Enhanced NOS-1 Expression with Gene Transfer Promotes Cardiac Vagal Gain of Function

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We tested the hypothesis that NOS-1 gene transfer into the right atrium would enhance vagally-induced bradycardia, since pharmacological evidence has implicated the NO-cGMP pathway in the facilitation of cholinergic neurotransmission. Percutaneous gene transfer to the atrial wall was performed in halothane-anaesthetised male guinea pigs, using replication-deficient adenoviruses encoding NOS-1 (Ad.NOS-1) or green fluorescent protein (eGFP) (Ad.eGFP; control virus). Animals received right atrial injections of $5x10^9$ – $5x10^{10}$ virus particles (Ad.NOS-1 or Ad.eGFP) in 300µl phosphate-buffered saline, or vehicle alone (sham). Isolated atria with intact right vagal nerve were removed following ~5 days incubation, and heart rate (HR) responses to 5Hz vagal nerve stimulation (VNS) were measured. Ad.NOS-1 treated right atria (n=8) showed greater expression of NOS-1 protein than those treated with Ad.eGFP (n=8; $p<0.05$). This was confirmed by NADPH-diaphorase staining of tissue cryosections. Expression of eGFP was seen only in atria from Ad.eGFP-treated animals, using both Western blotting and fluorescence microscopy. HR responses of Ad.NOS-1-treated atria to 5Hz VNS (-102±8 beats per minute (bpm), n=15) were greater ($p<0.05$) than those of both Ad.eGFP-treated (-67±5 bpm, n=17) and sham (-63±6 bpm, n=5) groups. Responses of Ad.eGFP-treated and sham preparations were not different. Treatment with the NOS inhibitor Nω-nitro-L-arginine significantly attenuated the enhanced vagal bradycardia in Ad.NOS-1-treated atria (from -102±8 to -74±9 bpm, n=15; $p<0.001$). NOS inhibition also attenuated ($p<0.01$) HR responses of Ad.eGFP-treated and sham preparations from -67±6 to -50±7

bpm (n=13), and from -67±6 to -58±4 bpm (n=5), respectively. Following NOS inhibition, responses of Ad.NOS-1, Ad.eGFP and sham-treated preparations to 5Hz VNS were not different. We also saw no significant difference among responses of the three groups to bath-applied carbamylcholine. This suggests that NOS-1 – derived NO acts pre-synaptically to enhance the bradycardia seen with VNS, and that up-regulation of NOS-1 via gene transfer may provide a novel method for increasing cardiac vagal tone.