Targeted overexpression of nNOS into cardiac noradrenergic
neurons attenuates sympathetic neurotransmission

Dan Li, Lijun Wang, Tom Dawson, Chee-Wan Lee, David J. Paterson
Department of Physiology, Anatomy and Genetics, University of Oxford, Parks Road, Oxford, U.K. OX1 3PT

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, but non-specific adenoviral vectors can cause promiscuous transduction. This problem can be circumvented by targeting the NO pathway into specific neuronal 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 NO-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 anesthesia. Adenoviral vectors encoding nNOS or eGFP were driven by a noradrenergic2 promoter. Animals received a right atrial injection of 5×10⁷ virus particles in phosphate-buffered saline.

Measurement of [3H]noradrenaline ([3H]NA) release

WKYs & SHRs (16-20 weeks) or gene transferred SD rats were used. Release of radiolabeled [3H]NA was measured using labelled [3H]NA isolated right atrium in response to field stimulation. NA 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) expression.

Measurement of nNOS activity

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-NAME (10⁻⁴ M), nitro-L-arginine, Dlhydroxocarotene.

Results

Fig 1

Summary

Fig 2

Summary

Fig 3

Results

Fig 4

Summary

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.

References


Conclusion

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