Differential restoration of functional hyperemia by antihypertensive drug classes in hypertension-related cerebral small vessel disease

Masayo Koide[1,2*], Osama F. Harraz[1,2], Fabrice Dabertrand[1,3,4], Thomas A. Longden[1,5], Hannah R. Ferris[1], George C. Wellman[1], David C. Hill-Eubanks[1], Adam S. Greenstein[1,2,6,7], Mark T. Nelson[1,2,6,7] *

[1] Department of Pharmacology, Larner College of Medicine University of Vermont, Burlington, VT, USA

[2] Vermont Center for Cardiovascular and Brain Health, Larner College of Medicine University of Vermont, Burlington, VT, USA

[3] Department of Anesthesiology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

[4] Department of Pharmacology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

[5] Department of Physiology, University of Maryland School of Medicine, Baltimore, MD, USA

[6] Division of Cardiovascular Sciences, School of Medical Sciences, University of Manchester, UK

[7] Geoffrey Jefferson Brain Research Centre, The Manchester Academic Health Science Centre, Northern Care Alliance NHS Group, University of Manchester

Supplementary materials

- Detailed Methods
- Supplemental Figure 1-3
- Supplemental Tables 1-7
- Supplemental References

Detailed Methods

Animal models

BPH/2J mice (The Jackson Laboratory, stock 3005), a murine model of polygenic hypertension, together with the corresponding normotensive control strain, BPN/3J (The Jackson Laboratory, stock 3004), were used in this study. Both mouse strains were developed from the same colony, which originated from the crossing of 8 inbred strains. BPH/2J mice were produced by selecting for elevated blood pressure over 23 generations, followed by sibling inbreeding over 20 generations. Breeding for the same number of generations without selecting for hypertensive animals yielded the normotensive BPN/3J mouse strain (1, 2). BPH/2J mice spontaneously develop lifelong hypertension (2), which has previously been reported as neurogenic, salt-insensitive, and driven by only a few gene loci (3-6). BPH/2J mice also exhibit significantly elevated blood pressure as early as 4 weeks of age (7) and cognitive impairment in adulthood (7, 8). Using these two strains of mice, we measured the impact of chronic hypertension on functional hyperemia and capillary-to-arteriole signaling at 1, 4, and 8 months of age. Antihypertensive treatment was started at 3 months of age and continued until the age of 8 months. The antihypertensives, amlodipine (50 µg/mL) and losartan (500 µg/mL), were administered orally by addition to the drinking water, yielding doses corresponding to a range of 5–15 mg/kg/d for amlodipine (9, 10) and 50–150 mg/kg/d for losartan (10, 11), based on changes in daily water intake over time (12). For experiments employing the mineralocorticoid receptor antagonist eplerenone, commercial chow containing eplerenone kneaded into an identical base diet (Prolab RMH 3000; 0.8 g eplerenone/kg pellets) was used; the estimated dose was ~100 mg/kg/d (13). Eplerenone is a second-generation mineralocorticoid receptor antagonist, with higher selectivity and fewer off-target effects (14). In subsets of animals, blood pressure and heart rate were measured in awake mice using tail-cuff plethysmography (Columbus Instruments) (15). Tail-cuff plethysmography was carried out by well-trained individuals for five consecutive days (4 days habituation training + 1 day of measurement), starting at approximately 10 am. All experimental protocols used in this study complied with ARRIVE guidelines and were in accord with guidelines approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Measurement of whisker stimulation-induced functional hyperemia using laser Doppler flowmetry

Whisker stimulation-induced functional hyperemia was measured in the somatosensory cortex using laser Doppler flowmetry as described previously (16, 17). Briefly, under isoflurane anesthesia (5% induction, 2% maintenance), a catheter was inserted into the femoral artery to allow blood pressure monitoring and blood sample collection. After the animal's head was immobilized on a custom-made stereotactic frame, a 2 x 2 mm cranial window was made over the somatosensory cortex, and the dura was slit opened to allow drug access to the brain parenchyma. The cortical

surface at the cranial window site was superfused with artificial cerebrospinal fluid (aCSF; 125 mM NaCl, 3 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 4 mM glucose, pH 7.3, ~37°C, gassed with 5% CO_2, 20% O_2, 75% N_2). To avoid the vasodilatory effects of isoflurane on blood pressure and CBF, we switched the anesthesia to α-chloralose (50 mg/kg, i.p.) and urethane (750 mg/kg, i.p.) during the experiment. Functional hyperemia was measured using laser Doppler flowmetry (PeriMed), and is expressed as the percent change in CBF in the somatosensory cortex in response to stroking contralateral vibrissae at a frequency of ~3 Hz for 1 minute (i.e., whisker stimulation). Barium chloride ($BaCl_2$, 100 μM), a Kir2.1 channel blocker, was topically applied by adding to the cortical superfusate. The difference in hyperemic response between superfusion of aCSF without and with $BaCl_2$ (referred to in the text as 'Ba^{2+}-sensitive component') was considered as the contribution of the Kir2.1 channel to whisker stimulation-induced functional hyperemia. Blood pressure was continuously recorded during CBF measurements via a femoral artery cannula, and body temperature was maintained at 37°C by a servo-controlled heating pad with a rectal temperature sensor probe. The depth of anesthesia was assessed by monitoring blood pressure and reflex responses to tail pinch. All data were recorded and analyzed using LabChart software (AD Instruments).

Evaluation of capillary-to-arteriole signaling in the capillary-parenchymal arteriole (CaPA) preparation

The CaPA preparation was developed in our laboratory as described previously in detail (17). Briefly, this novel preparation consists of a brain parenchymal arteriole branch from the middle cerebral artery isolated together with an attached intact capillary bed. One end of the precapillary arteriolar segment is cannulated on a glass micropipette and pressurized at 40 mmHg using a pressure servo controller with a mini peristaltic pump (Living Systems Instrumentation), and the other end of the arteriole is occluded by a tie. Capillary ends are sealed by the downward pressure of an overlying glass micropipette, after which segments are preconstricted by applying intraluminal pressure to induce intrinsic (i.e., myogenic) tone or by adding the thromboxane analogue, U46619 (100 nM), to the bath. In the experiments described here, the bath chamber was superfused with aCSF (125 mM NaCl, 3 mM KCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 4 mM glucose, pH 7.3, ~37°C, gassed with 5% CO_2, 20% O_2, 75% N_2). The superfusate contained no antihypertensive drugs. Capillary-to-arteriole signaling was determined as the vasodilatory response in the upstream arteriolar segment in response to stimulation of capillary extremities with 10 mM K^+ (17). We also examined Kir2.1 channel-mediated vasodilatory responses induced by application of 10 mM K^+ directly onto the arteriole segment. These K^+ stimulations were achieved by pressure ejection (~5 psi, 20 s) of 10 mM K^+-aCSF (prepared by isosmotic replacement of NaCl with KCl) through a glass micropipette with a ~5-μm tip attached to a Picospritzer III pressure ejection system (Parker). Arteriolar diameter was measured at 15 Hz using a CCD camera and analyzed using

IonWizard 6.2 edge-detection software (IonOptix). At the end of experiments, the maximum diameter of the arteriolar segment was obtained by superfusion of aCSF containing 0 mM Ca^{2+} and 100 μM diltiazem, an L-type Ca^{2+} channel blocker; no differences in maximum diameter were found among groups (Supplementary Fig. 1b).

Measurement of inward-rectifier K^+ (Kir) channel currents in capillary ECs

Isolation of single capillary ECs: Single capillary ECs were obtained from mouse brains as previously described (17, 18). Briefly, 160-μm–thick brain slices in ice-cold isolation solution (124 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 4 mM glucose) were mechanically disrupted using a Dounce homogenizer, and debris was removed by passing the homogenate through a 62-μm nylon mesh. Retained capillary fragments were washed into dissociation solution composed of 55 mM NaCl, 80 mM Na-glutamate, 5.6 mM KCl, 2 mM $MgCl_2$, 4 mM glucose, and 10 mM HEPES (pH 7.3) containing neutral protease (0.5 mg/ml), elastase (0.5 mg/ml; Worthington, USA) and 100 μM $CaCl_2$, and incubated for 24 minutes at 37°C. Then, 0.5 mg/ml collagenase type I (Worthington) was added and the solution was incubated for an additional 2 minutes at 37°C. The suspension was filtered and washed to remove enzymes, and single cells and small capillary fragments were dispersed by triturating 4–7 times with a fire-polished glass Pasteur pipette. Cells were kept in ice-cold solution and were used within ~6 h of dispersion.

Patch-clamp electrophysiology: Whole-cell currents were recorded in the conventional whole-cell configuration using a patch-clamp amplifier (Axopatch 200B; Molecular Devices), filtered at 1 kHz, and digitized at 5 kHz. Whole-cell capacitance was measured using the cancellation circuitry in the voltage-clamp amplifier. Recording pipettes (~4–5 MΩ) were backfilled with a solution consisting of 10 mM NaOH, 11.4 mM KOH, 128.6 mM KCl, 1.1 mM $MgCl_2$, 2.2 mM $CaCl_2$, 5 mM EGTA, and 10 mM HEPES (pH 7.2). Whole-cell inward-rectifier K^+ (Kir) channel currents were elicited with a 300-ms voltage-ramp from -140 to +40 mV from a holding potential of −50 mV. Detection of currents was facilitated by raising external [K^+] to 60 mM in the bath solution (80 mM NaCl, 60 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, 4 mM glucose, and 2 mM $CaCl_2$; pH 7.4). The difference current between before and after bath-superfusion of $BaCl_2$ (100 μM), a selective pore blocker of Kir2 channels, was considered as Kir currents. The obtained recordings were analyzed offline using Clampfit 10.3 software, and Kir current values at -140 mV were further utilized for statistical analyses.

Measurement of plasma aldosterone

Whole blood (~0.5 mL) was withdrawn through a cannula inserted into the femoral artery within a 2-h window (10 am to 12 pm) on the days of sample collection. Plasma was separated by centrifugation (2,000 x *g*, 20 minutes) and stored at -80°C until examined. Aldosterone concentrations in plasma were quantified using a

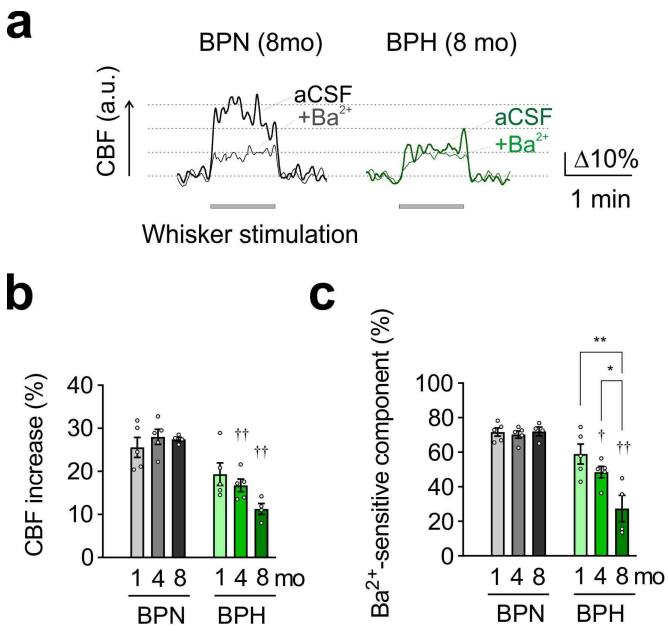
commercially available enzyme-linked immunosorbent assay (ELISA; Enzo Life Science, ADI-900-173). Briefly, standard solutions (3.9-250 pg/mL of non-conjugated aldosterone) or plasma samples (1:10 dilution using kit-provided diluent) were loaded into a 96-well plate coated with donkey anti-sheep IgG antibody. Alkaline phosphatase-conjugated aldosterone and a sheep polyclonal antibody against aldosterone were added to each well, and the plate was incubated at 4°C overnight. After washing three times with the kit-provided wash buffer, supplemented alkaline phosphate substrate (p-nitrophenyl phosphate) solution was added to wells and the plate was incubated at room temperature for 1 hour. The reaction was terminated by adding a kit-provided stop solution, and the optical density at 405 nm was measured using a Synergy H4 hybrid multi-mode microplate reader (BioTek).

Reagents

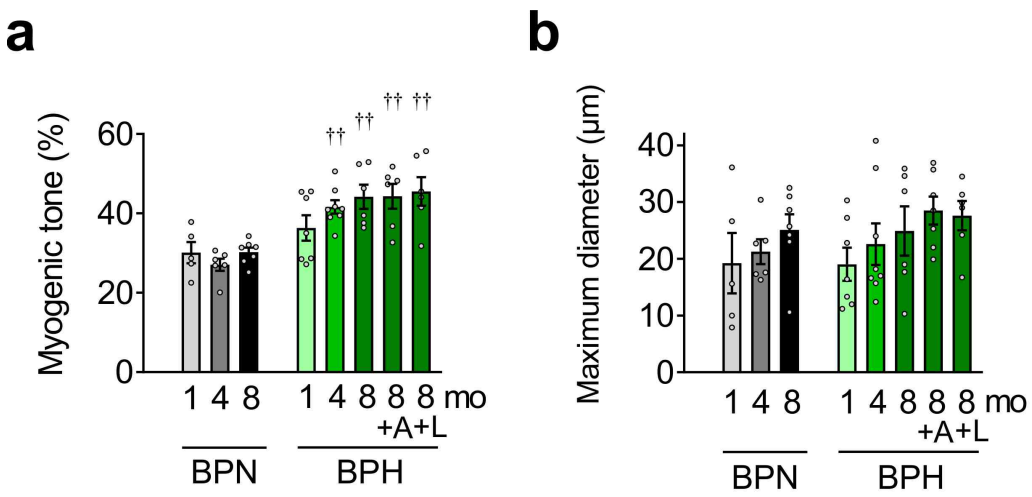
Pharmaceutical secondary standard grade antihypertensives (amlodipine besylate and losartan potassium) for treatments were obtained from Sigma-Aldrich (USA). U46619 was purchased from Cayman Chemical (USA). All other chemicals and reagents were obtained from Sigma-Aldrich (USA).

Statistics

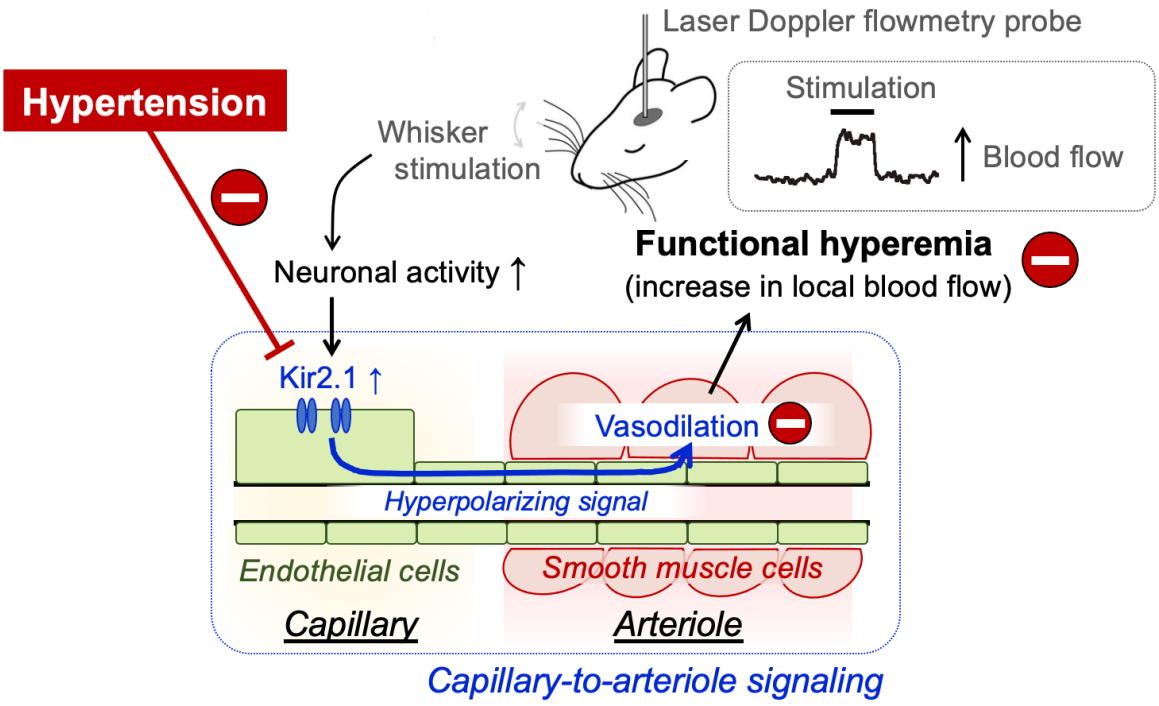
Data are presented as means ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism 8 software. One-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparisons test was used for multiple comparisons unless otherwise stated. Unpaired Student's t-test was used for two-group analyses. P-values < 0.05 were considered statistically significant.



Supplementary Figure 1: Chronic hypertension progressively impairs whisker stimulation-induced functional hyperemia in female mice. a. Representative traces of functional hyperemic responses before and after cortical superfusion of Ba^{2+}, a Kir2.1 channel blocker, in 8-month-old female BPN and BPH mice. Functional hyperemia was induced by contralateral side whisker stimulation and is presented as increases in CBF during whisker stimulation. **b.** Age-dependent deterioration of whisker stimulation-induced functional hyperemia in female hypertensive BPH mice **(**n = 4-5 mice/group; $^{\dagger\dagger}P < 0.01$ vs. BPN at corresponding ages by one-way ANOVA followed by Tukey's test). **c.** Decline in the Ba^{2+}-sensitive component of whisker stimulation-induced functional hyperemia in female BPH mice (n = 4-5 mice/group; $^{\dagger}P < 0.05$ $^{\dagger\dagger}P < 0.01$ vs. BPN at corresponding ages, $*P < 0.05$, $**P < 0.01$ between groups, by one-way ANOVA followed by Tukey's test).



Supplementary Figure 2: Properties of brain parenchymal arterioles isolated from BPN and BPH mice, and BPH mice with antihypertensive treatment. a. Myogenic tone (pressure-induced intrinsic vasoconstriction at 40 mmHg) was enhanced in parenchymal arterioles from BPH mice compared with those from BPN mice. This increase in tone was unaffected by treatment with either amlodipine (BPH+A) or losartan (BPH+L). Myogenic tone is expressed as percent constriction from maximum diameter (n = 5–8 mice/group; $^{\dagger\dagger}P < 0.01$ vs. BPN at corresponding ages). **b.** Maximum diameter of brain parenchymal arterioles obtained by superfusion of aCSF containing 0 mM Ca^{2+} and 100 μM diltiazem, an L-type Ca^{2+} channel blocker. Maximum arteriolar diameters were not significantly different among groups (n = 5–8 mice/group).



Supplemental Figure 3: Schematic illustration showing the mechanism of functional hyperemia deficits in hypertensive SVDs. Stroking mouse's vibrissae (whisker stimulation) results in capillary endothelial cell Kir2.1 channel activation due to astrocytic and neuronal release of K^+. By virtue of the activation of Kir2.1 channels and subsequent membrane hyperpolarization, hyperpolarizing signal rapidly propagates upstream, retrograde to flow, dilates the precapillary arteriole, and increases downstream tissue perfusion. This local increase in blood flow in the neuronally active region of the brain is termed functional hyperemia, which is detected using laser Doppler flowmetry in this study. Chronic hypertension disrupts the functional hyperemia in response to whisker stimulation by the mechanism causing the Kir2.1 channel dysfunction.

Supplementary Table 1: Blood pressure and heart rate, determined using a tail-cuff system, in male animals ± antihypertensive treatment

	3 months	4 months	8 months
Body weight (g)			
BPN	30.7 ± 1.2 (n = 6)	33.6 ± 1.6 (n = 6)	39.4 ± 1.8 (n = 6)$^{\dagger\dagger\,\S\S}$
BPH	22.5 ± 0.5 (n = 6)	24.5 ± 0.4 (n = 6)	29.5 ± 0.5 (n = 6)$^{\dagger\dagger\,\S\S}$
BPH+A	23.5 ± 0.4 (n = 8)$^{\#}$	24.8 ± 0.4 (n = 8)	30.3 ± 0.6 (n = 8)$^{\dagger\dagger\,\S\S}$
BPH+L	22.3 ± 0.4 (n = 11)$^{\#}$	24.5 ± 0.5 (n = 11)	30.3 ± 1.0 (n = 11)$^{\dagger\dagger\,\S\S}$
BPH+LE	23.1 ± 0.3 (n = 6)$^{\#}$	23.2 ± 0.3 (n = 6)	27.2 ± 0.4 (n = 6)$^{\dagger\dagger\,\S\S}$
Systolic blood pressure (mmHg)			
BPN	128.2 ± 3.4 (n = 6)	125.8 ± 2.7 (n = 6)	128.8 ± 2.1 (n = 6)
BPH	170.3 ± 4.0 (n = 6)	165.3 ± 5.8 (n = 6)	167.2 ± 5.8 (n = 6)
BPH+A	164.3 ± 3.2 (n = 8)$^{\#}$	133.9 ± 3.0 (n = 8)††	139.8 ± 3.5 (n = 8)††
BPH+L	169.5 ± 1.5 (n = 11)$^{\#}$	135.2± 1.7 (n = 11)††	141.0 ± 4.4 (n = 11)††
BPH+LE	171.2 ± 4.5 (n = 6)$^{\#}$	133.8 ± 1.7 (n = 6)††	128.0 ± 2.6 (n = 6)††
Diastolic blood pressure (mmHg)			
BPN	73.5 ± 3.5 (n = 6)	77.7 ± 3.4 (n = 6)	75.0 ± 1.9 (n = 6)
BPH	86.0 ± 3.2 (n = 6)	91.7 ± 4.7 (n = 6)	95.5 ± 4.9 (n = 6)
BPH+A	88.3 ± 2.6 (n = 8)$^{\#}$	74.9 ± 3.4 (n = 8)††	80.1 ± 2.3 (n = 8)
BPH+L	88.6 ± 2.1 (n = 11)$^{\#}$	73.2 ± 1.8 (n = 11)††	75.1 ± 2.2 (n = 11)††
BPH+LE	87.2 ± 2.1 (n = 6)$^{\#}$	75.0 ± 4.6 (n = 6)†	70.7 ± 2.3 (n = 6)††
Mean blood pressure (mmHg)			
BPN	91.7 ± 2.2 (n = 6)	93.8 ± 2.7 (n = 6)	93.2 ± 1.7 (n = 6)
BPH	114.2 ± 3.0 (n = 6)	116.2 ± 3.6 (n = 6)	119.3 ± 4.1 (n = 6)
BPH+A	113.5 ± 1.5 (n = 8)$^{\#}$	94.5 ± 2.1 (n = 8)††	99.9± 1.9 (n = 8)††
BPH+L	115.1 ± 1.1 (n = 11)$^{\#}$	93.7 ± 1.5 (n = 11)††	98.6 ± 2.7 (n = 11)††
BPH+LE	115.0 ± 2.6 (n = 6)$^{\#}$	94.8 ± 3.5 (n = 6)††	89.8 ± 2.2 (n = 6)††
Heart rate (bpm)			
BPN	668 ± 21 (n = 6)	666 ± 14 (n = 6)	663 ± 17 (n = 6)
BPH	749 ± 20 (n = 6)	756 ± 18 (n = 6)	772 ± 36 (n = 6)
BPH+A	733 ± 20 (n = 8)$^{\#}$	694 ± 14 (n = 8)	703± 13 (n = 8)
BPH+L	714 ± 9 (n = 11)$^{\#}$	702 ± 10 (n = 11)	701 ± 17 (n = 11)
BPH+LE	721 ± 34 (n = 6)$^{\#}$	679 ± 30 (n = 6)	695 ± 9 (n = 6)

*P < 0.05, **P < 0.01 between groups indicated by square brackets. Values for 3-month-old BPH mice with antihypertensive treatment, labeled with '#', were measured before starting treatment. †P < 0.05, ††P < 0.01 vs. 3-month-old animals of the same strain and treatment group; §P < 0.05, §§P < 0.01 vs. 4-month-old animals of the same strain and treatment group (two-way ANOVA followed by Tukey's multiple comparison test). Mean blood pressures were calculated with a pre-set program using the equation, (1/3 x systolic blood pressure + 2/3 x diastolic blood pressure).

Supplementary Table 2: Blood pressure and heart rate, determined using a tail-cuff system, in female animals

	3 months	4 months	8 months
Body weight (g)			
BPN	22.1 ± 0.8 (n = 8)	24.4 ± 1.1 (n = 8)	36.7 ± 1.3 (n = 8)$^{\dagger\dagger\ \S\S}$] **
BPH	19.8 ± 0.4 (n = 4)	21.7 ± 0.5 (n = 4)	26.1 ± 1.6 (n = 4)§
Systolic blood pressure (mmHg)			
BPN	126.5 ± 2.5 (n = 8)] **	127.0 ± 2.2 (n = 8)] **	124.0 ± 3.0 (n = 8)] **
BPH	165.3 ± 4.1 (n = 4)	162.3 ± 2.7 (n = 4)	174.5 ± 2.7 (n = 4)
Diastolic blood pressure (mmHg)			
BPN	73.5 ± 2.1 (n = 8)	71.4 ± 3.0 (n = 8)	65.1 ± 2.0 (n = 8)] **
BPH	87.3 ± 7.4 (n = 4)	85.5 ± 2.6 (n = 4)	82.8 ± 2.3 (n = 4)
Mean blood pressure (mmHg)			
BPN	91.3 ± 1.5 (n = 8)] **	90.0 ± 1.9 (n = 8)] **	84.6 ± 2.1 (n = 8)] **
BPH	113.3 ± 6.3 (n = 4)	111.3 ± 2.1 (n = 4)	113.3 ± 1.9 (n = 4)
Heart rate (bpm)			
BPN	636 ± 11 (n = 8)] *	630 ± 14 (n = 8)] *	645 ± 18 (n = 8)] *
BPH	727 ± 29 (n = 4)	723 ± 22 (n = 4)	737 ± 35 (n = 4)

*P < 0.05, **P < 0.01 between BPN and BPH groups indicated by square brackets; ††P < 0.01 vs. 3-month-old animals of the same strain; §P < 0.05, §§P < 0.01 vs. 4-month-old animals of the same strain (two-way ANOVA followed by Tukey's multiple comparison test). Mean blood pressure was calculated with the pre-set program using the equation, (1/3 x systolic blood pressure + 2/3 x diastolic blood pressure).

Supplementary Table 3: Heart rates (bpm) in anesthetized male animals in laser Doppler flowmetry studies

	1 month	4 months		8 months	
BPN	497 ± 22 (n = 5)	495 ± 8 (n = 6)	*	475 ± 13 (n = 9)	**
BPH	542 ± 13 (n = 5)	567 ± 24 (n = 5)		556 ± 6 (n = 9)	
BPH +A	N/A	N/A		515 ± 10 (n = 6)	
BPH +L	N/A	N/A		504 ± 14 (n = 5)	

*P < 0.05, **P < 0.01 between groups (one-way ANOVA followed by Turkey's multiple comparison test).

Supplementary Table 4: Body weight (g) in male BPN and BPH mice ± antihypertensive treatment in laser Doppler flowmetry studies

	1 month	4 months	8 months	
BPN	22.9 ± 0.8 (n = 5)	32.6 ± 2.3 (n = 6)††	41.2 ± 1.4 (n = 9)$^{\dagger\dagger\ \S\S}$	** ** **
BPH	17.5 ± 0.9 (n = 5)	25.7 ± 1.5 (n = 5)†	30.2 ± 1.1 (n = 9)††	
BPH+A	N/A	N/A	28.6 ± 0.8 (n = 6)	
BPH+L	N/A	N/A	29.6 ± 2.6 (n = 5)	

**P < 0.01 between groups indicated by square brackets; †P < 0.05, ††P < 0.01 vs. 1-month-old animals of the same strain; §§P < 0.01 vs. 4-month-old animals of the same strain (one-way ANOVA followed by Tukey's multiple comparison test).

Supplementary Table 5: Physiological parameters in anesthetized female animals in laser Doppler flowmetry studies

	1 month	4 months	8 months
MAP (mmHg)			
BPN	105.0 ± 3.8 (n = 5)] **	101.0 ± 4.3 (n = 5)] **	98.8 ± 5.6 (n = 4)] **
BPH	127.4 ± 4.5 (n = 5)	125.7 ± 3.1 (n = 5)	141.1 ± 3.8 (n = 4)
Heart Rate (bpm)			
BPN	511 ± 10 (n = 5)	500 ± 15 (n = 5)	459 ± 28 (n = 4)
BPH	555 ± 19 (n = 5)	537 ± 13 (n = 5)	527 ± 19 (n = 4)
Body weight (g)			
BPN	18.8 ± 0.5 (n = 5)	30.9 ± 1.1 (n = 5)††	32.2 ± 4.3 (n = 4)††
BPH	14.6 ± 0.1 (n = 5)	24.1 ± 1.0 (n = 5)††	29.9 ± 1.0 (n = 4)††

Abbreviations: MAP, mean arterial pressure; bpm, beats per minute. **P < 0.01 between strains (square brackets); ††P < 0.01 vs. 1-month-old animals of the same strain (two-way ANOVA followed by Tukey's multiple comparison test).

Supplementary Table 6: Body weight (g) in BPN and BPH mice with no experimental procedures

	1 month	3 months	4 month	8 months
Males				
BPN	22.9 ± 0.4 (n = 16)]**	30.4 ± 0.6 (n = 12)††]**	34.7 ± 0.9 (n = 14)$^{\dagger\dagger\ \S\S}$]**	39.4 ± 1.0 (n = 14)$^{\dagger\dagger\ \S\S\ \#\#}$]**
BPH	17.8 ± 0.5 (n = 16)]	23.6 ± 0.4 (n = 9)††]	25.7 ± 0.8 (n = 10)††]	32.5 ± 0.6 (n = 15)$^{\dagger\dagger\ \S\S\ \#\#}$]
Females				
BPN	18.5 ± 0.3 (n = 14)$^{!!}$]**	21.7 ± 0.6 (n = 9)$^{\dagger\ !!}$	24.3 ± 0.8 (n = 9)$^{\dagger\dagger\ !!}$	34.2 ± 1.7 (n = 12)$^{\dagger\dagger\ \S\S\ !!}$]**
BPH	15.5 ± 0.2 (n = 18)$^{!!}$]	20.3 ± 0.4 (n = 16)$^{\dagger\dagger\ !}$	23.5 ± 0.5 (n = 13)$^{\dagger\dagger\ \S\S}$	28.7 ± 0.7(n = 14)$^{\dagger\dagger\ \S\S\ \#\#\ !!}$]

**P < 0.01 between strains (square brackets); †P < 0.05, ††P < 0.01 vs. 1-month-old animals of the same strain; §§P < 0.01 vs. 3-month-old animals of the same strain; $^{\#\#}$P < 0.01 vs. 4-month-old animals of the same strain; $^{!}$P < 0.05, $^{!!}$P < 0.01 vs. male animals of the same strain (two-way ANOVA followed by Tukey's multiple comparison test).

Supplementary Table 7: Physiological parameters in anesthetized BPH mice that underwent combined losartan and eplerenone (BPH+LE group) treatment in laser Doppler flowmetry studies

	Body weight (g)	MAP (mmHg)	Heart rate (bpm)
BPH+LE	28.6 ± 0.9 (n = 5)	92.2 ± 0.7 (n = 5)	519 ± 18 (n = 5)

MAP: mean arterial pressure, bpm: beats per minute.

Supplemental References

1. Schlager G. Selection for blood pressure levels in mice. *Genetics.* 1974;76(3):537-49.
2. Schlager G, and Sides J. Characterization of hypertensive and hypotensive inbred strains of mice. *Lab Anim Sci.* 1997;47(3):288-92.
3. Jackson KL, et al. Major contribution of the medial amygdala to hypertension in BPH/2J genetically hypertensive mice. *Hypertension.* 2014;63(4):811-8.
4. Wright FA, et al. Genome scan for blood pressure loci in mice. *Hypertension.* 1999;34(4 Pt 1):625-30.
5. Jackson KL, et al. Mechanisms Responsible for Genetic Hypertension in Schlager BPH/2 Mice. *Front Physiol.* 2019;10:1311.
6. Leckie BJ. The action of salt and captopril on blood pressure in mice with genetic hypertension. *J Hypertens.* 2001;19(9):1607-13.
7. Faraco G, et al. Perivascular macrophages mediate the neurovascular and cognitive dysfunction associated with hypertension. *J Clin Invest.* 2016;126(12):4674-89.
8. Hartman RE, et al. Motor and cognitive deficits in mice bred to have low or high blood pressure. *Physiol Behav.* 2012;105(4):1092-7.
9. Devarajan S, et al. Depressor effect of chymase inhibitor in mice with high salt-induced moderate hypertension. *Am J Physiol Heart Circ Physiol.* 2015;309(11):H1987-96.
10. Nelson JW, et al. Endothelial transcriptomics reveals activation of fibrosis-related pathways in hypertension. *Physiol Genomics.* 2018;50(2):104-16.
11. Palma-Rigo K, et al. Renin-angiotensin and sympathetic nervous system contribution to high blood pressure in Schlager mice. *J Hypertens.* 2011;29(11):2156-66.
12. Jackson KL, et al. Energy metabolism in BPH/2J genetically hypertensive mice. *Hypertens Res.* 2014;37(5):413-21.
13. Diaz-Otero JM, et al. Mineralocorticoid receptor antagonism improves parenchymal arteriole dilation via a TRPV4-dependent mechanism and prevents cognitive dysfunction in hypertension. *Am J Physiol Heart Circ Physiol.* 2018;315(5):H1304-H15.
14. Struthers A, et al. A comparison of the aldosterone-blocking agents eplerenone and spironolactone. *Clin Cardiol.* 2008;31(4):153-8.
15. Sonkusare SK, et al. Inward rectifier potassium (Kir2.1) channels as end-stage boosters of endothelium-dependent vasodilators. *J Physiol.* 2016;594(12):3271-85.
16. Girouard H, et al. Astrocytic endfoot Ca^{2+} and BK channels determine both arteriolar dilation and constriction. *Proc Natl Acad Sci U S A.* 2010;107(8):3811-6.
17. Longden TA, et al. Capillary K^{+}-sensing initiates retrograde hyperpolarization to increase local cerebral blood flow. *Nat Neurosci.* 2017;20(5):717-26.
18. Harraz OF, et al. Endothelial GqPCR activity controls capillary electrical signaling and brain blood flow through PIP_2 depletion. *Proc Natl Acad Sci U S A.* 2018;115(15):E3569-E77.