Supplemental Material

Mice

$\beta arr2^{+/+}$ and $\beta arr2^{-/-}$ mice[1] on a C57BL/6 background were generously provided by Dr. Robert Lefkowitz (Duke university, Durham, NC). $\beta arr2^{-/-}$ mice were crossed with hyperlipidemic $ApoE^{-/-}$ mice (stock #002052, Jackson laboratories, Bar Harbor, ME) and the resulting mice ($ApoE^{+/-}$ /$\beta arr2^{+/-}$) were crossed to generate $ApoE^{-/-}$/$\beta arr2^{-/-}$ mice and their littermate controls ($ApoE^{-/-}$ /$\beta arr2^{+/+}$). Male, hyperlipidemic $ApoE^{-/-}$/$\beta arr2^{+/+}$ and $ApoE^{-/-}$/$\beta arr2^{-/-}$ mice at 8-10 weeks of age were use for measuring AngII-induced AAA incidence and severity and for mechanistic experiments. AngII-induced AAA incidence was also examined in 8-month old, male, normolipidemic $\beta arr2^{+/+}$ and $\beta arr2^{-/-}$ mice on a C57BL/6 background. All mice were housed under barrier conditions at the NIEHS animal husbandry facility and food and water were provided *ad libitum*. All studies were performed with the approval of the NIEHS Institutional Animal Care and Use Committee.

AAA quantitation

Mice were anesthetized by isoflurane inhalation and AngII (1000ng/kg/min; Sigma, St. Louis, MO) or saline was administered via subcutaneously implanted osmotic mini-pumps (Model 1004, Durect, Cupertino, CA) as described previously.[2, 3] Mice were euthanized after 28 days of AngII infusion and the aortas were perfused with ice-cold PBS followed by 10% formalin. The abdominal aortas were dissected out and immediately placed in PBS and cleaned of adventitial fat. The maximal abdominal aortic diameter was measured using a caliper under a dissecting microscope while the aortas were in PBS without physical stretching. A >50% increase in external diameter of the abdominal aorta was used to define the occurrence of an AAA. AAA severity was classified visually using a classification scheme similar to the one described previously,[3] where Type 1 represents a simple dilation of the abdominal aorta with an external diameter from 1.5-2 mm, Type 2 represents a remodeled AAA with the external diameter from 2-3 mm and Type 3 represents a pronounced bulbous containing a thrombus and an external aortic diameter of >3 mm. Mice in the Type 4 AAA category were those that died due to aneurysmal rupture and resultant bleeding in the peritoneal cavity. The presence of an AAA as well as the scoring of AAA pathology was determined by an investigator who was blinded to the mouse genotypes. Upon determination of the AAA incidence and classification, another investigator matched the scored AAAs to the genotypes of the mice. AAA severity was also determined by measuring the wet weights of the abdominal aortas.

Blood Pressure Measurements

Male $ApoE^{-/-}$/$\beta arr2^{+/+}$ and $ApoE^{-/-}$/$\beta arr2^{-/-}$ mice were used for the blood pressure measurements and were fed a normal chow diet with free access to water. Systolic blood pressure was measured in conscious, unrestrained mice by radio-telemetry using an indwelling transducer-tipped catheter (TA11PA-C20; Data Sciences International, St. Paul, MN, USA) that was surgically inserted into the right carotid artery.[4, 5] Mice were anesthetized with 2.5% isoflurane with a continuous flow of 100% O_2 and given 0.01 mg/kg Buprenorphine Hcl for analgesia, during the surgical insertion of the catheter. Mice were allowed to recover for 1 week following the surgery and baseline measurements were obtained continuously for 2 hours each morning, and averaged to yield a daily value. Ten days of baseline measurements were recorded. Mice were then implanted with osmotic mini-pumps containing AngII (1000ng/kg/min; Sigma, St. Louis, MO) and measurements were obtained for an additional 10 days. Daily values were averaged for statistical comparisons between genotypes.

Inhibition of ERK1/2 activation

The MAPK kinase 1(MEK1) inhibitor, CI1040 was used to examine the effects of inhibition of ERK1/2 activation. AngII-treated ApoE$^{-/-}$/βarr2$^{+/+}$ mice were injected subcutaneously with 100mg/kg/day CI1040 (Selleckchem, Houston, TX) or vehicle, 1 day prior to mini-pump implantation, and the injections were continued for 7 days simultaneously with the AngII infusion. CI1040 was suspended in an 8:1:1 PBS: ethanol: cremophore solution.

Histology and Immunohistochemistry

ApoE$^{-/-}$/βarr2$^{+/+}$ and ApoE$^{-/-}$/βarr2$^{-/-}$ mice were euthanized using CO_2 inhalation at the designated time-points (7 or 28 days) and the aortas were perfused with ice-cold PBS followed by 10% formalin solution. The abdominal aortas were dissected, cleaned of adventitial fat and fixed in 10% formalin solution overnight followed by processing and paraffin embedding. Serial sections (0.006 mm thickness) of the abdominal aortas were stained with hematoxylin and eosin (H&E) or Verhoeff van Geisen (VVG, elastin) for histological analysis. Sections were also subject to immunohistochemical analysis for COX-2 (Vector labs, Burlingame, CA), Mac-3 (BD Biosciences, San Jose, CA), or p-ERK1/2 (Cell Signaling Technology, Danvers, MA) expression. Antibody binding was detected using the Vectastain Elite ABC kit and di-amino benzidine (DAB) staining using manufacturer's instructions (Vector labs, Burlingame, CA). Quantitation of immuno-positive cells was performed by determining the ratio of the number of positive cells (COX-2, p-ERK1/2 or Mac-3) to the total number of hematoxylin positive cells in a defined field (at 400X magnification) on multiple slides (a minimum of 5 slides per mouse per genotype and treatment).

Quantitation of mRNA expression

Abdominal aortic tissue from ApoE$^{-/-}$/βarr2$^{+/+}$ and ApoE$^{-/-}$/βarr2$^{-/-}$ mice was dissected as described above and stored immediately in RNAlater solution (Ambion, Austin, TX) at 4°C. Total RNA was extracted from individual abdominal aortic segments using the Qiagen RNeasy fibrous mini kit (Qiagen, Alameda, CA) followed by reverse transcription using the Superscript III First-Strand Synthesis Supermix (Life Technologies, Grand Island, NY). Real time quantitative PCR analysis was performed for COX-2, CD68, MCP-1, MIP1α, MMP2 or MMP9, using Taqman gene expression assays (Applied Biosystems/Life Technologies, Carlsbad, CA). Fold differences in gene expression were calculated using the ΔΔCt method using hypoxanthine phosphoribosyl transferase (HPRT) as the endogenous control.

Statistics

Data are shown as the mean ± SEM of independent experiments. Fisher's exact test was used to compare AAA incidence among different groups. Two-way ANOVA was use to examine changes in blood pressure between genotypes. One-way ANOVA followed by Bonferroni's multiple comparison tests were used to determine the statistical significance of the data in all other experiments. Values with $P<0.05$ were considered statistically significant.

References

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