ONLINE SUPPLEMENT

AUGEMENTATION OF HYPERTENSION AND PERIPHERAL INFLAMMATION BY REDUCTION OF EXTRACELLULAR SUPEROXIDE DISMUTASE IN THE CENTRAL NERVOUS SYSTEM

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Short Title: Central SOD3 in Hypertension

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Supplemental Methods:

Power Spectral Analysis of Heart rate and blood pressure: Data were extracted with a sampling rate of 500 Hz using the Hemolab Software Suite Version 8.3 (http://www.haraldstauss.com/HemoLab). Artifact free heart rate and systolic pressure beat-to-beat data were resampled at a frequency of 25 Hz and converted from non-equidistant to equidistant time series. From this equidistant data a spectral analysis was performed using the Fast Fourier Transformation technique as previously described. [10, 11]

Flow cytometric analysis of inflammatory cells: Aortas were cleared of blood by perfusion with phosphate-buffered saline (PBS), excised, and digested using collagenase type IX (125 U/ml), collagenase type IV (450 U/ml), and hyaluronidase IS (60 U/ml) dissolved in 20 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-PBS buffer containing calcium. The digested tissue was then passed through a 70 mm sterile cell strainer (Falcon, BD), yielding single cell suspensions.

Peripheral blood mononuclear cells (PBMC) were isolated from whole heparinized blood after osmotic lysis of red blood cells. Cells were then centrifuged (800 g), washed twice with PBS and 0.5% BSA (FACS buffer), counted, resuspended in 1% BSA/PBS and stored on ice. Within 30 min, 1x106 cells were stained for 15 min at 4°C with antibodies and washed twice with FACS buffer.

Cell labeling was performed using the following antibodies (all from BD Pharmingen): fluorescein isothiocyanate (FITC) anti-CD45 (30-F11); FITC anti-CD69 (H1.2F3); FITC γ/δ (GL3); FITC CD44 (IM7); PerCP anti-CD45 (30-F11); PerCP CD4 (RM4-5); PerCP anti-CD8 (53–6.7); PE anti-CD4 (GK1.5); PE anti-CD195 (CCR5); PE CD25 (PC61); PE TcR β chain (H57-597); APC anti-CD4 (RM4-5); APC anti-CD4 (GK1.5); APC anti-CD3 (145-2C11). Cells were washed twice with 1% bovine serum albumin-PBS buffer. After immunostaining, cells were resuspended in FACS buffer and analyzed immediately on a LSR-II flow cytometer with DIVA software (Becton Dickinson). For aortic tissue an initial gate was applied to exclude cell debris from further analysis, and CD45 staining was used to identify leukocytes within the aortic cell suspension. In this case, all data were analyzed as total cell number.

Quantitative real-time polymerase-chain-reaction: Aortas were removed immediately after euthanasia, snap-frozen in liquid nitrogen and homogenized. The lysate was cleaned with a QIAshredderTM column and RNA was extracted using the RNeasy Mini Kit (Qiagen, USA) according to the manufacturer's protocol. RNA with an A260/280-ratio between 1.6 and 2.0 was used for reverse transcription using the High Capacity cDNA Reverse Transcription Kit from Applied Biosystems. Quantitative real-time PCR was performed in an ABI 7500

Fast Thermocycler (Applied Bioscience, USA) using SYBR Green (SuperArray Bioscience). Primers for Rantes, ICAM-1, IL-17A, VCAM-1 and GAPDH were obtained from SuperArray Bioscience. Total mRNA copy numbers were normalized to GAPDH.