



Cardiac Gene Transfer with nNOS into Sympathetic Nerves Reverses Abnormal Neurotransmission in Spontaneous Hypertensive Rats

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Introduction

Neuronal production of nitric oxide (NO) can influence cardiovascular homeostasis through its action as a neuromodulator within the autonomic nervous system. Sympathetic over-activity is a feature of early hypertension. Adenoviral gene transfer of neuronal NO synthase (nNOS) can decrease central sympathetic outflow¹, but non-specific adenoviral vectors can cause promiscuous transduction. This problem can be circumvented by targeting the NO pathway in vivo in selected cellular populations using cell specific viral vectors².

We tested the hypothesis that

1. A significant component of cardiac sympathetic hyper-responsiveness in hypertension occurs at the end organ level due to impaired NO-cyclic nucleotide signaling.
2. Gene transfer with a noradrenergic cell specific promoter coupled to nNOS increases the bioactivity of nNOS and the production of cGMP that results in restoring sympathetic transmission levels in spontaneously hypertensive rats (SHR) to similar responses seen in normotensive Wistar-Kyoto (WKY) rats.

Methods

Gene transfer to the right atrium of the rat

Percutaneous gene transfer to the right atrium was performed in male WKY & SHR (16-20 weeks), under isoflurane anaesthesia. Adenovirus encoding either nNOS or eGFP driven by PRS promoter was used. Animals received a right atrial injection of 5×10^{10} virus particles in phosphate-buffered saline.

Measurement of [³H]norepinephrine ([³H]NE) release

Neurotransmitter NE release was measured using labelled [³H]NE isolated right atrium in response to 5Hz field stimulation. [³H] outflow was expressed as a percentage of the total radioactivity released at the different time point.

Cardiac sympathetic neuron isolation and transduction

Middle cervical stellate sympathetic ganglia were isolated and transduced by adenoviral vector encoding eGFP or nNOS driven by PRS promoter. Sympathetic neurons were identified by anti-tyrosine hydroxylase (TH)

Measurement of tissue cGMP levels and nNOS activity

Atrial cGMP amount was measured by radioimmunoassay kit (Amersham UK). NOS activity in atria was quantified by measuring the conversion of [³H]-L-arginine to [³H]-L-citrulline in the presence of saturating concentrations of the cofactors of the enzyme with calcium and eNOS inhibitor, L-N⁵-(1-Iminoethyl)ornithine, Dihydrochloride.

Results

Fig 1. SHR has sympathetic hyper-responsiveness

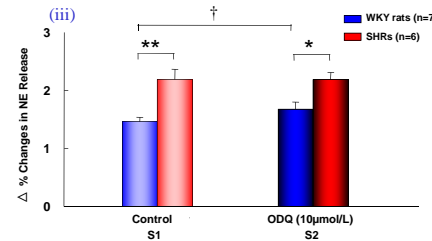
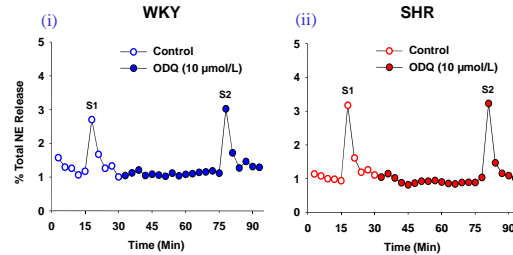


Fig 2. Cardiac sympathetic neuron transduced with Ad.PR5-nNOS expressed nNOS

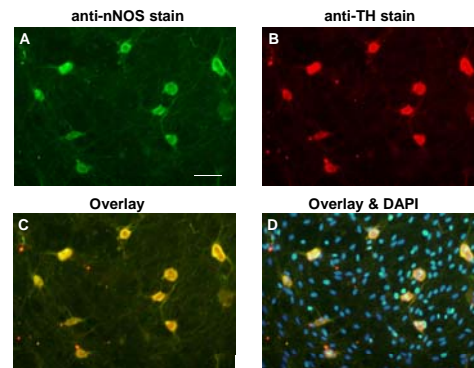


Fig 3. Ad.PR5-nNOS decreases NE

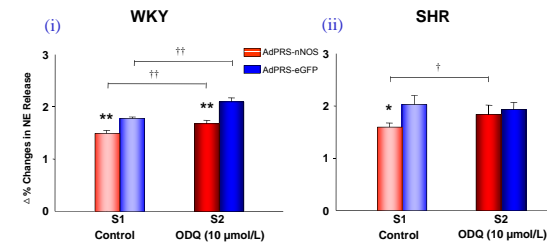


Fig 4. Ad.PR5-nNOS increases cGMP

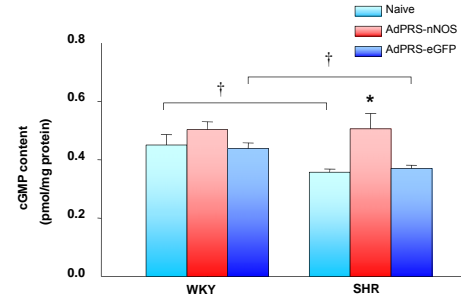
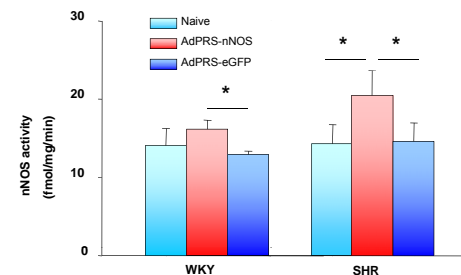
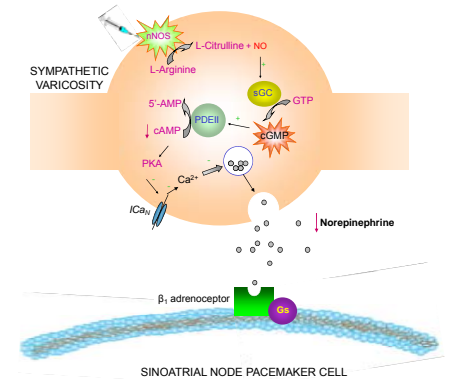


Fig 5. Ad.PR5-nNOS increases nNOS activity



Summary



Adapted from Paton et al 2002³

Conclusion

Noradrenergic cell specific nNOS gene transfer into the SHR right atrial wall upregulates bioavailability of NO in cardiac sympathetic neurons and restores impaired NO-cGMP signalling associated with hypertension, and normalises cardiac sympathetic function. This targeted technique may provide a novel method for reducing sympathetic hyperactivity in pathological states such as hypertension.

References

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2. Wang L, Li D, Plested, CP, Dawson, TD, Teschemacher, A and Paterson, DJ (2006). Noradrenergic neuron-specific overexpression of nNOS in cardiac sympathetic nerves decreases neurotransmission. *J Mol Cell Cardiol*. 41(2):364-370
3. Paton JF, Kasparov S, Paterson DJ (2002). Nitric oxide and autonomic control of heart rate: a question of specificity. *Trends Neurosci*. 25(12): 626-631.