Cardiac nNOS gene transfer decreases beta-adrenergic hyper-responsiveness and enhances vagal function in spontaneously hypertensive rats

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INTRODUCTION

Hypertension is associated with cardiac sympathetic hyper-responsiveness and impaired vagal function. NO derived from nNOS plays an important role in the autonomic regulation of cardiac excitability, directly enhancing the negative chronotropic effect of cholinergic stimulation (e.g.: 1-3), by activating the guanylate cyclase / cGMP pathway to facilitate release of ACh (4). NO also exerts complementary actions on cardiac sympathetic responsiveness, inhibiting the heart rate (HR) response to both sympathetic nerve stimulation and bath-applied norepinephrine (5). We recently showed in the guinea pig that adenoviral-mediated gene transfer of nNOS to the right atrium results in increased vagal neurotransmission and gain of function (6), combined with blunted beta-adrenergic responsiveness (7). We attempted to apply this technique to the spontaneously hypertensive rat (SHR), an animal model which displays blunted cardiac vagal function and sympathetic hyper-responsiveness.

Hypothesis:

We tested the hypothesis that nNOS gene transfer to the right atrium of the SHR would blunt beta-adrenergic hyper-responsiveness and enhance vagal function, producing a beneficial switch in cardiac autonomic phenotype.

RESULTS

Molecular phenotype following gene transfer

Beta-adrenergic responsiveness of SHR atria normalised by gene transfer of nNOS

Parasympathetic phenotype of transfected SHRs

Conclusions

- The SHR displays autonomic dysfunction, characterised by beta-adrenergic hyper-responsiveness relative to the normotensive WKY rat.
- Right atrial gene transfer of nNOS normalizes beta-adrenergic responsiveness of the SHR. This effect is partially reversed by pharmacological nNOS inhibition.
- nNOS gene transfer also enhances vagal function in the SHR. This occurs pre-synaptically, since the heart rate response to carbachol is unaffected.
- This beneficial switch in autonomic phenotype may have cardioprotective implications.

METHODS

Gene transfer to the right atrium of the rat

Percutaneous gene transfer to the right atrium was performed in male SHRs (20-24 weeks old) and age-matched normotensive WKY rats, under halothane anaesthesia. A suspension of replication-deficient adenovirus encoding either nNOS (Ad.nNOS) or eGFP (Ad.eGFP; control virus) was used. Animals received a right atrial injection of 5x10¹⁵ virus particles in phosphate-buffered saline.

Autonomic phenotyping of transfected animals

Following an incubation period of ~5 days, HR responses to 0.1-5.0µM norepinephrine were measured in isolated atria, before and after NOS inhibition with Nω-nitro-L-arginine (L-NA, 100µM). Isolated atria were also used to measure HR responses to the muscarinic agonist carbachol (0.1-0.2µM). HR responses to 3-10Hz (15V, 1ms pulse duration) vagal nerve stimulation were measured in anaesthetised animals following bilateral vagotomy. Expression of nNOS and eGFP protein in isolated right atria was measured using Western blotting.