Exacerbation of adverse cardiovascular effects of aircraft noise in an animal model of arterial hypertension

Steven, Sebastian; Frenis, Katie; Kalinovic, Sanela; Kvandova, Miroslava; Oelze, Matthias; Helmstädter, Johanna; Hahad, Omar; Filippou, Konstantina; Kus, Kamil; Trevisan, Chiara; Schlüter, Klaus Dieter; Boengler, Kerstin; Chlopicki, Stefan; Frauenknecht, Katrin; Schulz, Rainer; Sorensen, Mette; Daiber, Andreas; Kröller-Schön, Swenja; Münzel, Thomas

Published in:
Redox Biology

DOI:
10.1016/j.redox.2020.101515

Publication date:
2020

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Steven, S., Frenis, K., Kalinovic, S., Kvandova, M., Oelze, M., Helmstädter, J., Hahad, O., Filippou, K., Kus, K., Trevisan, C., Schlüter, K. D., Boengler, K., Chlopicki, S., Frauenknecht, K., Schulz, R., Sorensen, M., Daiber, A., Kröller-Schön, S., & Münzel, T. (2020). Exacerbation of adverse cardiovascular effects of aircraft noise in an animal model of arterial hypertension. *Redox Biology*, *34*, Article 101515. https://doi.org/10.1016/j.redox.2020.101515

Redox Biology 34 (2020) 101515

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Research Paper

Exacerbation of adverse cardiovascular effects of aircraft noise in an animal model of arterial hypertension

Sebastian Steven[a,b,1], Katie Frenis[a,1], Sanela Kalinovic[a], Miroslava Kvandova[a], Matthias Oelze[a], Johanna Helmstädter[a], Omar Hahad[a,i], Konstantina Filippou[a], Kamil Kus[c], Chiara Trevisan[d], Klaus-Dieter Schlüter[e], Kerstin Boengler[e], Stefan Chlopicki[c,f], Katrin Frauenknecht[d], Rainer Schulz[e], Mette Sorensen[g,h], Andreas Daiber[a,i,*], Swenja Kröller-Schön[a,1], Thomas Münzel[a,i,*,1]

[a] Center for Cardiology, Cardiology I – Laboratory of Molecular Cardiology, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany
[b] Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany
[c] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland
[d] Institute of Neuropathology, University Hospital, Zurich, Switzerland
[e] Department of Physiology, Justus-Liebig University Gießen, Germany
[f] Chair of Pharmacology, Jagiellonian University Medical College, Krakow, Poland
[g] Danish Cancer Society, Copenhagen, Denmark
[h] Department of Natural Science and Environment, Roskilde University, Roskilde, Denmark
[i] German Center for Cardiovascular Research (DZHK), Partner Site Rhine-Main, Mainz, Germany

ARTICLE INFO

Keywords:
Environmental noise exposure
Arterial hypertension
Vascular oxidative stress
Inflammation
Endothelial function

ABSTRACT

Arterial hypertension is the most important risk factor for the development of cardiovascular disease. Recently, aircraft noise has been shown to be associated with elevated blood pressure, endothelial dysfunction, and oxidative stress. Here, we investigated the potential exacerbated cardiovascular effects of aircraft noise in combination with experimental arterial hypertension. C57BL/6J mice were infused with 0.5 mg/kg/d of angiotensin II for 7 days, exposed to aircraft noise for 7 days at a maximum sound pressure level of 85 dB(A) and a mean sound pressure level of 72 dB(A), or subjected to both stressors. Noise and angiotensin II increased blood pressure, endothelial dysfunction, oxidative stress and inflammation in aortic, cardiac and/or cerebral tissues in single exposure models. In mice subjected to both stressors, most of these risk factors showed potentiated adverse changes. We also found that mice exposed to both noise and ATII had increased phagocytic NADPH oxidase (NOX-2)-mediated superoxide formation, immune cell infiltration (monocytes, neutrophils and T cells) in the aortic wall, astrocyte activation in the brain, enhanced cytokine signaling, and subsequent vascular and cerebral oxidative stress. Exaggerated renal stress response was also observed. In summary, our results show an enhanced adverse cardiovascular effect between environmental noise exposure and arterial hypertension, which is mainly triggered by vascular inflammation and oxidative stress. Mechanistically, noise potentiates neuroinflammation and cerebral oxidative stress, which may be a potential link between both risk factors. The results indicate that a combination of classical (arterial hypertension) and novel (noise exposure) risk factors may be deleterious for cardiovascular health.

1. Introduction

Arterial hypertension represents a cardiovascular risk factor that has been shown to substantially increase mortality and morbidity [1]. A meta-analysis of 42 trials and 144 220 patients demonstrated a direct linear relationship between each increase of 5 mmHg in systolic pressure and risk of cardiovascular mortality [2]. In addition to the strong correlation with cardiovascular events and mortality, arterial hypertension also has a high prevalence of 1.3 billion worldwide [3]. For this reason, it is important to establish the precise pathophysiological

* Corresponding authors. Center for Cardiology, Cardiology I – Laboratory of Molecular Cardiology, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany.
E-mail addresses: daiber@uni-mainz.de (A. Daiber), tmuenzel@uni-mainz.de (T. Münzel).
[1] S.S., K.F. and S.K.-S., T.M. contributed equally and should be considered joint first or senior authors.

https://doi.org/10.1016/j.redox.2020.101515
Received 19 February 2020; Received in revised form 18 March 2020; Accepted 19 March 2020
Available online 18 April 2020

List of abbreviations

3-NT	3-nitrotyrosine
4-HNE	4-hydroxynonenal
ACh	acetylcholine
ATII	angiotensin II
CAD	coronary artery disease
cGMP	cyclic guanosine monophosphate
dB(A)	decibel
DHE	dihydroethidium
ET1	endothelin-1
HO-1	heme oxygenase-1
IL	interleukin
L-NAME	N(gamma)-nitro-L-arginine methyl ester
NIBP	non-invasive blood pressure
NOX	NADPH oxidase
PAI-1	plasminogen activator inhibitor-1
ROS	reactive oxygen species
VCAM-1	vascular cell adhesion molecule-1

interactions between arterial hypertension and other cardiovascular risk factors.

Environmental noise pollution has been identified as a cardiovascular risk factor and has been associated with arterial hypertension [4–7]. Translational studies in humans both with and without established coronary artery disease have found that in particular, nighttime noise is associated with an increase in blood pressure, endothelial dysfunction, and an increase in stress hormone levels [8,9]. The genesis of these noise-dependent pathophysiological changes is likely in part related to an increase in oxidative stress and the subsequent decreased nitric oxide bioavailability within the vasculature [8,9]. Importantly, the adverse effects of aircraft noise on vascular function were more pronounced in patients with coronary artery disease (CAD) compared to healthy subjects [10], suggesting that in the presence of preexisting CAD and/or cardiovascular risk factors, aircraft noise may have additional or even potentiating negative impact on the cardiovascular system [10]. In a recently established animal model, we provided molecular insight into the vascular and cerebral consequences of aircraft noise [11,12]. In particular, nighttime noise exposure was associated with increased oxidative stress within the vasculature and the brain, with increased stress hormone levels, endothelial dysfunction and increased evidence for inflammation [11,12]. Phagocytic NADPH oxidase (NOX-2) and uncoupled nitric oxide synthase have also been identified as significant sources of superoxide [11,12]. NADPH oxidase is believed to be the mediator by which angiotensin II (ATII) causes arterial hypertension and oxidative stress [13–15], suggesting that a combination of ATII and noise may have additive or even potentiating adverse effects on arterial hypertension. Thus, the specific question being addressed in the present study is whether aircraft noise may further enhance oxidative stress in the vessels and the brain, inflammatory reactions, endothelial dysfunction when applied simultaneously with ATII.

2. Methods

2.1. Animals

All animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the U.S. National Institutes of Health with approval granted by the Ethics Committee of the University Medical Center Mainz and the Landesuntersuchungsamt Rheinland-Pfalz (Koblenz, Germany; permit number: 23 177–07/G 15-1-094 and G 18-1-084). All mice were male C57BL/6J between 6 and 12 weeks old, purchased from Janvier. The mice were housed in a standard 12-h light/dark cycle with free access to food and water. A total number of 255 mice were used for the study. Four groups of mice were used: untreated controls (CTR), noise-exposed for 7 days (Noise), ATII infusion for 7 days (ATII), and ATII infusion for 7 days plus simultaneous noise exposure for 7 days (ATII + Noise). Prior to the start of the experiment, mice were trained three times and a baseline blood pressure measurement was taken. Following mini-pump implantation and subsequent noise exposure, animals were sacrificed by cardiac puncture under isoflurane anesthesia. The heart, aorta, kidney, brain and plasma were harvested.

2.2. Minipump implantation

Alzet minipumps were loaded with ATII obtained from Bachem AG as acetate salt (prod no. 4006473) in 0.9% NaCl solution to deliver a dose of 0.5 mg/kg/d. Pumps were implanted subcutaneously in mice anesthetized with isoflurane as described [16,17]. Briefly, an incision of approximately 1 cm was made on the dorsal flank to allow for the insertion of the minipump. Immediately following the pump implantation, the wounds were treated with lidocaine ointment and mice were moved to the noise exposure chamber.

2.3. Noise exposure

The noise chamber maintains the same housing conditions as the institutional animal facility in order to minimize transfer stress. Mice were exposed to 43-s-long noise events, irregularly distributed over a 2-h sequence, repeated constantly over a 7-day period. Noise was applied through downward-facing speakers positioned approximately 30 cm above open mouse cages as described [11,12]. The sound system is a Grundig MS 540 with a total output of 65 W. The noise events had a maximum sound pressure level of 85 dB(A) and a mean of 72 dB(A) and were separated by silent periods with irregular distribution to prevent early adaptation. Sound pressure levels were calibrated with a Class II Sound level meter (Casella CEL-246) within one of the cages at initial setup.

2.4. Non-invasive blood pressure measurements (NIBP)

NIBP measurements were performed on days 0, 2, 4, and 6 of the treatment regimen using a CODA High Throughput 2 Noninvasive Blood Pressure System (Kent Scientific, Torrington, USA). This method has been proofed in accuracy in comparison to radiotelemetric measurement by Feng et al. [18]. Measurements were preceded by three training sessions to acclimate the animal before the baseline measurement. Conscious animals were allowed to enter the restraining tube freely, placed in restraining tubes on a preheated platform (32 °C), and allowed to rest for 15 min. Fifteen NIBP measurements were taken per animal, the first five of which were discarded as acclimation cycles. The mean values of the remaining 10 NIBP measurements were used in calculations. Measurements took place at a set time each day to account for diurnal variation of blood pressure.

2.5. Immunohistochemistry for brain astrocytes

Immunohistochemistry was performed on 4–5 μm thick de-paraffinized sagittal brain sections as described previously [11]. In brief, the slices were first incubated in Ventana buffer, followed by automatic staining procedure using the NEXEX immunohistochemistry robot (Ventana Instruments). Primary antibody for astrocytes (GFAP; 1:300; polyclonal, rabbit, DAKO, Glostrup) was used and the sections were developed using iVIEW DAB Detection Kit (Ventana). For negative

controls, the primary antibodies were omitted. Sections were scanned under equal lighting conditions using a Hamamatsu tissue scanner (Nanozoomer). Afterwards, Hamamatsu NDPI images of each scanned slide were transferred to a computer screen (Viewer Version DIH 4.0.0-20130402-63).

2.6. Detection of astrogliosis

Representative micrographs (TIF) from the corpus callosum (cc) were captured at 20x magnification. The micrographs were processed by using ImageJ software (https://imagej.net/ImageJ [19]) as follows. Color deconvolution function (vector H DAB) was used to unmix RGB images. Micrographs from channel two (brown signal) were then processed by using the threshold function. The GFAP immunoreactive area in % was then quantified in two adjacent subfields (size 400 × 200 µm each) of the cc by using the analysis function and were then normalized to control level (baseline = 100%).

2.7. Statistical analysis

Results are expressed as mean ± SEM. All statistical calculations were performed with GraphPad Prism 8.01. Two-way ANOVA with Bonferroni's correction were used for comparisons of concentration-relaxation curves and blood pressure over time. For endpoint blood pressure, Western blot, dot blot, rtPCR, flow cytometry, immunohistochemical, and oxidative stress parameters, one-way ANOVA with Bonferroni's or Tukey's correction was used for the comparison of multiple means. In cases of failed normality, the Kruskal-Wallis test (Dunn multiple comparison) was used. All $p < 0.05$ are considered significant denoted by: * vs. Control, # vs. Noise, $ vs. ATII, and § vs. ATII + Noise. The number of replicates in the different assays may vary since not all animals were used in all assays.

2.8. Supplementary methods

Detailed methodological descriptions for all other methods are described in the online-only supplement: Vascular function by isometric tension studies [20,21]; protein expression by immunohistochemistry [22]; detection of oxidative stress in aortic cryo-sections by dihydroethidium fluorescence microtopography [23–25], by dot blot analysis of oxidative stress markers [11,12], by dihydroethidium-based HPLC analysis (aorta, heart, or frontal cortex) [23,26,27], by mitoSOX-based HPLC analysis of cardiac mitochondria [28,29] and by S-glutathionylation of eNOS using immunoprecipitation [30,31]; protein expression by Western blotting of aortic [11,12] and cardiac tissues [32]; mRNA expression by quantitative reverse-transcriptase PCR of aortic [16,22] and renal tissues [33,34]; quantification of nitrite in plasma by HPLC [35]; vascular infiltration of immune cells by flow cytometry [14,36].

3. Results

3.1. Effects of aircraft noise and ATII treatment on blood pressure

Using tail cuff plethysmography, we observed a significantly higher systolic blood pressure in the mice that were exposed to both ATII and

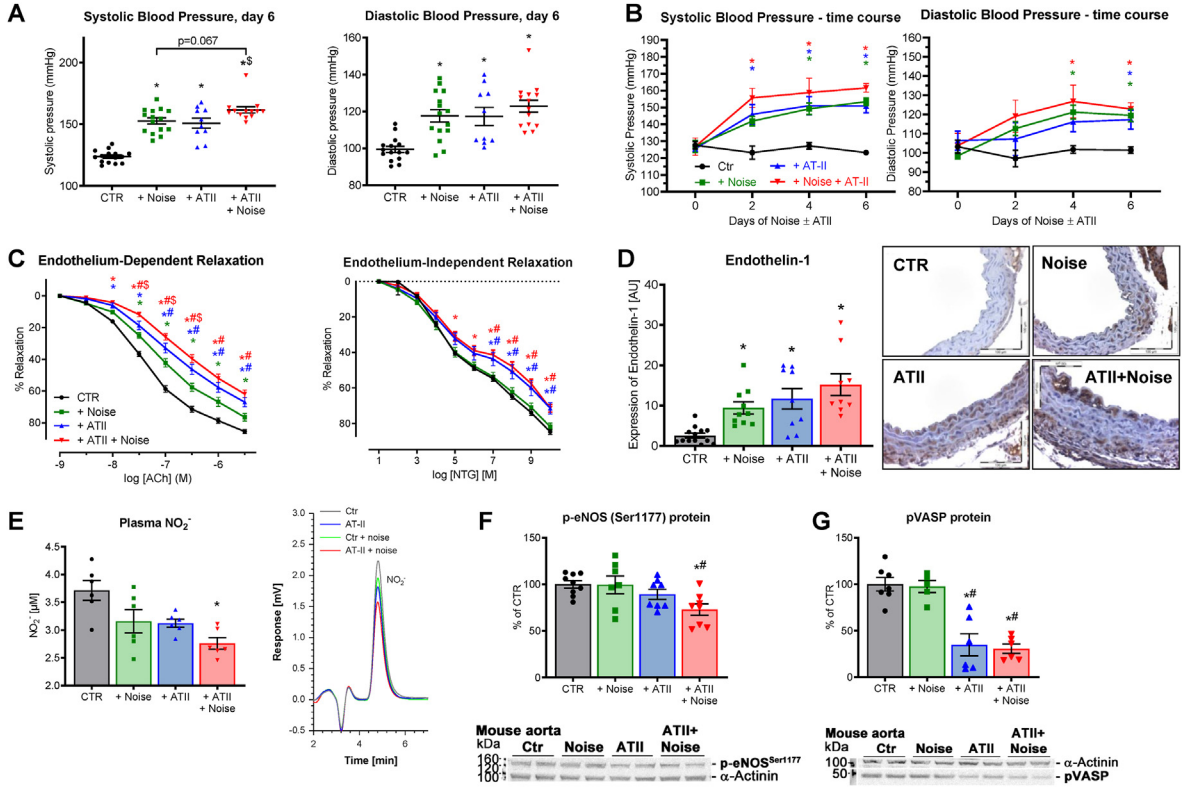

Fig. 1. Blood pressure, vascular function and NO signaling show exacerbated impairment in hypertensive mice exposed to noise. (A) Systolic and diastolic arterial blood pressure measured on the final day of the treatment. (B) The respective time courses of blood pressure over entire treatment period. (C) Endothelium-dependent (ACh) and -independent (NTG) relaxation of thoracic aortic rings was measured by isometric tension method. (D) Densitometric analysis and representative pictures of immunohistochemical stainings for endothelin-1 in aortic sections. Magnification 40x, scale bars represent 50 µm. (E) HPLC quantification of plasma nitrite concentrations with representative chromatograms. (F,G) Levels of eNOS phosphorylated at Ser1177 and VASP phosphorylated at Ser239 with representative Western blots. Data points are measurements from individual samples; 2-way ANOVA with Bonferroni's multiple comparison test (B,C) or 1-way ANOVA with Tukey's multiple comparison test (A,D-G); n = 10–15 (A), 4–17 (B), 15–42 (C) or 9–13 mice per group (D) or n = 5–9 samples (each pooled from 2 to 3 animals) (E–G). *$P < 0.05$ vs. untreated controls; $^{\#}P < 0.05$ vs. +Noise; $^{\$}P < 0.05$ vs. +ATII.

noise as compared to the ATII-only group and a borderline significant increase (p = 0.067) as compared to the noise-only group (Fig. 1A). For diastolic blood pressure, we observed no significant differences between the three exposure regimens (ATII, Noise and ATII + Noise), but the systolic and diastolic blood pressure of all treatment groups were increased compared to untreated mice (Fig. 1A). These effects were stable and were observed over the course of the treatment period in mice that underwent both ATII and noise treatments (Fig. 1B).

3.2. Effects of aircraft noise and ATII treatment on endothelium-dependent and -independent vasodilation

Noise exposure significantly impaired endothelium-dependent vasodilation (acetylcholine [ACh]-response) with no effect on endothelium-independent direct vasodilation by the nitrovasodilator nitroglycerine (NTG-response) (Fig. 1C). ATII-only treatment negatively impacted vasodilation of both types stronger than noise-only treatment. The negative effect on the endothelium-dependent vasodilation of ATII + Noise exposure was significantly higher than in the noise-only group. Endothelium-independent vasodilation was impaired in all ATII-treated groups without additional adverse effects by noise exposure (Fig. 1C). Alongside our isometric tension studies, immunohistochemical stainings revealed markedly increased levels of the vasoconstrictor endothelin-1 in aortic tissue of noise- or ATII-only groups with a minor tendency for exacerbated effects in the mice that underwent both treatments (Fig. 1D).

3.3. Effects of aircraft noise and ATII treatment on vascular NO signaling

Levels of plasma nitrite were decreased in all treatment groups compared to controls mice, with lowest levels in the ATII + Noise group (Fig. 1E). Levels of pSer^{1177}-eNOS were slightly and insignificantly decreased in the ATII-only group, whereas in the ATII + noise-treated group we found a statistically significant decrease in pSer^{1177}-eNOS levels (Fig. 1F). The surrogate parameter of NO/cGMP/PKG signaling, phosphorylated Ser^{239}-Vasodilator-Stimulated Phosphoprotein (P-VASP), was decreased in both ATII treated groups without additional effects by noise exposure (Fig. 1G).

3.4. Effects of aircraft noise and ATII treatment on vascular ROS formation

Both ATII- and noise-exposed mice had higher levels of oxidative stress in plasma as well as aortic and cardiac tissue (4-hydroxynonenal, dihydroethidium aortic cryo staining and HPLC assays for superoxide), compared to untreated controls (Fig. 2). These levels tended to be exacerbated in the ATII + Noise group (Fig. 2A and B), which was most evident when superoxide was measured by HPLC assays, illustrating significant enhanced effects (Fig. 2C and D) and indicating heightened generation of ROS and/or impairment in oxidant clearance. The marker of nitro-oxidative stress, 3-nitrotyrosine, was increased in all treatment groups in plasma and the aorta, with potentiated aggravation in the ATII + Noise group (suppl. Fig. S1).

3.5. Effects of aircraft noise and ATII treatment on NOX-2 expression and eNOS uncoupling

Aortic NOX-2 protein levels were increased in ATII treated group and in the ATII + Noise group as compared to controls. There was a tendency towards highest levels in the ATII + Noise group, indicating it as a likely source for the increased oxidant burden (Fig. 3A). NOX-2 protein data were confirmed by NOX2 mRNA expression that was increased in all treatment groups and by trend further aggravation in the ATII + Noise group (suppl. Fig. S2). Furthermore, to investigate the functional status of eNOS, we examined eNOS S-glutathionylation, a

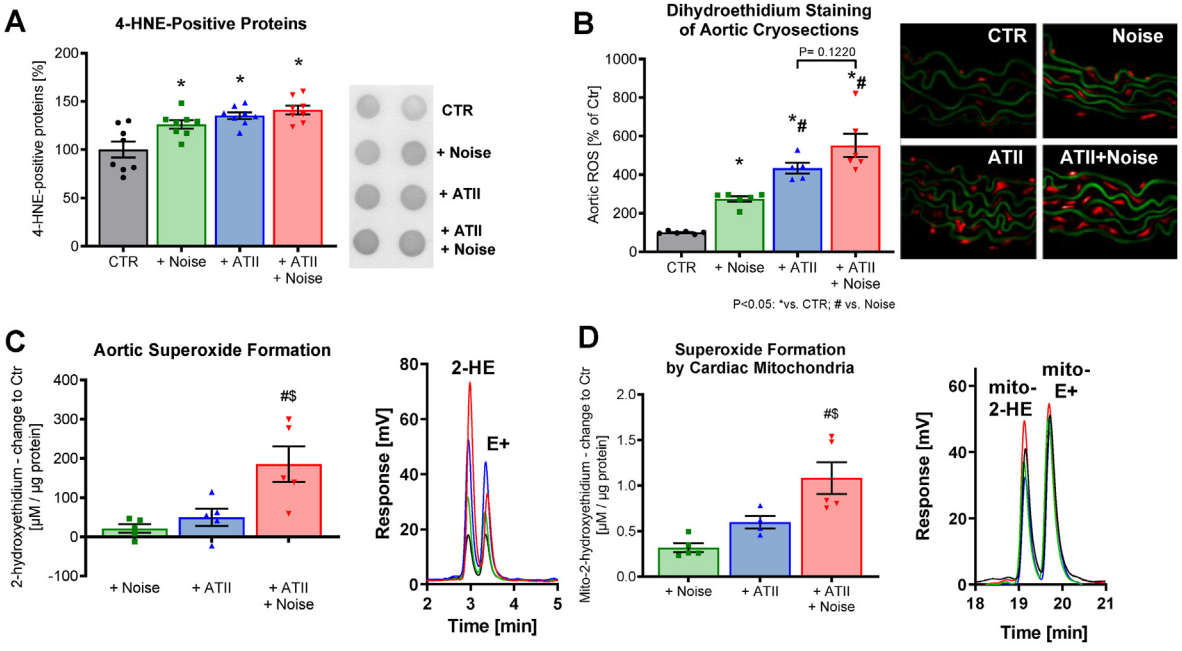

Fig. 2. Oxidative stress is increased by both hypertension and noise exposure and shows potentiated aggravation in the presence of both stressors. (A) Densitometry and representative dot blot of 4-hydroxynonenal (4HNE)-positive proteins in plasma denoting lipid peroxidation as a consequence of superoxide formation. (B) Dihydroethidium stainings of aortic cryosections and their representative photomicrographs show ROS formation as red fluorescence and autofluorescence from aortic laminae as green. (C) Quantification and representative chromatograms of superoxide levels in aortic tissue as measured by HPLC analysis of 2-hydroxyethidium formation and expressed as changes to untreated control. (D) MitoSOX was used in HPLC analysis of isolated cardiac mitochondria as an indicator of mitochondria-specific superoxide formation expressed as changes to untreated control. Data points from (A) are measurements from 8 individual samples (each pooled from 4 animals), (B) represents individual animals, and (C–D) represent 4–6 individual samples (each pooled from 2 to 3 mice); 1-way ANOVA with Tukey's multiple comparison test; $^{*}P < 0.05$ vs. untreated controls; $^{\#}P < 0.05$ vs. +Noise; $^{\$}P < 0.05$ vs. +ATII. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

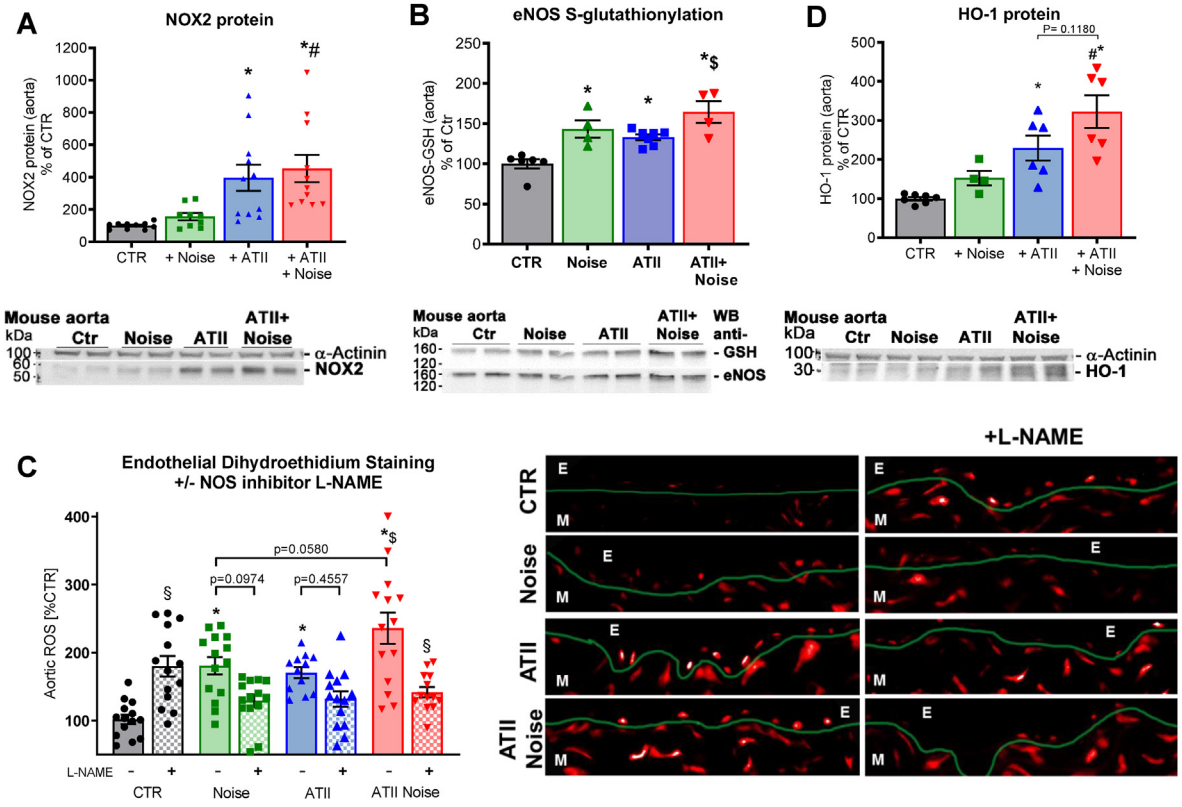

Fig. 3. Sources of reactive oxygen species and stress-response pathway show enhanced activation in the presence of both stressors. (A) NADPH oxidase isoform 2 (NOX-2, mainly in phagocytes) protein expression via Western blot in aortic tissue (representative Western blots below the densitometry). (B) *S*-glutathionylation of eNOS was measured by immunoprecipitation of eNOS, followed by Western blot against *S*-glutathionylated proteins as an assessment for the presence of the uncoupled ROS-generating enzyme form (representative Western blots below the densitometry). (C) Aortic cryosections were stained with DHE for ROS formation, with and without the specific NOS inhibitor L-NAME, which suppresses DHE signal from uncoupled eNOS in the aortic endothelium (representative staining images besides the densitometry). (D) Heme oxygenase-1 protein expression was measured in aortic tissue to evaluate the antioxidant stress response to the increase in ROS generation (representative Western blots below the densitometry). Data points from (A) are measurements from 5 to 8 samples (each pooled from 2 to 4 mice), (B) 4 samples (each pooled from 2 to 4 mice), (C) 13–14 individual animals and (D) 4–7 samples (each pooled from at least 2 mice); Kruskal-Wallis non-parametric test with Dunn's multiple comparison test (A,D) or 1-way ANOVA with Tukey's multiple comparison test (B,C); $*P < 0.05$ vs untreated controls, $\#P < 0.05$ vs + Noise, $\$P < 0.05$ vs + ATII, $\#P < 0.05$ vs + ATII + Noise.

mechanism for eNOS uncoupling. Noise, ATII and combination of both stressors significantly increased eNOS S-glutathionylation with a significant exacerbated effect in the ATII + Noise mice as compared to the ATII-only group (Fig. 3B). DHE staining showed increased oxidative stress in the endothelial cell layer of all treatment groups with a significant difference between ATII and ATII + Noise as well as borderline significance between Noise and ATII + Noise (Fig. 3C). Furthermore, a reduction in dihydroethidium (DHE) signal upon incubation with nitric oxide synthase inhibitor L-NAME implicates an uncoupled eNOS as a source of ROS with the most pronounced effect of L-NAME in the double treatment group (Fig. 3C). The protein expression of heme oxygenase-1 (HO-1) was also increased in all treatment groups and showed an significant potentiated increase with both stressors as compared to the noise-only group (Fig. 3D), mirroring the compensatory upregulation of this antioxidant enzyme by enhanced ROS levels as well as the expression of NOX-2 in the ATII + Noise group.

3.6. Inflammation is exacerbated in hypertension after noise exposure

Flow cytometry analysis revealed a significantly increased presence/infiltration of inflammatory immune cells in aorta of the ATII + Noise group. Inflammatory monocytes ($Ly6G^{-}Ly6C^{+}$), neutrophils ($Ly6G^{+}Ly6C^{+}$), and T-cells ($TCR\beta^{+}$) were increased markedly in count upon ATII + Noise treatment, with smaller increases in counts in the ATII-only and noise-only groups (Fig. 4A and B). *Plasminogen-activator inhibitor-1* (*PAI-1*), *cluster of differentiation 68* (*CD68*) and *vascular cell adhesion molecule 1* (*VCAM1*) gene expression was increased in aortic tissue of all treated mice with an exaggerated effect in *VCAM1*'s and *CD68*'s expression in the ATII + Noise group over noise-only exposure (Fig. 4C and D). A central role of activated monocytes in the pathogenesis of vascular dysfunction was also supported by higher expression levels of monocyte chemotactic protein-1 (MCP-1) in the presence of both stressors (suppl. Fig. S3).

3.7. Cerebral ROS and inflammation are increased following hypertensive noise exposure

ROS levels were elevated in cerebral tissue of all treatment groups, shown by DHE staining of cryosections, with a significant increase in the ATII + Noise group as compared to the noise-only group (Fig. 5A). A similar pattern was found in HPLC measurements of cerebral superoxide (Fig. 5B), with highest levels in the ATII + Noise group, which was significantly higher than in the noise-only group. Similarly, IL-1β and IL-6 levels, which indicate the presence of inflammation within the cortex, also increased step-wise in the order noise-only, ATII-only and ATII + Noise treatment (Fig. 5C and D). Significant induction of neuroinflammation was shown by immunohistochemical detection of astrocyte activation in the ATII + Noise group, whereas noise-only and ATII-only treatments showed minor and insignificant increases in activated astrocytes as compared to the untreated control (Fig. 5E).

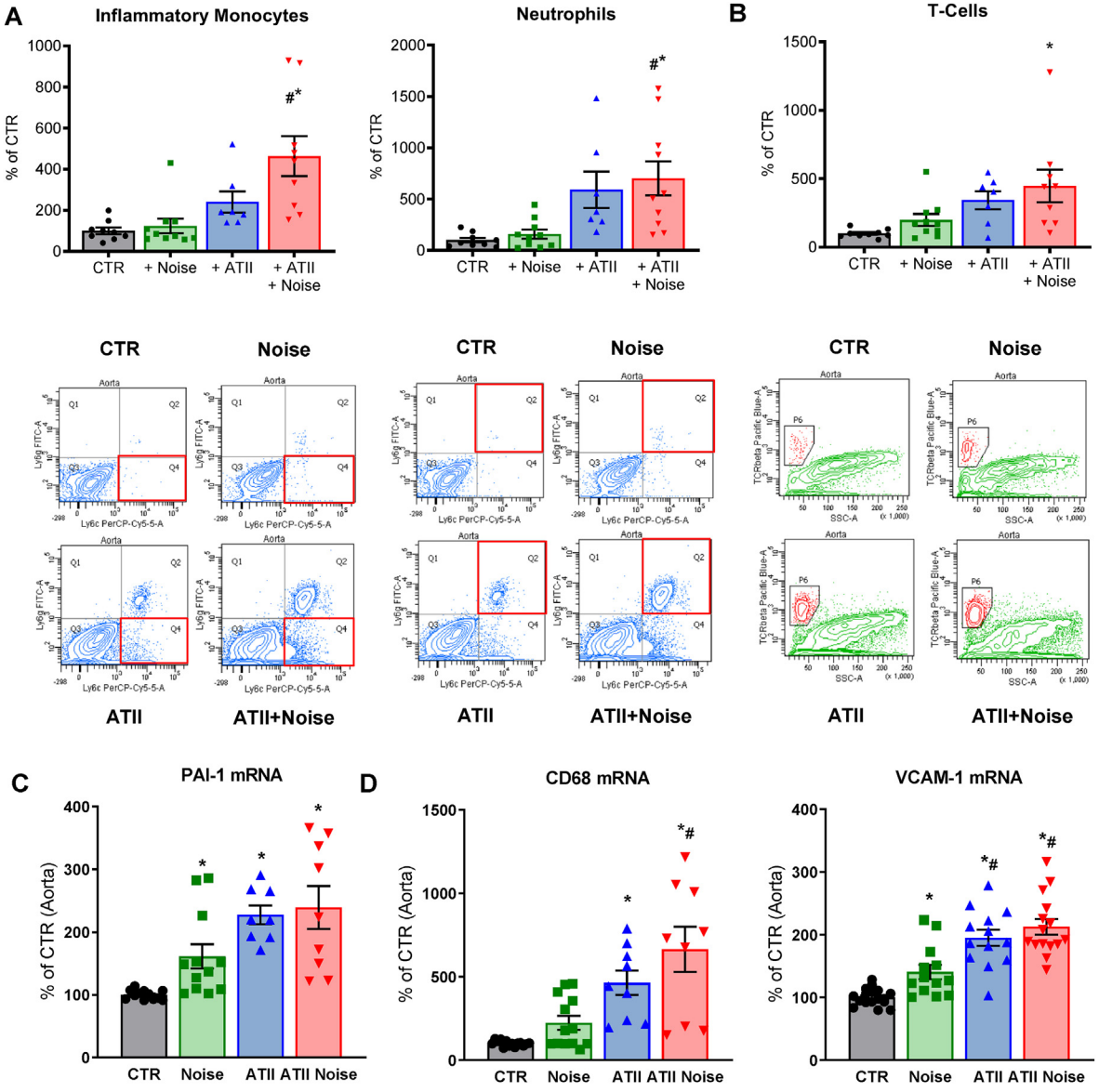

Fig. 4. Immune cell infiltration into the vasculature and markers of thrombo-inflammation show exaggerated activation in the presence of both stressors. (A,B) Quantification of flow cytometry of vascular infiltrates $Ly6G^{-}Ly6C^{+}$ (inflammatory monocytes), $Ly6G^{+}Ly6C^{+}$ (neutrophils), and TCRβ (T-cells) in aortic lysate are shown with representative flow cytometric plots. The gating strategy is shown in detail in suppl. Fig. S6. (C) *PAI*-1 mRNA expression was also evaluated by rtPCR as a risk factor for thrombosis and atherosclerosis. (D) Expression of *CD68* and *VCAM-1* were measured by quantitative rtPCR as readout of the inflammatory response to ATII and noise treatment. Data points are measurements from n = 7–10 (A,B) and 8–18 (C,D) animals; 1-way ANOVA with Tukey's multiple comparison test; $^{*}P < 0.05$ vs. untreated controls, $^{\#}P < 0.05$ vs. +Noise.

3.8. Evidence for structural and metabolic changes in the heart and kidney by hypertension and noise exposure

As shown in suppl. Fig. S4, there was a clear trend for down-regulation of proteins that account for structural processes, calcium handling and antioxidant defense, mostly in the heart of ATII exposed mice: sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA2a, −60%), mitochondrial manganese-superoxide dismutase (MnSOD, −45%), phospho-connexin 43 (pS325/328/330-Cx43, -20%) and total connexin 43 (Cx43, -30%). There was no evidence for synergistic effects on these parameters but overall, these changes are indicative of structural alterations and changes in calcium handling in the heart. As shown in suppl. Fig. S5, there was a clear trend for synergistic upregulation of genes involved in the regulation of metabolic processes in the kidney of ATII + Noise mice: uncoupling protein 3 (*UCP-3*, 9-fold), parathormone-related protein (*PTHRP*, 3-fold and significant versus control) and arginase 2 (*ARG2*, 3-fold). In addition, there was a clear trend for synergistic downregulation of the matrix metalloproteinase 12 gene (*MMP12*, -40%) in the kidney of ATII + Noise mice. These changes are indicative of early stress adaptation of the kidney.

4. Discussion

In the present study we sought to determine whether mice with experimental arterial hypertension (ATII-infusion for 7d) had exacerbated adverse cardiovascular and cerebral effects by co-exposure to aircraft noise. We found that noise applied to animals with previously established hypertension exaggerated endothelial dysfunction, increased oxidative stress and inflammation, particularly in the brain. Further, we found that the phagocytic NADPH oxidase (NOX-2)-mediated superoxide formation, immune cell infiltration (inflammatory monocytes, neutrophils and T cells) in the aortic wall and astrocyte

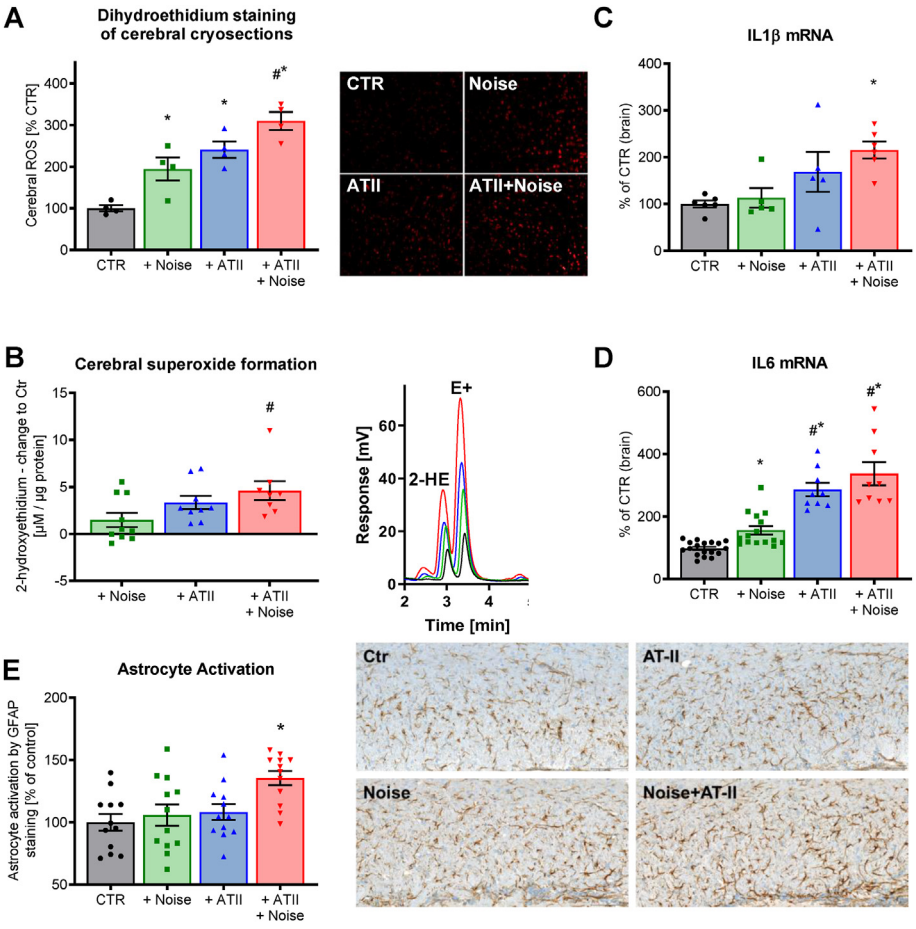

Fig. 5. Cerebral oxidative stress and neuroinflammation are aggravated in the presence of both stressors. (A) Cryosections of the frontal cortex were stained with DHE to yield red fluorescence in the presence of ROS (representative staining images besides the densitometry). (B) Cerebral lysate was subjected to HPLC analysis with DHE in order to measure the formation of the superoxide-specific 2-hydroxyethidium product (representative chromatograms besides the densitometry). (C,D) mRNA expression of IL-1β and IL-6 in cortical tissue was measured via quantitative rtPCR. (E) Astrocyte activation was determined by immunohistochemistry in the corpus callosum. Representative images for immunohistochemical staining for GFAP are shown at the level of the corpus callosum (CC). Suppl. Fig. S7 illustrates the workflow of the GFAP analysis. Data points from (A,B) are measurements from n = 4–10 individual animals, points from (C,D) are from 5 to 6 samples (IL-1β, each pooled from 2 to 4 mice) or 9–18 (IL-6) mice/group and points from (E) are from 12 individual animals; 1-way ANOVA with Tukey's multiple comparison test; $^{*}P < 0.05$ vs. untreated controls, $^{\#}P < 0.05$ vs. +Noise. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

activation in the brain and cytokine signaling were also increased after exposing the hypertensive ATII mice to noise. Mechanistically, vascular and cerebral inflammation and oxidative stress may represent key factors of the underlying pathophysiology.

Transportation noise, in particular road and aircraft noise, are known to be associated with an increased incidence of cardiovascular disease (for review see Ref. [10]). Recent studies indicate that each 10 dB increase in transportation noise is associated with a 6–8% increase in risk for ischemic heart disease (e.g. heart attacks or other cardiovascular disease events) [37,38] and that in the setting of pre-existing coronary artery disease, aircraft noise may exaggerate the negative effects on vascular (endothelial) function and blood pressure responses [8,9]. Animal and clinical studies have indicated that cardiovascular risk factors, such as hypercholesterolemia, arterial hypertension, diabetes mellitus, and smoking lead to endothelial dysfunction and increased oxidative stress (for review see Ref. [39,40]). Vascular and phagocytic NADPH oxidase [14] and uncoupled NO synthase (for review see Ref. [41]) have been identified as significant superoxide sources contributing to the pro-oxidant state induced by cardiovascular risk factors.

Recent experimental studies with transportation noise identified similar pathomechanisms accounting for endothelial dysfunction in response to noise and to cardiovascular risk factors. Simulated sleep phase aircraft noise, rather than awake phase noise was able to induce hypertension, increased release of stress hormones, endothelial dysfunction and increased vascular oxidative stress and inflammation [11,12]. Likewise, the phagocytic NADPH oxidase and an uncoupled NO synthase were identified as significant superoxide sources [11,12].

In the present study, we established that exposure to noise in hypertensive mice (induced by ATII) was associated with higher blood pressure, deterioration of endothelial function, a decrease in vascular NO levels representing reduced vascular NO bioavailability and increased vascular superoxide production. Correspondingly, we found that noise exposure increased NOX-2 protein expression in hypertensive ATII-treated animals, and increases in eNOS uncoupling as demonstrated by the increased levels in *S*-glutathionylated eNOS and the significantly increased inhibitory effects of the NOS inhibitor L-NAME on vascular superoxide production. In addition, eNOS activity was significantly inhibited by the combination of ATII and noise, as indicated by the decrease in the eNOS phosphorylation at Ser^{1177}. As a consequence, HO-1 levels increased, which can be considered to be a compensatory response to increased vascular oxidative stress via redox-sensitive activation of NRF-2 [42]. However, this compensatory upregulation of HO-1 was obviously futile since the inflicted damage by both stressors was not normalized. We also found a significant increase in mitochondrial superoxide production by noise in animals treated with ATII, which may impair left ventricular relaxation via decreased myocardial NO bioavailability [43].

We found that vascular inflammation was exacerbated in hypertensive ATII mice in response to aircraft noise. Flow cytometric analysis revealed a significantly increased presence/infiltration of immune cells in aorta of the mice that underwent both treatments. Numbers of T-cells, neutrophils, and inflammatory monocytes were increased upon ATII + Noise treatment versus ATII alone. Oxidative stress-sensitive thrombosis/atherosclerosis risk factor plasminogen-activator inhibitor-1 (*PAI-1*), cluster of differentiation 68 (*CD68*) and vascular cell adhesion molecule 1 (*VCAM1*) gene expression was increased in aortic tissue of all treated groups with a small potentiated effect by both stressors over noise-only exposure in *VCAM1* and *CD68* expression, suggesting the strong inflammatory phenotype in response to ATII treatment represents a ceiling effect and may have partially masked the additional adverse consequences of noise on vascular

inflammation. Nevertheless, our data suggests that noise exposure results in an exacerbation of the hypertensive inflammatory phenotype.

Substantial adverse effects of aircraft noise were seen with respect to oxidative stress and inflammatory responses in the brain. ROS levels were markedly elevated in cerebral tissue of all treatment groups, as evidenced by DHE staining of cryosections, with a significant increase in the mice with both treatments as compared to the ATII-only group and increase by trend as compared to the noise-only group. Likewise, IL-1β and IL-6 levels were higher and astrocytes were more activated in the ATII + noise group versus the noise-only and ATII-only groups pointing to a neuroinflammatory phenotype. The activation of stress hormone signaling pathways is the most likely explanation for the link between adverse cerebral effects of noise and the subsequent cardiovascular damage [11]. In support, a recent study found that chronic exposure to aircraft and road traffic noise was associated with higher amygdala activity, vascular inflammation and increased cardiovascular event rates, emphasizing a neurobiological basis by which transportation noise may induce cardiovascular damage [44,45]. Moreover, chronic aircraft noise exposure has been shown to be associated with cognitive impairment in children [46] and mental health conditions in adults [47], most likely due to increased cerebral oxidative stress due to downregulation and uncoupling of neuronal NOS [11] mainly located in the prefrontal cortex, which regulates autonomic and neuroendocrine stress signaling and thus may contribute to noise-induced cerebral dysfunction [48]. In line with this, numerous studies have demonstrated that hypertension is associated with increased risk of cognitive impairment and vascular dementia [49].

Sustained effects of ATII and/or noise are supported by clear trends of altered expression of cardiac/renal proteins and genes that are involved in structural or metabolic processes. The decreased SERCA2a protein levels may be indicative of slower sarcoplasmic reticulum-calcium reuptake and reduced end-diastolic sarcoplasmic reticulum-calcium content, whereas diminished levels of Cx43 expression and phosphorylation at serine residues targeted by casein kinase 1 are associated with alterations in gap junction formation, electrical remodeling and increased susceptibility to arrhythmias [50]. It is known that ATII treatment reduces SERCA2a expression [51]. Decreased amounts of the MnSOD may contribute to the enhanced ROS formation detected in cardiac ROS subjected to ATII and/or noise. The mitochondrial membrane transporter UCP3 decreases mitochondrial membrane potential and thereby limits excessive ROS formation. Compensatory upregulation of UCP3 was reported for hypertensive, ATII-infused mice and significantly suppressed ROS formation in cardiomyocytes [52]. PTHRP displays endocrine, autocrine, paracrine and intracrine hormone activities and acts as a vasodilator and mitogenic agent. Increased expression of PTHRP and kidney damage was reported for hypertensive (ATII-infused) mice, which was prevented by AT1-receptor blocker therapy [53]. MMP-12 is also known as macrophage metallo-elastase and is involved in the breakdown of extracellular matrix. Although, genetic deficiency of *MMP-12* was protective in ATII-induced hypertensive mice and limited heart fibrosis [54], MMP-12 was also discussed as an endogenous factor of resolution and clearance (e.g. after myocardial infarction) [55]. Arginases catalyze the break-down of arginine to ornithine and urea, probably contributing to altered nitric oxide synthesis and signaling. Increased arginase activity was identified as a major trigger of diminished nitric oxide bioavailability in ATII-induced hypertensive mice [56] and ARG2 was proposed as a marker of macrophage M1 polarization in aldosterone/salt-induced hypertension [57]. UCP-3 and ARG2 are located in mitochondria, whereas MMP12 affects the extracellular tissue. PTHRP is affecting ion transporters but also regional perfusion. In summary, this represents early stress adaptation in the kidney.

5. Conclusion and clinical implications

The present study found that exposure to aircraft noise in mice with ATII-induced hypertension resulted in additional cardiovascular and cerebral adverse effects, showing that hypertensive mice are further challenged by aircraft noise. Taking into account previously established ameliorating effects of NOX-2 deletion in the setting of noise exposure [11] and the similar effects of depletion of $LysM^+$ immune cells in the setting of ATII-induced hypertension [14], it is tempting to speculate that phagocytic NOX-2 indeed represents the key enzyme in mediating adverse effects in the setting of noise exposure and ATII hypertension. These observations may explain at least in part why patients with existing cardiovascular disease may be more sensitive to additional noise-induced cardiovascular effects [8]. Our data on aircraft noise-exacerbated cardiovascular damage in hypertensive mice may have implications for traffic noise-mediated adverse cardiovascular health effects in the general population, especially in subjects with pre-established hypertension.

Funding

This work was supported by a vascular biology research grant from the Else-Kröner-Fresenius Foundation for “Noise and arterial hypertension” (2017_A106 to S.S.) and from the Boehringer Ingelheim Foundation for the collaborative research group ‘Novel and neglected cardiovascular risk factors: molecular mechanisms and therapeutic implications’ to study the effects of aircraft noise exposure on vascular function and oxidative stress (A.D., S.S., and T.M.). K.B. was supported by the German Research Foundation (DFG Bo 2955-4/1). Part of the measurements was supported by the Foundation for Polish Science from the resources of the TEAM TECH–Core Facility program (S.CH.). K.Fra. is recipient of a career development award of the Stavros Niarchos Foundation. The collaboration of the authors was supported by European COST Action EU-CARDIOPROTECTION (CA16225). T.M. is PI of the DZHK (German Center for Cardiovascular Research), Partner Site Rhine-Main, Mainz, Germany.

Declaration of competing interest

Nothing to declare.

Acknowledgements

We are indebted to Jörg Schreiner, Bettina Mros, Nicole Glas and Angelica Karpi for their expert technical assistance. The following data are part of doctoral/master thesis projects: HPLC-based ROS measurements (Sanela Kalinovic), the immunohistochemical stainings of aorta (Konstantina Filippou) and brain (Chiara Trevisan), all other data (Katie Frenis). Katie Frenis and Sanela Kalinovic hold PhD stipends of the TransMed PhD Program at the University Medical Center Mainz.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2020.101515.

References

[1] A.J. Cohen, M. Brauer, R. Burnett, H.R. Anderson, J. Frostad, K. Estep, K. Balakrishnan, B. Brunekreef, L. Dandona, R. Dandona, V. Feigin, G. Freedman, B. Hubbell, A. Jobling, H. Kan, L. Knibbs, Y. Liu, R. Martin, L. Morawska, C.A. Pope 3rd, H. Shin, K. Straif, G. Shaddick, M. Thomas, R. van Dingenen, A. van Donkelaar, T. Vos, C.J.L. Murray, M.H. Forouzanfar, Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015, Lancet 389 (2017) 1907–1918, https://doi.org/10.1016/S0140-6736(17)30505-6.

[2] J.D. Bundy, C. Li, P. Stuchlik, X. Bu, T.N. Kelly, K.T. Mills, H. He, J. Chen, P.K. Whelton, J. He, Systolic blood pressure reduction and risk of cardiovascular disease and mortality: a systematic review and network meta-analysis, JAMA Cardiol 2 (2017) 775–781, https://doi.org/10.1001/jamacardio.2017.1421.

[3] M.J. Bloch, Worldwide prevalence of hypertension exceeds 1.3 billion, J Am Soc

Hypertens 10 (2016) 753–754, https://doi.org/10.1016/j.jash.2016.08.006.
[4] L. Jarup, W. Babisch, D. Houthuijs, G. Pershagen, K. Katsouyanni, E. Cadum, M.L. Dudley, P. Savigny, I. Seiffert, W. Swart, O. Breugelmans, G. Bluhm, J. Selander, A. Haralabidis, K. Dimakopoulou, P. Sourtzi, M. Velonakis, F. Vigna-Taglianti, H.s. team, Hypertension and exposure to noise near airports: the HYENA study, Environ. Health Perspect. 116 (2008) 329–333, https://doi.org/10.1289/ehp.10775.
[5] W. Babisch, Cardiovascular effects of noise, Noise Health 13 (2011) 201–204, https://doi.org/10.4103/1463-1741.80148.
[6] W. Babisch, G. Wolke, J. Heinrich, W. Straff, Road traffic noise and hypertension–accounting for the location of rooms, Environ. Res. 133 (2014) 380–387, https://doi.org/10.1016/j.envres.2014.05.007.
[7] M. Basner, W. Babisch, A. Davis, M. Brink, C. Clark, S. Janssen, S. Stansfeld, Auditory and non-auditory effects of noise on health, Lancet 383 (2014) 1325–1332, https://doi.org/10.1016/S0140-6736(13)61613-X.
[8] F. Schmidt, K. Kolle, K. Kreuder, B. Schnorbus, P. Wild, M. Hechtner, H. Binder, T. Gori, T. Munzel, Nighttime aircraft noise impairs endothelial function and increases blood pressure in patients with or at high risk for coronary artery disease, Clin. Res. Cardiol. 104 (2015) 23–30, https://doi.org/10.1007/s00392-014-0751-x.
[9] F.P. Schmidt, M. Basner, G. Kroger, S. Weck, B. Schnorbus, A. Muttray, M. Sariyar, H. Binder, T. Gori, A. Warnholtz, T. Munzel, Effect of nighttime aircraft noise exposure on endothelial function and stress hormone release in healthy adults, Eur. Heart J. 34 (2013) 3508–3514a, https://doi.org/10.1093/eurheartj/eht269.
[10] T. Munzel, F.P. Schmidt, S. Steven, J. Herzog, A. Daiber, M. Sorensen, Environmental noise and the cardiovascular system, J. Am. Coll. Cardiol. 71 (2018) 688–697, https://doi.org/10.1016/j.jacc.2017.12.015.
[11] S. Kroller-Schon, A. Daiber, S. Steven, M. Oelze, K. Frenis, S. Kalinovic, A. Heimann, F.P. Schmidt, A. Pinto, M. Kvandova, K. Vujacic-Mirski, K. Filippou, M. Dudek, M. Bosmann, M. Klein, T. Bopp, O. Hahad, P.S. Wild, K. Frauenknecht, A. Methner, E.R. Schmidt, S. Rapp, H. Mollnau, T. Munzel, Crucial role for Nox2 and sleep deprivation in aircraft noise-induced vascular and cerebral oxidative stress, inflammation, and gene regulation, Eur. Heart J. 39 (2018) 3528–3539, https://doi.org/10.1093/eurheartj/ehy333.
[12] T. Munzel, A. Daiber, S. Steven, L.P. Tran, E. Ullmann, S. Kossmann, F.P. Schmidt, M. Oelze, N. Xia, H. Li, A. Pinto, P. Wild, K. Pies, E.R. Schmidt, S. Rapp, S. Kroller-Schon, Effects of noise on vascular function, oxidative stress, and inflammation: mechanistic insight from studies in mice, Eur. Heart J. 38 (2017) 2838–2849, https://doi.org/10.1093/eurheartj/ehx081.
[13] H. Mollnau, M. Wendt, K. Szocs, B. Lassegue, E. Schulz, M. Oelze, H. Li, M. Bodenschatz, M. August, A.L. Kleschyov, N. Tsilimingas, U. Walter, U. Forstermann, T. Meinertz, K. Griendling, T. Munzel, Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling, Circ. Res. 90 (2002) E58–E65, https://doi.org/10.1161/01.RES.0000012569.55432.02.
[14] P. Wenzel, M. Knorr, S. Kossmann, J. Stratmann, M. Hausding, S. Schuhmacher, S.H. Karbach, M. Schwenk, N. Yogev, E. Schulz, M. Oelze, S. Grabbe, H. Jonuleit, C. Becker, A. Daiber, A. Waisman, T. Munzel, Lysozyme M-positive monocytes mediate angiotensin II-induced arterial hypertension and vascular dysfunction, Circulation 124 (2011) 1370–1381, https://doi.org/10.1161/CIRCULATIONAHA.111.034470.
[15] U. Landmesser, H. Cai, S. Dikalov, L. McCann, J. Hwang, H. Jo, S.M. Holland, D.G. Harrison, Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II, Hypertension 40 (2002) 511–515, https://doi.org/10.1161/01.HYP.0000032100.23772.98.
[16] M. Hausding, K. Jurk, S. Daub, S. Kroller-Schon, J. Stein, M. Schwenk, M. Oelze, Y. Mikhed, J.G. Kerahrodi, S. Kossmann, T. Jansen, E. Schulz, P. Wenzel, A.B. Reske-Kunz, C. Becker, T. Munzel, S. Grabbe, A. Daiber, CD40L contributes to angiotensin II-induced pro-thrombotic state, vascular inflammation, oxidative stress and endothelial dysfunction, Basic Res. Cardiol. 108 (2013) 386, https://doi.org/10.1007/s00395-013-0386-5.
[17] T. Jansen, S. Kroller-Schon, T. Schonfelder, M. Foretz, B. Viollet, A. Daiber, M. Oelze, M. Brandt, S. Steven, M. Kvandova, S. Kalinovic, J. Lagrange, J.F. Keaney Jr., T. Munzel, P. Wenzel, E. Schulz, alpha1AMPK deletion in myelomonocytic cells induces a pro-inflammatory phenotype and enhances angiotensin II-induced vascular dysfunction, Cardiovasc. Res. 114 (2018) 1883–1893, https://doi.org/10.1093/cvr/cvy172.
[18] M. Feng, S. Whitesall, Y. Zhang, M. Beibel, L. D'Alecy, K. DiPetrillo, Validation of volume-pressure recording tail-cuff blood pressure measurements, Am. J. Hypertens. 21 (2008) 1288–1291, https://doi.org/10.1038/ajh.2008.301.
[19] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis, Nat. Methods 9 (2012) 671–675.
[20] T. Munzel, A. Giaid, S. Kurz, D.J. Stewart, D.G. Harrison, Evidence for a role of endothelin 1 and protein kinase C in nitroglycerin tolerance, Proc. Natl. Acad. Sci. Unit. States Am. 92 (1995) 5244–5248.
[21] M. Oelze, M. Knorr, S. Kroller-Schon, S. Kossmann, A. Gottschlich, R. Rummler, A. Schuff, S. Daub, C. Doppler, H. Kleinert, T. Gori, A. Daiber, T. Munzel, Chronic therapy with isosorbide-5-mononitrate causes endothelial dysfunction, oxidative stress, and a marked increase in vascular endothelin-1 expression, Eur. Heart J. 34 (2013) 3206–3216, https://doi.org/10.1093/eurheartj/ehs100.
[22] M. Oelze, S. Kroller-Schon, P. Welschof, T. Jansen, M. Hausding, Y. Mikhed, P. Stamm, M. Mader, E. Zinssius, S. Agdauletova, A. Gottschlich, S. Steven, E. Schulz, S.P. Bottari, E. Mayoux, T. Munzel, A. Daiber, The sodium-glucose co-transporter 2 inhibitor empagliflozin improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering with oxidative stress and glucotoxicity, PloS One 9 (2014) e112394, , https://doi.org/10.1371/journal.pone.0112394.
[23] P. Wenzel, E. Schulz, M. Oelze, J. Muller, S. Schuhmacher, M.S. Alhamdani, J. Debrezion, M. Hortmann, K. Reifenberg, I. Fleming, T. Munzel, A. Daiber, AT1-receptor blockade by telmisartan upregulates GTP-cyclohydrolase I and protects eNOS in diabetic rats, Free Radic. Biol. Med. 45 (2008) 619–626, https://doi.org/10.1016/j.freeradbiomed.2008.05.009.
[24] M. Oelze, M. Knorr, S. Schuhmacher, T. Heeren, C. Otto, E. Schulz, K. Reifenberg, P. Wenzel, T. Munzel, A. Daiber, Vascular dysfunction in streptozotocin-induced experimental diabetes strictly depends on insulin deficiency, J. Vasc. Res. 48 (2011) 275–284, https://doi.org/10.1159/000320627.
[25] M. Oelze, A. Daiber, R.P. Brandes, M. Hortmann, P. Wenzel, U. Hink, E. Schulz, H. Mollnau, A. von Sandersleben, A.L. Kleschyov, A. Mulsch, H. Li, U. Forstermann, T. Munzel, Nebivolol inhibits superoxide formation by NADPH oxidase and endothelial dysfunction in angiotensin II-treated rats, Hypertension 48 (2006) 677–684, https://doi.org/10.1161/01.HYP.0000239207.82326.29.
[26] H. Zhao, J. Joseph, H.M. Fales, E.A. Sokoloski, R.L. Levine, J. Vasquez-Vivar, B. Kalyanaraman, Detection and characterization of the product of hydroethidine and intracellular superoxide by HPLC and limitations of fluorescence, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 5727–5732.
[27] P. Wenzel, H. Mollnau, M. Oelze, E. Schulz, J.M. Wickramanayake, J. Muller, S. Schuhmacher, M. Hortmann, S. Baldus, T. Gori, R.P. Brandes, T. Munzel, A. Daiber, First evidence for a crosstalk between mitochondrial and NADPH oxidase-derived reactive oxygen species in nitroglycerin-triggered vascular dysfunction, Antioxidants Redox Signal. 10 (2008) 1435–1447, https://doi.org/10.1089/ars.2007.1969.
[28] J. Zielonka, S. Srinivasan, M. Hardy, O. Ouari, M. Lopez, J. Vasquez-Vivar, N.G. Avadhani, B. Kalyanaraman, Cytochrome c-mediated oxidation of hydroethidine and mito-hydroethidine in mitochondria: identification of homo- and heterodimers, Free Radic. Biol. Med. 44 (2008) 835–846, https://doi.org/10.1016/j.freeradbiomed.2007.11.013.
[29] S. Kalinovic, M. Oelze, S. Kroller-Schon, S. Steven, K. Vujacic-Mirski, M. Kvandova, I. Schmal, A. Al Zuabi, T. Munzel, A. Daiber, Comparison of mitochondrial superoxide detection ex vivo/in vivo by mitoSOX HPLC method with classical assays in three different animal models of oxidative stress, Antioxidants 8 (2019), https://doi.org/10.3390/antiox8110514.
[30] S. Schuhmacher, M. Oelze, F. Bollmann, H. Kleinert, C. Otto, T. Heeren, S. Steven, M. Hausding, M. Knorr, A. Pautz, K. Reifenberg, E. Schulz, T. Gori, P. Wenzel, T. Munzel, A. Daiber, Vascular dysfunction in experimental diabetes is improved by pentaerithrityl tetranitrate but not isosorbide-5-mononitrate therapy, Diabetes 60 (2011) 2608–2616, https://doi.org/10.2337/db10-1395.
[31] S. Kroller-Schon, S. Steven, S. Kossmann, A. Scholz, S. Daub, M. Oelze, N. Xia, M. Hausding, Y. Mikhed, E. Zinssius, M. Mader, P. Stamm, N. Treiber, K. Scharffetter-Kochanek, H. Li, E. Schulz, P. Wenzel, T. Munzel, A. Daiber, Molecular mechanisms of the crosstalk between mitochondria and NADPH oxidase through reactive oxygen species-studies in white blood cells and in animal models, Antioxidants Redox Signal. 20 (2014) 247–266, https://doi.org/10.1089/ars.2012.4953.
[32] K. Boengler, P. Bencsik, J. Paloczi, K. Kiss, M. Pipicz, J. Pipis, P. Ferdinandy, K.D. Schluter, R. Schulz, Lack of contribution of p66shc and its mitochondrial translocation to ischemia-reperfusion injury and cardioprotection by ischemic preconditioning, Front. Physiol. 8 (2017) 733, https://doi.org/10.3389/fphys.2017.00733.
[33] R. Schreckenberg, A.M. Horn, R.M. da Costa Rebelo, S. Simsekyilmaz, B. Niemann, L. Li, S. Rohrbach, K.D. Schluter, Effects of 6-months' exercise on cardiac function, structure and metabolism in female hypertensive rats-the decisive role of lysyl oxidase and collagen III, Front. Physiol. 8 (2017) 556, https://doi.org/10.3389/fphys.2017.00556.
[34] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[35] K. Kus, M. Walczak, E. Maslak, A. Zakrzewska, A. Gonciarz-Dytman, P. Zabielski, B. Sitek, K. Wandzel, A. Kij, A. Chabowski, R.J. Holland, J.E. Saavedra, L.K. Keefer, S. Chlopicki, Hepatoselective nitric oxide (NO) donors, V-pyrro/NO and V-proli/NO, in nonalcoholic fatty liver disease: a comparison of antisteatotic effects with the biotransformation and pharmacokinetics, Drug Metab. Dispos. 43 (2015) 1028–1036, https://doi.org/10.1124/dmd.115.063388.
[36] S. Kossmann, M. Schwenk, M. Hausding, S.H. Karbach, M.I. Schmidgen, M. Brandt, M. Knorr, H. Hu, S. Kroller-Schon, T. Schonfelder, S. Grabbe, M. Oelze, A. Daiber, T. Munzel, C. Becker, P. Wenzel, Angiotensin II-induced vascular dysfunction depends on interferon-gamma-driven immune cell recruitment and mutual activation of monocytes and NK-cells, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 1313–1319, https://doi.org/10.1161/ATVBAHA.113.301437.
[37] D. Vienneau, C. Schindler, L. Perez, N. Probst-Hensch, M. Roosli, The relationship between transportation noise exposure and ischemic heart disease: a meta-analysis, Environ. Res. 138 (2015) 372–380, https://doi.org/10.1016/j.envres.2015.02.023.
[38] E.V. Kempen, M. Casas, G. Pershagen, M. Foraster, WHO environmental noise guidelines for the European region: a systematic review on environmental noise and cardiovascular and metabolic effects: a summary, Int. J. Environ. Res. Publ. Health 15 (2018) 379, https://doi.org/10.3390/ijerph15020379.
[39] T. Gori, T. Munzel, Oxidative stress and endothelial dysfunction: therapeutic implications, Ann. Med. 43 (2011) 259–272, https://doi.org/10.3109/07853890.2010.543920.
[40] T. Munzel, T. Gori, R.M. Bruno, S. Taddei, Is oxidative stress a therapeutic target in cardiovascular disease? Eur. Heart J. 31 (2010) 2741–2748, https://doi.org/10.1093/eurheartj/ehq396.
[41] U. Forstermann, T. Munzel, Endothelial nitric oxide synthase in vascular disease: from marvel to menace, Circulation 113 (2006) 1708–1714.

[42] A. Cuadrado, G. Manda, A. Hassan, M.J. Alcaraz, C. Barbas, A. Daiber, P. Ghezzi, R. Leon, M.G. Lopez, B. Oliva, M. Pajares, A.I. Rojo, N. Robledinos-Anton, A.M. Valverde, E. Guney, H. Schmidt, Transcription factor NRF2 as a therapeutic target for chronic diseases: a systems medicine approach, Pharmacol. Rev. 70 (2018) 348–383, https://doi.org/10.1124/pr.117.014753.

[43] T. Munzel, T. Gori, J.F. Keaney Jr., C. Maack, A. Daiber, Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications, Eur. Heart J. 36 (2015) 2555–2564, https://doi.org/10.1093/eurheartj/ehv305.

[44] M.T. Osborne, A. Radfar, M.Z.O. Hassan, S. Abohashem, B. Oberfeld, T. Patrich, B. Tung, Y. Wang, A. Ishai, J.A. Scott, L.M. Shin, Z.A. Fayad, K.C. Koenen, S. Rajagopalan, R.K. Pitman, A. Tawakol, A neurobiological mechanism linking transportation noise to cardiovascular disease in humans, Eur. Heart J. (2019), https://doi.org/10.1093/eurheartj/ehz820.

[45] T. Munzel, S. Steven, O. Hahad, A. Daiber, The sixth sense is involved in noise-induced stress responses and vascular inflammation: evidence for heightened amygdalar activity in response to transport noise in man, Eur. Heart J. (2019), https://doi.org/10.1093/eurheartj/ehz867.

[46] S.A. Stansfeld, B. Berglund, C. Clark, I. Lopez-Barrio, P. Fischer, E. Ohrstrom, M.M. Haines, J. Head, S. Hygge, I. van Kamp, B.F. Berry, R.s. team, Aircraft and road traffic noise and children's cognition and health: a cross-national study, Lancet 365 (2005) 1942–1949, https://doi.org/10.1016/S0140-6736(05)66660-3.

[47] S.A. Stansfeld, M.M. Haines, M. Burr, B. Berry, P. Lercher, A review of environmental noise and mental health, Noise Health 2 (2000) 1–8.

[48] S. Manikandan, M.K. Padma, R. Srikumar, N. Jeya Parthasarathy, A. Muthuvel, R. Sheela Devi, Effects of chronic noise stress on spatial memory of rats in relation to neuronal dendritic alteration and free radical-imbalance in hippocampus and medial prefrontal cortex, Neurosci. Lett. 399 (2006) 17–22, https://doi.org/10.1016/j.neulet.2006.01.037.

[49] W.S. Aronow, Hypertension and cognitive impairment, Ann. Transl. Med. 5 (2017) 259, https://doi.org/10.21037/atm.2017.03.99.

[50] B.F. Remo, J. Qu, F.M. Volpicelli, S. Giovannone, D. Shin, J. Lader, F.Y. Liu, J. Zhang, D.S. Lent, G.E. Morley, G.I. Fishman, Phosphatase-resistant gap junctions inhibit pathological remodeling and prevent arrhythmias, Circ. Res. 108 (2011) 1459–1466, https://doi.org/10.1161/CIRCRESAHA.111.244046.

[51] K. Gusev, A.A. Domenighetti, L.M. Delbridge, T. Pedrazzini, E. Niggli, M. Egger, Angiotensin II-mediated adaptive and maladaptive remodeling of cardiomyocyte excitation-contraction coupling, Circ. Res. 105 (2009) 42–50, https://doi.org/10.1161/CIRCRESAHA.108.189779.

[52] D. Liu, L. Huang, Y. Wang, W. Wang, X.H. Wehrens, T. Belousova, M. Abdelrahim, G. DiMattia, D. Sheikh-Hamad, Human stanniocalcin-1 suppresses angiotensin II-induced superoxide generation in cardiomyocytes through UCP3-mediated antioxidant pathway, PloS One 7 (2012) e36994, , https://doi.org/10.1371/journal.pone.0036994.

[53] O. Lorenzo, M. Ruiz-Ortega, P. Esbrit, M. Ruperez, A. Ortega, S. Santos, J. Blanco, L. Ortega, J. Egido, Angiotensin II increases parathyroid hormone-related protein (PTHrP) and the type 1 PTH/PTHrP receptor in the kidney, J. Am. Soc. Nephrol. 13 (2002) 1595–1607, https://doi.org/10.1097/01.asn.0000015622.33198.bf.

[54] L. Stawski, P. Haines, A. Fine, L. Rudnicka, M. Trojanowska, MMP-12 deficiency attenuates angiotensin II-induced vascular injury, M2 macrophage accumulation, and skin and heart fibrosis, PloS One 9 (2014) e109763, , https://doi.org/10.1371/journal.pone.0109763.

[55] A.J. Mouton, O.J. Rivera Gonzalez, A.R. Kaminski, E.T. Moore, M.L. Lindsey, Matrix metalloproteinase-12 as an endogenous resolution promoting factor following myocardial infarction, Pharmacol. Res. 137 (2018) 252–258, https://doi.org/10.1016/j.phrs.2018.10.026.

[56] A. Shatanawi, T. Lemtalsi, L. Yao, C. Patel, R.B. Caldwell, R.W. Caldwell, Angiotensin II limits NO production by upregulating arginase through a p38 MAPK-ATF-2 pathway, Eur. J. Pharmacol. 746 (2015) 106–114, https://doi.org/10.1016/j.ejphar.2014.10.042.

[57] B. Martin-Fernandez, A. Rubio-Navarro, I. Cortegano, S. Ballesteros, M. Alia, P. Cannata-Ortiz, E. Olivares-Alvaro, J. Egido, B. de Andres, M.L. Gaspar, N. de Las Heras, V. Lahera, J.A. Moreno, Aldosterone induces renal fibrosis and inflammatory M1-macrophage subtype via mineralocorticoid receptor in rats, PloS One 11 (2016) e0145946, , https://doi.org/10.1371/journal.pone.0145946.