhibitor on high K^+ evoked $[Ca^{2+}]_i$

5A shows an example recording from Ad.PRS-nNOS transduced sympathetic neurons responds to nNOS specific inhibitor (4S)-N-(4-Amino-5-[aminoalkyl]aminopentyl)-N-nitroguanidine (NNNG). **5B** are statistic data from 5 independent transduction in 11 neurons. The average increase of $[Ca^{2+}]_i$ after apply nNOS inhibitor NNNG is 42.3%. $P<0.001$, paired T test.

Conclusions



1. **Sympathetic neuron-specific nNOS gene transfer increased cGMP and decreased cAMP levels. PDE2 specific inhibitor Bay60 (50uM) partially reversed this effect.**
2. **nNOS gene transfer reduced high K^+ evoked increase of $[Ca^{2+}]_i$ in sympathetic neurons. nNOS specific inhibitor NNNG (5uM) reversed this effect.**

These findings support our hypothesis that nNOS gene transfer with neuron-specific adenoviral vector decreases cardiac sympathetic neurotransmission via activation of cGMP-stimulated PDE2 and subsequent inhibition of N-type Ca^{2+} channel.

References:

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