CARDIAC nNOS GENE TRANSFER DECREASES BETA-ADRENEGRIC HYPER-RESPONSIVENESS AND ENHANCES VAGAL FUNCTION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Hypertension is associated with cardiac sympathetic hyper-responsiveness and impaired vagal function. We tested the hypothesis that cardiac nNOS gene transfer would restore autonomic balance in the spontaneously hypertensive rat (SHR), since pharmacological evidence suggests that NO exerts a beneficial effect on sympathovagal activity. Percutaneous gene transfer to the right atrium was performed in 20-22 week old SHRs, using $5x10^{10}$ particles of replication-deficient adenovirus encoding nNOS (Ad.nNOS) or green fluorescent protein (Ad.eGFP; control virus). After ~5 days animals were re-anesthetised and heart rate (HR) responses to 3-10Hz right vagal nerve stimulation (VNS) were measured. Isolated atria were used to measure HR responses to norepinephrine (NE; 0.1-5.0μM) and carbachol (0.1-0.2μM). Western blotting showed increased right atrial expression of nNOS protein in the Ad.nNOS group. A comparison of dose-response curves to 0.1-5.0μM NE in age-matched SHRs (n=20) and normotensive Wistar-Kyoto rats (WKY, n=6) showed beta-adrenergic hyper-responsiveness in the SHR (0.1μM: 66±7 beats per minute (bpm) vs 25±5bpm (WKY); 5μM: 164±6 vs 130±5 bpm (WKY), $p<0.01$). Treatment with Ad.nNOS (n=8) blunted responses of SHR atria to NE relative to the Ad.eGFP group (n=20) (0.1μM: 22±8 vs 66±7 bpm, $p<0.01$; 5μM: 124±5 vs 164±6 bpm, $p<0.001$), suggesting a post-synaptic antagonism of beta-adrenergic signalling. Partial reversal was seen with the NOS inhibitor Nω-nitro-L-arginine at NE doses >0.5μM. In contrast, HR responses of Ad.nNOS treated SHRs to VNS *in vivo* were

significantly enhanced relative to the Ad.eGFP group at all frequencies tested, for example: 3Hz: -42±3 vs -19±4 bpm ($p<0.01$), 10Hz: -133±10 vs -65±3 bpm ($p<0.001$). The *in vitro* HR response to carbachol was unaffected suggesting a pre-synaptic effect of Ad.nNOS on vagal transmission. These results show that artificial up-regulation of cardiac nNOS via gene transfer may provide a novel intervention for correcting peripheral autonomic imbalance in hypertension.