Online Supplement

Supplementation of nitric oxide attenuates AβPP and BACE1 protein levels in cerebral microcirculation of eNOS-deficient mice

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Contents:

Supplemental Methods

Animals

Male wild type (C57BL6) and eNOS$^{-/-}$ (Nos3^{tm1Unc}/J) mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice had free access to food and water. Wild type and eNOS$^{-/-}$ mice, 4 months of age, were treated with 30 mg/kg nitroglycerine b.i.d. or vehicle, via subcutaneous injections, for 3 days[1]. Upon completion of 3 day treatment, mice were sacrificed by lethal dose of pentobarbital. All animal care and use were approved by Mayo institutional Animal Care and Use Committee.

Glucose and Cholesterol measurements

Glucose was measured in whole blood using Accu Check (Roche Diagnostics, Indianapolis, IN). Blood was centrifuged (2,000 rpm, 10 mins, 4°C) and stored at -80° until all samples were collected. Total cholesterol levels were measured using the Hitachi 912 chemistry analyzer (Roche Diagnostics).

Blood pressure

Mice were trained for blood pressure measurements. Systolic blood pressure was measured before and at the end of treatment in non-anesthetized mice using the tail cuff method as previously described[2](Harvard Apparatus Ltd, Kent, England).

Cerebral microvessel isolation

Cerebral microvessels were isolated from brain tissue, devoid of large vessels, as previously described[3]. Tissue was homogenized in ice cold PBS with Dounce homogenizer and rinsed

twice in PBS. The resulting pellet was resuspended and layered over 15% Dextran/PBS (Sigma, St. Louis, MO) and centrifuged at 4500g for 30 minutes at 4°C. The supernatant was discarded and the final pellet was resuspended in 1% bovine serum albumin (BSA), the suspension was then passed through a 40 μm nylon mesh (BD Falcon). Microvessels were washed with 1% BSA/PBS and collected by centrifugation. Microvessels were resuspended in lysis buffer according to assay instructions.

Aβ ELISA

$A\beta_{40}$ and $A\beta_{42}$ from brain tissue lysates was measured using a commercially available colorimetric ELISA kit following manufacturer's instructions (Covance, Princeton, NJ).

Supplemental Table 1. Characteristics of wild type and eNOS$^{-/-}$ mice treated with nitroglycerin

	Wild type		eNOS -/-	
Systolic Blood pressure (mm Hg)	Vehicle	Nitroglycerin	Vehicle	Nitroglycerin
Before treatment	115.31±4.97	116.11±4.42	134.90±3.53*	133.87±3.58*
After treatment	116.09±2.12	112.92±1.95	131.54±3.67*	130.04±2.38*

	Wild type		eNOS -/-	
Parameter	Vehicle	Nitroglycerin	Vehicle	Nitroglycerin
Body weight (g)	31.32±2.21	31.69±2.35	28.62±2.08*	28.69±2.12*
Total Cholesterol (mg/dL)	79.13±9.94	73.63±13.83	79.87±14.95	84.00±11.93
HDL (mg/dL)	63.25±10.21	58.75±12.45	66.50±13.58	71.86±16.97
Glucose (mg/dL)	178.75±63.32	165.00±39.47	226.07±62.05	177.31±58.73
Triglycerides (mg/dL)	82.63±28.99	77.63±33.67	74.63±14.02	70.43±16.82

Data presented as mean ± SD (n=7-17)
Statistical significance based on genotype (*wild type versus eNOS$^{-/-}$; P<0.001)

Figure Legends:

Supplemental Table 1. Characteristics of wild type and eNOS$^{-/-}$ mice treated with nitroglycerin. Body weight, blood pressure, total cholesterol, HDL, glucose and triglycerides were measured in wild type and eNOS$^{-/-}$ mice treated with or without nitroglycerine. Data is presented as mean ± SD (n=7-17, $p<0.001$).

Supplementary References

[1] Otto A, Fontaine J, Tschirhart E, Fontaine D, Berkenboom G (2006) Rosuvastatin treatment protects against nitrate-induced oxidative stress in eNOS knockout mice: implication of the NAD(P)H oxidase pathway. *Br J Pharmacol* **148**, 544-552.

[2] d'Uscio LV, Smith LA, Katusic ZS (2011) Differential effects of eNOS uncoupling on conduit and small arteries in GTP-cyclohydrolase I-deficient hph-1 mice. *Am J Physiol Heart Circ Physiol*.

[3] Austin SA, Santhanam AV, Katusic ZS (2010) Endothelial nitric oxide modulates expression and processing of amyloid precursor protein. *Circ Res* **107**, 1498-1502.