Targeted overexpression of nNOS into cardiac noradrenergic neurons attenuates sympathetic neurotransmission

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Introduction

It is increasingly recognized that neuronal production of nitric oxide (NO) can influence cardiovascular homeostasis through its action as a neuromodulator within the autonomic nervous system. Sympathetic over-activity has been most clearly demonstrated in early hypertension. Adenoviral gene transfer of neuronal NO synthase (nNOS) can decrease central sympathetic outflow[1], but non-specific adenoviral vectors can cause promiscuous transduction. This problem can be circumvented by targeting the NO pathway in-vivo into selected cellular populations using cell specific viral vectors.

Aims of this study

1. To establish whether noradrenergic neuro-specific gene transfer with nNOS into the cardiac sympathetic innervation can reduce sympathetic neurotransmission via aNO-dependent pathway.
2. To demonstrate whether noradrenaline (NA) release is significantly increased in spontaneously hypertensive rats (SHR) compared with 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 SD rats (16-20 weeks), under isoflurane anaesthesia. Adenovirus encoding either nNOS or eGFP was driven by a noradrenergic[2] promoter. Animals received a right atrial injection of $5x10^{10}$ virus particles in phosphate-buffered saline.

Measurement of [^{3}H]noradrenaline ([^{3}H]NA) release

WKYs & SHRs (16-20 weeks) or gene transferred SD rats were used. Neurotransmitter NA release was measured using labelled [^{3}H]NA isolated right atrium in response to 5Hz field stimulation. [^{3}H] 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 nNOS activity

NOS activity in atria was quantified by measuring the conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline in the presence of saturating concentrations of the cofactors of the enzyme with calcium and eNOS inhibitor, L-N^5-(1-Iminoethyl)ornithine, Dihydrochloride.

References

1. Li YF, Roy SK, Channon KM, Zucker IH, Kaushik P. Patel KP (2002). Effect of in vivo gene transfer of nNOS in the PVN on renal nerve discharge in rats. *Am J Physiol Heart Circ Physiol*. 282(2): H594-601.
2. Hwang DY, Carlezon WA Jr, Isacson O, Kim KS (2002). A high-efficiency synthetic promoter that drives transgene expression selectively in noradrenergic neurons. *Hum Gene Ther*. 12(14): 1731-40.
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.

Results

Fig 1

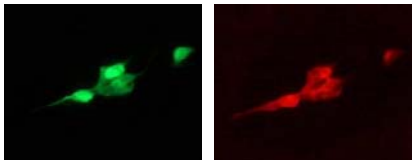


Fig. 1 Cardiac sympathetic neruon transduced with Ad.PRS-nNOS expressed nNOS.

Fig 2

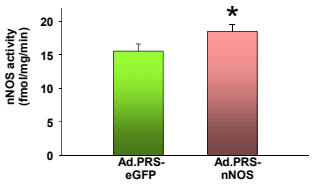


Fig. 2. nNOS activity in Ad.PRS-nNOS was 18.88% higher than Ad.PRS-eGFP group (P<0.05, unpaired t test, n=6 in each group).

Fig 3

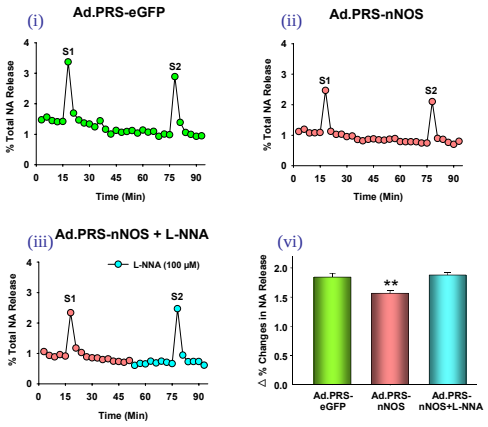


Fig. 3. Representative raw data of [^{3}H]NA release in (i) Ad.PRS-eGFP, (ii) Ad.PRS-nNOS and (iii) Ad.PRS-nNOS with NO synthase inhibitor, Nω-Nitro-L-arginine (L-NNA) (100 µmol/L). (vi) Ad.PRS-nNOS treatment significantly decreased (**P<0.01, unpaired t test, n=15) the [3H]NA release compared with Ad.PRS-eGFP control (n=11). L-NNA (100 µmol/L) can reverse this response (**P<0.01, unpaired t test, compared with AdPRS-nNOS, n=6).

Fig 4

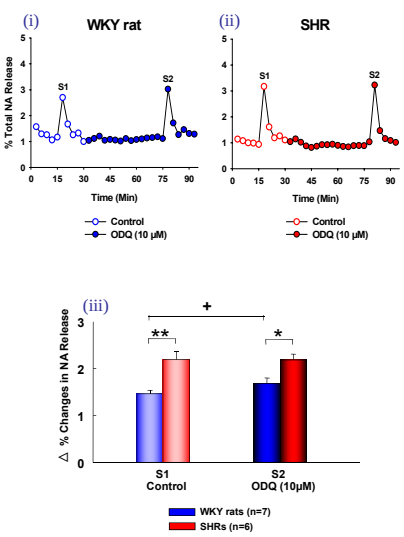
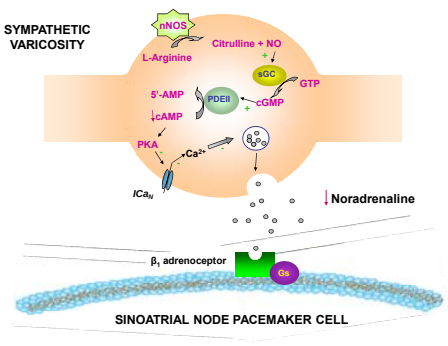


Fig. 4. Raw data trace (i) (ii) and group data (iii) showing [^{3}H]NA release is significantly enhanced in SHRs (n=6) compared with WKY rats (n=7). The soluble guanylyl cyclase inhibitor, 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 µM) significantly enhanced the [^{3}H] NA release in WKY rats, but no changes in SHRs (*P<0.05, **P<0.01,unpaired t test; +P<0.05, paired t test).

Summary



Adapted from Paton et al 2002[3]

Conclusion

NA release is significantly elevated in SHR compared with WKY rats. Noradrenergic cell specific gene transfer with nNOS can increase NOS activity resulting in inhibition of cardiac sympathetic transmission in normotensive SD rats. This targeted technique may provide a novel method for reducing sympathetic hyperactivity in pathological state such as hypertension.

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The Wellcome Trust