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Molecular adaptations in vasoactive systems during acute stroke in salt-induced hypertension

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Abstract Investigations regarding hypertension and dietary sodium, both factors that influence stroke risk, have previously been limited to using genetically disparate treatment and control groups, namely the stroke-prone, spontaneously hypertensive rat and Wistar-Kyoto rat. In this investigation, we have characterized and compared cerebral vasoactive system adaptations following stroke in genetically identical, salt-induced hypertensive, and normotensive control mice. Briefly, $ANP^{+/-}$ (C57BJ/6 × SV129 background) mice were fed chow containing either 0.8 % NaCl (NS) or 8.0 % NaCl (HS) for 7 weeks. Transient cerebral ischemia was induced by middle cerebral artery occlusion (MCAO). Infarct volumes were measured 24-h post-reperfusion and the mRNA expression of five major vasoactive systems was characterized using qPCR. Along with previous publications, our data validate a salt-induced hypertensive state in $ANP^{+/-}$ mice fed HS chow as they displayed left ventricular hypertrophy, increased systolic blood pressure, and increased urinary sodium excretion. Following MCAO, mice fed HS exhibited larger infarct volumes than their dietary counterparts. In addition, significant up-regulation in *Et-1* and *Nos3* mRNA expression in response to salt and stroke suggests implications with increased cerebral damage in this group. In conclusion, our data demonstrate increased cerebral susceptibility to stroke in salt-induced hypertensive mice. More importantly, however, we have characterized a novel method of investigating hypertension and stroke with the use of genetically identical treatment and control groups. This is the first investigation in which genetic confounding variables have been eliminated.

Keywords Stroke · Hypertension · Salt-sensitivity · Dietary sodium · Natriuretic peptide

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Background

Hypertension is the most important preventable risk factor for premature death worldwide [1], increasing the risk of developing coronary artery disease [2] and stroke [3]. Dietary salt intake is a known contributor to hypertensive disease [4–6].

Approximately, one-third of patients with essential hypertension are sensitive to dietary changes in salt [6]. Increased sodium has been linked to vessel remodeling [7],

increased left ventricular (LV) mass, increased blood pressure (BP) [5, 8], and increased urinary sodium excretion [1, 8]. Epidemiological studies have shown a positive correlation between salt intake and increased risk of stroke [6, 9, 10], while others have demonstrated correlations between BP and essential hypertension on stroke [3, 11–13]. No direct relationship between salt-induced hypertension and stroke injury has previously been examined. Vasoactive systems, such as the natriuretic peptide (NP), nitric oxide synthase (NOS), renin–angiotensin (RAS), and endothelin (ET) systems, possess homeostatic roles in normal states to regulate BP. In salt-induced hypertension these systems show adaptive changes that may affect stroke injury.

Atrial natriuretic peptide (ANP) is modulated by volume status and is responsible for the homeostatic control of sodium and water content in the body. Heterozygous ANP gene-disrupted mice ($ANP^{+/-}$) display normal cardiac mass and BP under normal conditions. Due to the salt-sensitivity of this genotype, $ANP^{+/-}$ mice display left ventricular hypertrophy (LVH) and increased BP when fed high salt (HS) [14, 15]. In the current study, we have used the $ANP^{+/-}$ mouse to investigate the effects of salt-induced hypertension on stroke particularly with interest in cerebral adaptations in the NP, NOS, RAS, ET, and the vascular endothelial growth factor (VEGF) vasoactive systems.

Methods

Animal model and genotyping

Experimental protocols pertaining to the use of mice in this study were approved by the University Animal Care Committee (UACC) of Queen's University in accordance with the guidelines of the Canadian Council of Animal Care (CCAC). All mice were bred and cared for in the Animal Care Facility at Queen's University. Mice were housed in cages (up to 4 animals per cage) at room temperature on a 12 h light/dark cycle. Heterozygous ($ANP^{+/-}$) offspring were obtained by crossing wild-type ($ANP^{+/+}$, C57BJ/6) females with homozygous ANP gene-disrupted ($ANP^{-/-}$, C57BJ/6 × SV129 background) [14] males. Mice were weaned at 3 weeks and ear, and tail tissue biopsies were collected for genotyping. The genotype of each mouse was determined using an AccuStart™II mouse genotyping kit (Quanta Biosciences, Gaithersburg, MD, USA) and previously published PCR methods [16].

Dietary treatment and non-invasive tail-cuff BP measurements

Male $ANP^{+/-}$ mice were fed either normal-salt (NS; 0.8 % NaCl) or high-salt chow (HS; 8.0 % NaCl, Lab Diet 5001®, Brentwood, MO, USA) for 7 weeks. A total of 22 NS-treated and 23 HS-treated mice (between 13 and 15 weeks of age) were used. Chow diets and tap water were provided ad libitum. At the beginning of the fourth week of salt treatment, a randomized subset of mice (NS; $n = 4$, HS; $n = 5$) underwent a 3-week training period to acquire BP measurements using a CODA non-invasive tail-cuff system (Kent Scientific, Connecticut, USA) [17]. Following training, systolic BP measurements were recorded and the mean value from two separate days at the end of salt treatment was calculated.

To ensure successful salt treatment, a separate randomized subset of mice was used for urine collection (0.8 % NaCl, $n = 4$; 8.0 % NaCl, $n = 4$). Urine was collected from each mouse by metabolic cages over a morning, 4-h time period, and analyzed for urinary Na^+ excretion. Urinary Na^+ concentration was measured using a sodium specific electrode (Model #: K-27504-30, Cole Palmer, Montreal, Canada).

Induction of transient cerebral ischemia

Transient cerebral ischemia was induced by temporary occlusion of the proximal left middle cerebral artery (MCA) using the intraluminal filament method previously described by Longa et al. [18] and later modified by Barber et al. [19]. Mice were anesthetized with isoflurane (3 % initial, 1–1.5 % maintenance), in oxygen to air ratio of 20:80 %. Core body temperature was monitored and maintained at 36.5 °C using a rectal probe with feedback to a heated surgical stage. Transcranial measurements of cerebral blood flow were obtained by laser Doppler flowmetry (Perimed, Periflux System 5010, North Royalton, OH, USA). A reduction of 70 % or more in regional MCA cerebral blood flow was considered adequate to induce ischemia. Occlusion was maintained for 30 min after which reperfusion was achieved. All mice recovered in a cage warmed to 34 °C by a circulating water blanket for 24 h and received 2 mg/kg of bupivacaine subcutaneous (SQ), 0.5 mL of 0.9 % saline SQ and 2 mg/kg of meloxicam SQ for analgesia immediately following surgery. Sham mice were used as controls; all surgical procedures were conducted with exception of MCA occlusion.

Vascular casting

Mice ($n = 4$) were transcardially perfused with 140 mmol/L NaCl, 10 mmol/L KCl, and 5 mmol/L ethylenediaminetetraacetic acid (EDTA) (pH 7.5). Batson's #17 polymer (Polysciences Inc., Warrington, PA, USA) (2 mL) was injected (retrograde) through the thoracic aorta and left to polymerize for 24 h. The skull was isolated and the soft

and bony tissue digested in 1 mol/L NaOH and 5 % Contrad 70 (Fisher Scientific, Pittsburgh, PA, USA) detergent.

Tissue collection

Mice were anesthetized via an intraperitoneal (IP) injection of euthanyl (100 mg/kg body weight) 24-h post-reperfusion. One millimeter cerebral sections were obtained. The fourth slice (Bregma ± 1 mm) was isolated and snap-frozen for molecular analysis. All other slices were stained in 0.5 % 2,3,5-triphenyltetrazolium chloride (TTC) diluted in 1× phosphate buffered saline (PBS). Infarct volumes were measured using ImageJ software (http://rsbweb.nih.gov/ij/). To account for the overestimation of infarct size due to cerebral swelling, infarct volume measurements were calculated according to Swanson et al. [20]. The heart was excised and dissected into individual chambers. Organ tissue weights were normalized to tibia length.

RNA isolation

Total RNA was isolated using a combination of Trizol (Tri Reagent, Molecular Research Centre, Inc. Burlington, ON, Canada) and a high pure RNA isolation kit (Roche Scientific Co., Laval, QC, Canada). All wash and elution steps were carried out according to the manufacturer's instructions. RNA was measured using Nano-Drop 2000 spectrometer (Thermo Scientific, Wilmington, DE, USA).

Reverse transcription

Total RNA (1 μg) was reverse transcribed into cDNA using a high capacity cDNA reverse transcription kit (LifeTechnologies, Carlsbad, CA). Protocol was carried out according to the manufacturer's instructions. Samples were incubated for 1 h with oligo-dT_{18} and an additional hour with random hexamers.

Real-time quantitative PCR (qPCR)

Oligonucleotide primers were designed using Primer Design 2.0 software (Scientific & Educational Software, Cary, NC, USA) from published mRNA sequences from NIH GenBank (www.ncbi.nlm.nih.gov/Genbank) (National Center for Biotechnical Information, Bethesda, MD, USA) (Table 1). Primer sets yielding a standard curve with efficiency within 1.7–2.0 were deemed adequate. Levels of mRNA expression were measured using the standard curve method for each gene with hypoxanthine–guanine phosphoribosyltransferase (HPRT) as a reference gene. All qPCR was performed using the LightCycler® 480 system II (Roche Scientific, Laval, QU, USA) and all samples were run in triplicates.

Data and statistical analysis

All statistical analyses were performed using Prism 6.0 Software (GraphPad Software Inc., La Jolla, CA, USA). Organ tissue weights to tibia length ratios, systolic blood pressure (SBP) measurements for NS and HS treatment groups, and infarct volumes were compared by unpaired, two-tailed Student's t test and plotted as mean ± SD. Target gene mRNA expressional data were compared by two-way ANOVA with multiple comparisons and Tukey post hoc test. Data are presented as mean ± SEM. $P \leq 0.05$ was deemed statistically significant. Potential outliers were determined for all statistical analyses using Grubb's test.

Results

$ANP^{+/-}$ mice exhibit salt-induced hypertension, LVH and a complete circle of Willis

Body weight and cardiac mass to tibia length ratios are presented in Table 2. Previously, we have demonstrated that $ANP^{+/-}$ mice are salt sensitive [14, 15]. When fed HS chow over a 7 week period, $ANP^{+/-}$ mice exhibited significant LVH, a clear indicator for cardiac changes associated with the development of hypertension. Tibia lengths were used as a non-confounding variable to normalize all physical masses. As compared to $ANP^{+/-}$ mice fed NS chow, those fed a HS diet, demonstrated a 12 % increase in cardiac mass by the end of the dietary treatment period. Furthermore, consistent cerebrovascular casting demonstrated a complete circle of Willis in all mice with the presence of posterior communicating arteries (PCommAs) in both right and left cerebral hemispheres (Fig. 1).

Salt-induced hypertensive $ANP^{+/-}$ mice exhibit larger infarct volumes

A subset of $ANP^{+/-}$ mice underwent non-invasive tail-cuff BP measurements to ensure the development of hypertension. As expected, a significant increase (22 %) in SBP was shown in $ANP^{+/-}$ mice fed HS chow (159.5 ± 4.5 mmHg) as compared to the NS (130.8 ± 10.2 mmHg) group (Fig. 2a). Only SBP is being reported as other investigators have demonstrated inaccuracies in measuring diastolic blood pressure (DBP) using non-invasive tail-cuff methods [21]. In addition, mice fed HS chow excrete more urinary Na^+ as compared to those fed NS (Fig. 2b).

Table 1 qPCR primer sequences, annealing temperatures (T_a), and standard curve efficiency values for genes assessed in the NP, NOS, RAS, ET and VEGF systems

Peptide name	Gene	Sequence		T_a (°C)	Standard curve efficiency
NP system					
C-type natriuretic peptide	*Nppc*	S	TGCGTGTGGACACCAAGT	62	1.746
		AS	GATGCTGGAGGCTGATGAC	62	
Natriuretic peptide receptor 2	*Npr2*	S	GAACAATGACCGCGAGAC	62	1.762
		AS	TCTCCAGCATCAGCTTCC	61	
NOS system					
Neuronal nitric oxide synthase	*Nos1*	S	AGTCATGCTTGCCATCAGTC	63	1.959
		AS	TCAACTACATCTGTAACCACGTC	63	
Endothelial-derived nitric oxide synthase	*Nos3*	S	TTGAGGATGTGGCTGTGTG	63	1.787
		AS	GAGTTAGGCTGCCTGAGATG	63	
Soluble guanylyl cyclase alpha 1	$sGC_{\alpha 1}$	S	TGTGATCGCATCATGGTG	61	1.799
		AS	CTCTGTTGGCTCCTTAGGAA	62	
Soluble guanylyl cyclase beta 1	$sGC_{\beta 1}$	S	TTCGTCTTCTGCCAGGAGT	63	1.771
		AS	CCGAGTAGTAGTGCAGGATGA	63	
RAS system					
Angiotensin converting enzyme	*Ace*	S	AACGGCAAGGACTTCAGG	62	1.895
		AS	AAGGCGATCTTGTCGAGG	62	
Angiotensinogen	*Agt*	S	CAATGATCGCCAACTTCG	62	1.839
		AS	GAGCCAGGCTCTGAACAA	62	
Angiotensin receptor 1	*Agtr1a*	S	GGCCTAACCAAGAACATCC	62	1.717
		AS	CAGCTGAATCAGCACATCC	62	
Angiotensin receptor 2	*Agtr2*	S	ACCTTCTTGGATGCTCTGAC	62	1.955
		AS	CCAATGGCTAGGCTGATTAC	62	
ET system					
Endothelin-1	*Et-1*	S	CGCTGTTCCTGTTCTTCCT	63	1.869
		AS	CCTGGTCTGTGGCCTTATT	63	
Endothelin receptor A	*Et-A*	S	TGCCTCTGTTGCTGTTGTC	62	1.802
		AS	GCATCTGTGGCGTAATGGT	64	
VEGF system					
Vascular endothelial growth factor A	*Vegfa*	S	AACACAGACTCGCGTTGC	62	2.019
		AS	CGTGGTGGTGACATGGTT	62	
Vascular endothelial growth factor receptor 1	*Flt-1*	S	TGGTCCTATGGCGTGTTG	63	2.009
		AS	CTGTTGGACGTTGGCTTG	63	
Vascular endothelial growth factor receptor 2	*Flk-2*	S	TAGCTGTCGCTCTGTGGTT	62	1.983
		AS	AATCACGCTGAGCATTGG	62	
CTRL					
Hypoxanthine–guanine phosphoribosyltransferase	*Hprt*	S	TGCTCGAGATGTCATGAAGG	64	1.863
		AS	GTAATCCAGCAGGTCAGCAA	64	

S Sense forward direction, *AS* antisense reverse direction, *CTRL* control gene

Following middle cerebral artery occlusion (MCAO), mice treated with 8.0 % NaCl exhibited an approximate threefold increase in infarct size as compared to the 0.8 % NaCl group ($P = 0.0002$) (Figs. 2c, 3). Thus, the development of salt-induced hypertension in $ANP^{+/-}$ mice significantly impacted cerebral ischemic infarct size.

Cerebral vasoactive gene expression in response to salt-induced hypertension and transient cerebral ischemia

The mRNA expression levels of various vasoactive system genes were assessed by qPCR methods. Sham operated mice were used as controls to better assess gene expression differences in response to stroke and/or dietary treatment.

Table 2 Body weight and cardiac mass physical data for 0.8 and 8.0 % NaCl treatment groups

Treatment group	*n*	Body weight:tibia length (g/mm)	Heart weight:tibia length (mg/mm)	Left ventricle weight:tibia length (mg/mm)
0.8 % NaCl	14	1.58 ± 0.154	8.86 ± 1.18	5.94 ± 0.56
8.0 % NaCl	14	1.57 ± 0.152	9.96 ± 1.53*	6.62 ± 0.83*

* Indicates that the 0.8 % NaCl treatment group is significantly different as compared to the 8.0 % NaCl treatment group at $P \leq 0.05$

Natriuretic peptide (NP) system

Gene mRNA expression levels of C-type natriuretic peptide (*Nppc*) and its specific receptor, natriuretic peptide receptor 2 (*Npr2*) demonstrated no significant differences across dietary treatment or across surgical intervention (refer to Fig. S-1 in the Supplemental Figures). All components of the NP system, including ANP (*Nppa*), B-type natriuretic peptide (*Nppb*) and natriuretic peptide receptor 1 (*Npr1*) were assessed; however, expression levels of these peptides and receptor were undetectable by qPCR methods.

Nitric oxide synthase (NOS) system

Figure 4 summarizes the changes in mRNA expression of neuronal nitric oxide synthase (*Nos1*) (Fig. 4a), endothelial-derived nitric oxide synthase (*Nos3*) (Fig. 4b) and receptors soluble guanylyl cyclase alpha 1 ($sGC_{1\alpha 1}$) (Fig. 4c) and beta-1 ($sGC_{1\beta 1}$) (Fig. 4d). A general, non-significant trend toward increased expression in response to HS was observed in both receptor genes as well as *Nos1* with no influence from stroke. The expression of *Nos3* significantly increased in stroked, HS-treated (HS-MCAO) mice as compared to the NS, stroke (NS-MCAO), and HS-treated sham (HS-Sham) groups. *Nos3* levels appear to change in response to the combined effect of HS treatment and stroke.

Renin–angiotensin (RAS) system

The resultant mRNA expression of angiotensinogen (*Agt*), angiotensin-converting enzyme (*Ace*), angiotensin II receptor type 1a (*Agtr1a*), and angiotensin II receptor type 2 (*Agtr2*) did not show significant changes although some trends were observed. Both *Agt* and *Agtr1* target genes demonstrated a pattern of decreased gene expression in response to stroke in both dietary groups equally, thus increased salt did not influence these genetic changes.

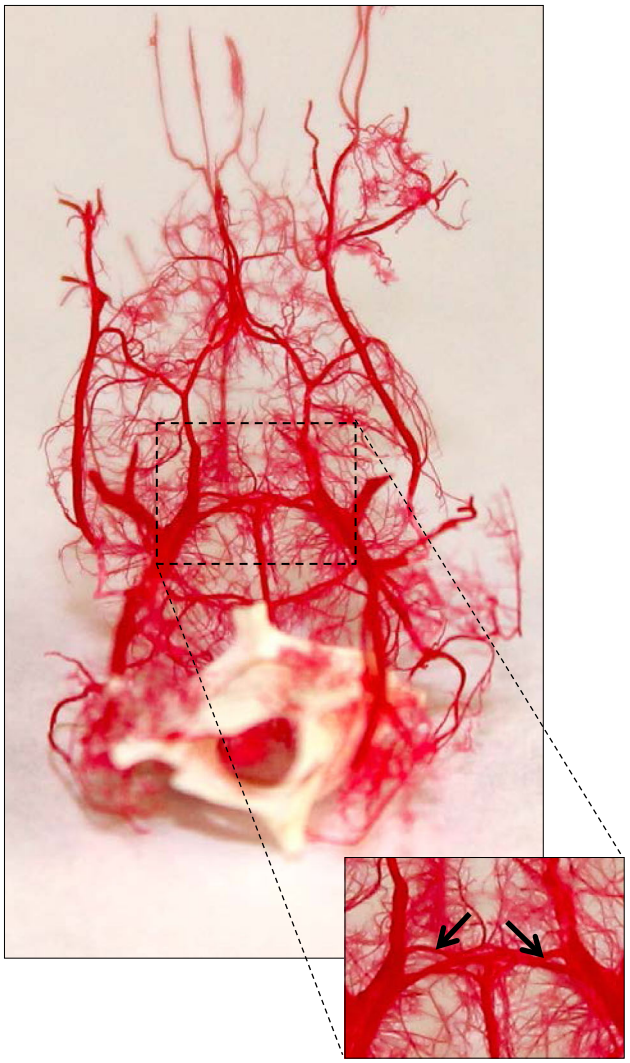

Fig. 1 Representative cerebrovascular cast. *Arrows* demonstrate presence of posterior communicating arteries forming a complete circle of Willis ($n = 4$)

Agtr2 mRNA expression increased in response to high salt alone. (refer to Fig. S-2 in the Supplemental Figures).

Endothelin (ET) system

Endothelin-1 (*Et-1*) mRNA levels demonstrated a trend toward increased expression following stroke in normotensive mice (Fig. 5a). A significant increase in *Et*-1 expression was observed in the HS-MCAO group as compared to all other treatment groups (Fig. 5a). This pattern of expression suggests that the combined presence of increased sodium, hypertension and stroke stimulates this system, above all, to respond. Although, no significant changes were observed, a similar trend of increased endothelin receptor A (*Et-A*) mRNA expression was seen

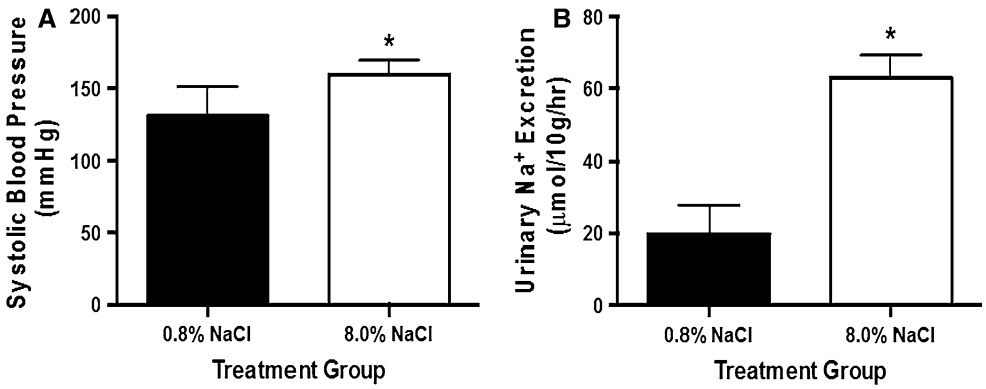

Fig. 2 Systolic blood pressure (**a**) and urinary sodium excretion (**b**) measurements in NS and HS treatment groups. **a** Data represented as mean ± SEM, $n = 4$–5; **b** data represented as mean ± SD, $n = 4$. $^*P \leq 0.05$ using unpaired, two-tailed Student's *t* test

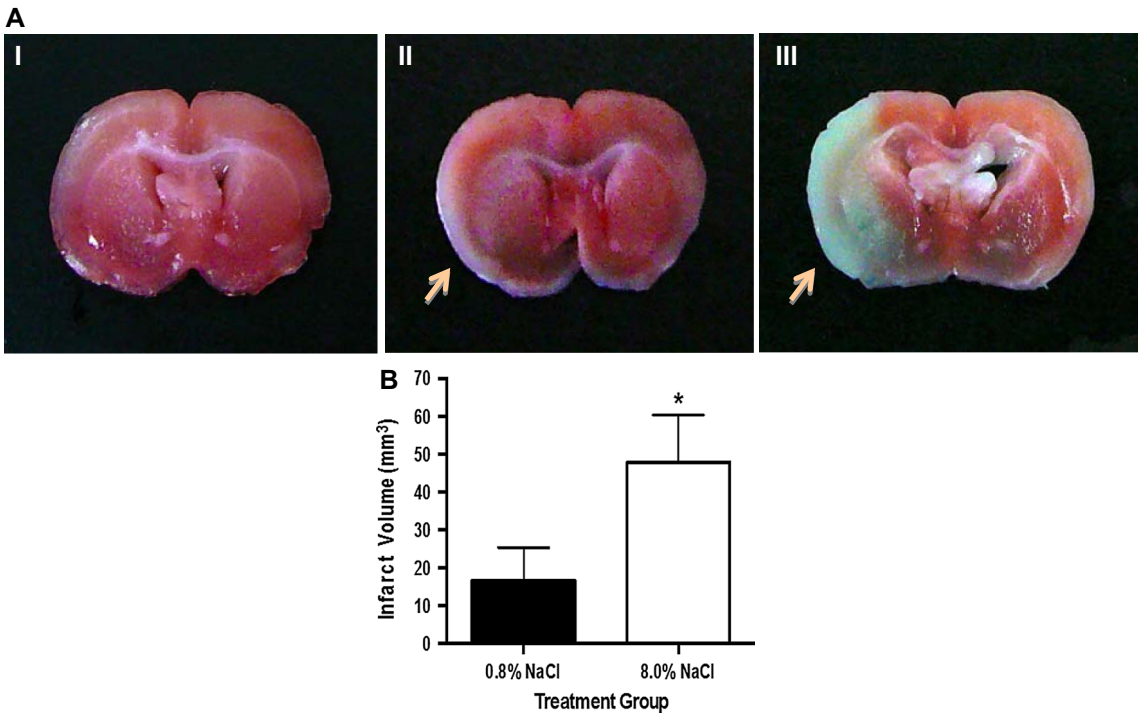

Fig. 3 Representative brain images stained with TTC 24 h post reperfusion (**a**) and infarct volume measurements (**b**) demonstrating the significant infarct volume differences across $ANP^{+/-}$ treatment groups; **a** control sham (*I*), 0.8 % NaCl (*II*) and 8.0 % NaCl (*III*). All brain sections (1 mm) were stained with TTC to measure infarct volume. *Arrows* locate infarcted area. **b** Data are represented as mean ± SD, $n = 6$. $^*P \leq 0.05$ using unpaired, two-tailed Student's *t* test

in the HS-MCAO group compared to all others, which corresponds to *Et-1* levels (Fig. 5b).

Vascular endothelial growth factor (VEGF) system

Vascular endothelial growth factor type A (VEGFA), vascular endothelial growth factor receptor 1 (Flt-1), and vascular endothelial growth factor receptor 2 (Flk-1) were assessed by qPCR to determine whether cerebral ischemia and/or hypertension stimulates this physiological response. A general trend to increased *Vegfa* expression (Fig. 6a) in response to salt was seen. Receptors *Flt-1* (Fig. 6b) and *Flk-2* (Fig. 6c) exhibited similar expression patterns. Both receptors showed an increase in mRNA expression in the HS-MCAO group; however, only the *Flk-1* expression was significantly increased. As a result, these data demonstrate angiogenic responses to a combined salt-induced hypertensive and cerebral ischemic event.

Discussion

We have used a novel approach to study the effects of salt-induced hypertension on adaptations in vasoactive systems

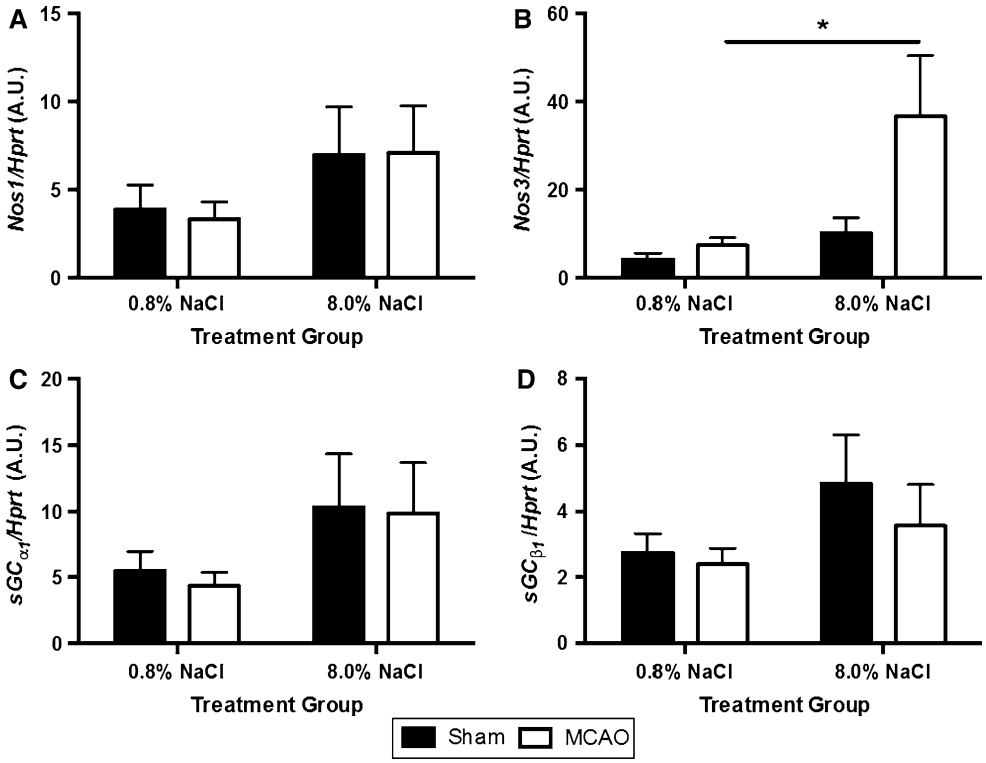

Fig. 4 Changes in mRNA expression of *Nos1* (**a**), *Nos3* (**b**), sGC_{a1} (**c**), and sGC_{b1} (**d**) in left hemispheric brain tissue of sham and left MCAO mice fed either NS or HS diet. Data are presented as mean ± SEM. $^*P \leq 0.05$, using two-way ANOVA with Tukey's post hoc test. $n = 6$

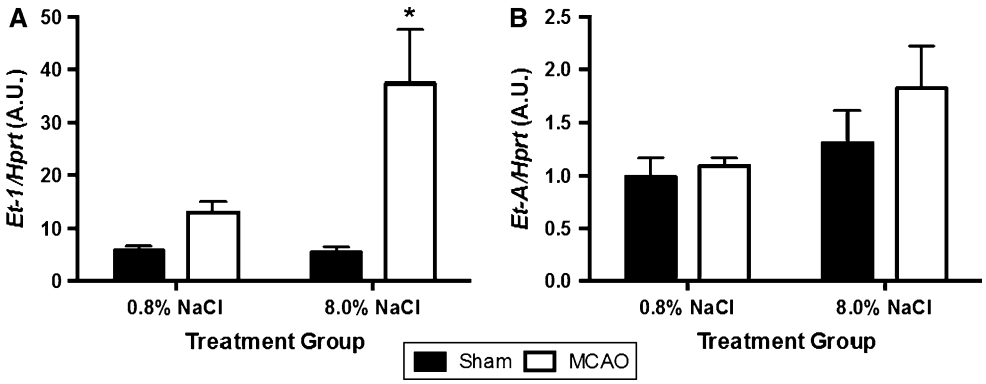

Fig. 5 Changes in mRNA expression of *Et-1* (**a**) and *Et-A* (**b**) in left hemispheric brain tissue of sham and left MCAO mice fed either NS or HS diet. Data are presented as mean ± SEM. $^*P \leq 0.05$, using two-way ANOVA with Tukey's post hoc test. $n = 6$

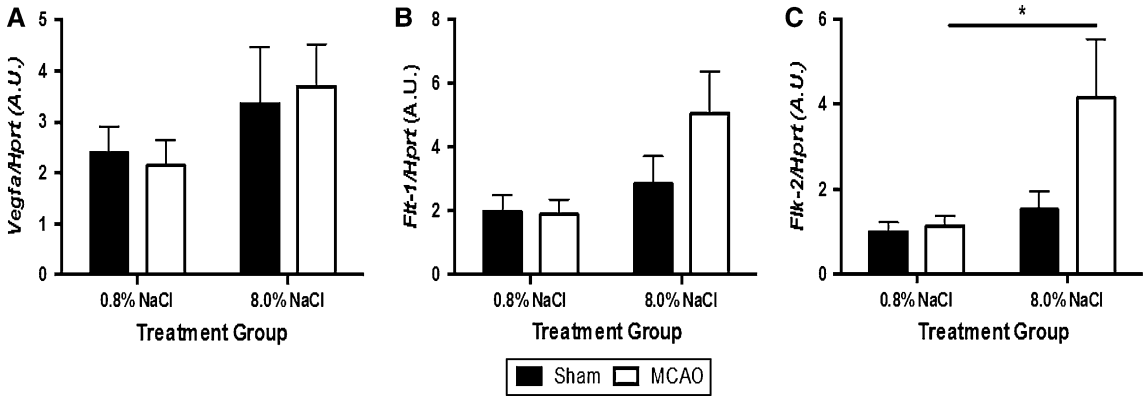

Fig. 6 Changes in mRNA expression of *Vegfa* (**a**), *Flt-1* (**b**), and *Flk-2* (**c**) in left hemispheric brain tissue of sham and left MCAO mice fed either NS or HS diet. Data are presented as mean ± SEM. $^*P \leq 0.05$, using two-way ANOVA with Tukey's post hoc test. $n = 6$

in acute stroke. The genotype expressed by the $ANP^{+/-}$ mouse allowed us to examine the effects of salt-sensitivity without confounding from genetic influences. Experiments using the spontaneously hypertensive rat (SHR) and spontaneously hypertensive stroke-prone (SHR-SP) rat developed by Okamoto et al. [22], often use the Wistar-Kyoto (WKY) rat as the control group, a genetically disparate animal from SHR and SHR-SPs [23]. The genetic factors by which these rats develop hypertension or become more vulnerable to stroke is still poorly understood. This is also the case with Dahl salt-sensitive and Dahl salt-resistant rat models [24, 25] for which the genetic basis of salt-sensitivity has also not been elucidated. This is the first study in which the influence of salt-induced hypertension on stroke tissue injury has been examined using treatment and control groups with well-defined and identical genotypes. Through evaluation of urinary sodium excretion, SBP and cardiac hypertrophy, we have validated the establishment of salt-induced hypertension in the $ANP^{+/-}$ mouse. This is in accordance with previously published work [14, 15, 26].

Vascular casting in the $ANP^{+/-}$ mouse demonstrated an intact circle of Willis without anastomotic variations, thus allowing comparison of cerebral responses across treatment groups following stroke. As a result, we can be confident that the increased infarct size we have observed in the HS group is due to mechanistic changes that have been influenced by the acquired salt-induced hypertensive state.

Cerebrovascular adaptations, resulting from increased salt and high BP, could contribute to the large infarct volumes experienced by hypertensive $ANP^{+/-}$ mice. The NP and RAS systems did not show significant changes in mRNA expression of the peptides or receptors investigated implying that these systems are not responsible for cerebrovascular changes in this state. However, changes involving NOS1, NOS3, ET-1, and VEGF were seen.

Nitric oxide (NO) production is known to play a pathophysiological role for various vascular diseases, in particular, those related to aberrant blood flow and inflammatory response. Increases in NOS1 have been implicated with larger infarct volumes due to its neurotoxic effects, while increases in NOS3 play a neuroprotective and angiogenic role [27, 28]. In response to the HS dietary treatment, cerebral mRNA expression of NOS1 increased in $ANP^{+/-}$ mice, suggesting that NOS1-associated neurotoxicity may be stimulated in the presence of high sodium. Cerebral NOS3 expression increased markedly in response to the combined presence of high sodium and stroke, perhaps reflecting an induced endogenous protective response.

The effect of NO on vascular smooth muscle cells is calcium dependent. Binding of NO to sGC receptors catalyzes the conversion of GTP to cGMP causing further alterations in calcium availability leading to vasodilation. Gene mapping of sGC receptor isoforms has revealed a connection between expression of these receptors, salt-sensitivity, and BP [29]. Our molecular analysis demonstrates a similar role for the α-1 and β-1 sGC isoforms as trending increased mRNA expression was seen in the hypertensive animal.

The ET system is responsible for vasoconstriction of blood vessels, in particular, small arterioles, and controlling myogenic tone of capillary beds. The plasma concentration of ET-1 is increased in acute stroke patients [30, 31] and salt-sensitive/hypertensive individuals [32]. In addition, ET-1 has been shown to disrupt microcirculation in the brain rendering the cerebrovasculature at risk for damage [33]. Although not significant, *increased Et*-1 mRNA expression was seen in response to stroke. In contrast, there was a significant rise in cerebral mRNA expression of *Et-1* in the HS-MCAO group alluding to a potential role *for Et-1* in contributing to the large infarct volumes in this subset of mice. Future experimentation is required to better understand the mechanism of these alterations and the role of ET-1.

VEGF is involved in angiogenesis [34] and enhanced vascular permeability [35–37]. Ischemic conditions induce the production of VEGF to promote new vessel formation [38], but it remains unclear if this is beneficial or detrimental to stroke injury. Increased *Vegfa*, *Flt-1*, and *Flk-2* in response to HS may reflect a propensity for increased vascularization and possibly contribute to larger infarct size. Further experimentation would be needed to clarify this.

Conclusion

We have successfully examined potential influences of salt-induced hypertension on stroke with genetically identical treatment and control groups using the $ANP^{+/-}$ mouse. Our data indicate that a salt-induced state of hypertension renders the brain more susceptible to tissue damage following an ischemic event. We provide evidence for the notion that the large infarct volumes observed are due to alterations in vasoactive systems, in particular the NOS, ET, and VEGF systems, which change the response and activity of the cerebrovasculature. Further investigation is required to identify the mechanistic role the proposed vasoactive systems play with respect to salt-induced hypertension and stroke.

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Conflict of interest None.

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