DYSFUNCTIONAL REGULATION OF NEURONAL NITRIC OXIDE SYNTHASE (nNOS) IN RESPONSE TO EXERCISE-TRAINING IN MICE LACKING ONE nNOS ALLELE

Implications for neural control of heart rate

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Background: Recent evidence suggests that regulation of eNOS during exercise requires the presence of two intact alleles of the gene. We tested the hypothesis that a single gene polymorphism of nNOS would impair the neural control of heart rate following physical training, and that this phenotype could be rescued following targeted gene transfer of nNOS.

Methods and Results: Voluntary wheel-running for 5 weeks in male heterozygous nNOS knockout mice (nNOS+/-,+EX; 8-12 weeks old; n=12; peak performance 9.1$\pm$1.8 km/day) was undertaken and compared to age-matched wild-type mice (WT,+EX; n=12; 9.5$\pm$0.8 km/day). In WT, +EX mice, baroreflex sensitivity increased by 80% and HR responses to direct vagal nerve stimulation (VNS) *in-vitro* were also significantly enhanced compared to WT,-EX atria ($P<0.05$); whereas autonomic responses were absent in nNOS+/-,+EX compared to nNOS+/-,-EX . Inhibition of nNOS with L-VNIO attenuated HR responses to VNS in all atria *in-vitro* ($P<0.05$) and normalized the responses in WT, +EX with respect to WT,-EX atria. Effects of L-VNIO on VNS responses were reversed by L-arginine. Western Blot analysis confirmed that expression of nNOS protein in atria was only increased in WT, +EX compared to WT,-EX (by 78%; $P<0.05$). Basal expression of nNOS in nNOS+/-,-EX was not different compared to WT,-EX atria and nNOS was not increased in response to exercise in nNOS+/- atria. *In-vivo* nNOS gene transfer using adenoviruses to intracardiac ganglia enhanced choline acetyltransferase/nNOS colocalization and increased baroreflex sensitivity and vagal bradycardia *in-vitro* compared to gene transfer of eGFP in nNOS+/- mice ($P<0.01$). This difference was abolished by nNOS inhibition using L-VNIO *in-vitro*.

Conclusion: Genomic regulation of NO-bioavailability from nNOS in cardiac autonomic ganglia in response to physical training is dependent on both alleles of the gene. Although basal expression of nNOS is normal, polymorphisms of nNOS may prevent adaptations to the neural control of heart rate

following training associated with reduced arrhythmia. Targeted gene transfer of nNOS can rescue this impairment in the nNOS+/- mouse.