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Blood brain barrier dysfunction and a role for inflammation in age-dependent salt-sensitive hypertension

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BLOOD BRAIN BARRIER DYSFUNCTION AND A ROLE FOR INFLAMMATION IN AGE-DEPENDENT SALT-SENSITIVE HYPERTENSION

by

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DEDICATION

Mom and Dad- thank you for always being down for whatever crazy adventure I come up with next. I can always count on your love, support, and a healthy dose of perspective.

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BLOOD BRAIN BARRIER DYSFUNCTION AND A ROLE FOR INFLAMMATION IN AGE-DEPENDENT SALT-SENSITIVE HYPERTENSION

KAYLA MARIE NIST

ABSTRACT

Hypertension is the principal risk factor for chronic kidney disease, myocardial infarction, and stroke, and is responsible for 10% of deaths globally. Normal aging and elevated dietary sodium intake have both been identified as contributors to hypertension, the salt sensitivity of blood pressure, and elevations in sympathetic tone. Normal aging has also been identified as a contributor to vascular dysfunction and inflammation. We hypothesize that normal aging and increased dietary sodium intake contribute to the development and maintenance of age-related salt-sensitive hypertension. We anticipate with age that there are elevations in sympathetic tone, decreases in the vascular integrity of the blood brain barrier, and an increase in the inflammatory response of the innate immune system of the brain by astrocytes and microglia.

Using male Sprague-Dawley (SD) rats aged 3-, 8- and 16-months-old as a model of normal aging, rodents were randomly assigned to a normal salt (NS; 0.6% NaCl) or high salt (HS; 4% NaCl) diet. We then assessed the development of hypertension, the salt sensitivity of blood pressure, changes in central vascular integrity, and a role for central inflammation in age-dependent hypertension.

We observed a robust age-dependent increase in blood pressure in rodents on a NS diet suggesting male SD rats develop age-dependent hypertension. We also observed that aged rats develop the salt sensitivity of blood pressure. We saw significant elevations in

global sympathetic tone, sympathetic tone to the vasculature, and sympathetic tone to the kidney in response to normal aging. Further, there was a dramatic extravasation of intravascularly infused dextrans in the paraventricular nucleus of the hypothalamus (PVN) with age, suggesting a loss in vascular integrity of the blood brain barrier, regardless of dietary sodium intake. Astrocytic density in the PVN was maintained in animals on a NS diet. In rodents on a HS diet, both young and middle aged rats, but not in aged rats showed increased levels of astrocytic density. There was no difference in interstitial astrocyte densities, which excluded perivascular astrocytes, compared to total astrocyte density, suggesting that perivascular astrocytes did not play a significant role in astrocyte activation. Microglial branching complexity was maintained in all groups regardless of salt intake, suggesting that microglial activation was the same through all groups. However, microglial morphology showed a trending increase in activation level on a NS with age. On a HS diet, there is an increase in activation state at 8-months-old.

Overall, normal aging and increases in dietary sodium intake contribute to increases in blood pressure, elevations in sympathetic tone, and ultimately a loss in vascular integrity of the blood brain barrier. Importantly, the following studies reveal a novel breakdown in the blood brain barrier in a normal aging model of hypertension by which increased blood pressure and aging evokes an increase in blood brain barrier permeability.

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LIST OF ABBREVIATIONS

ANGII .... Angiotensin II

ANOVA .... Analysis of Variance

BBB.. Blood Brain Barrier

BUSM .... Boston University School of Medicine

CNS .... Central Nervous System

FITC10 .... Fluorescein Isothiocyanate-Dextran

HS .... High Salt

IACUC .... Institutional Animal Care and Use Committee

i.p. .... Intraperitoneal

i.v. .... Intravenous

MAP .... Mean Arterial Pressure

NE .... Norepinephrine

NS .... Normal Salt

NVU .... Neurovascular Unit

PBS .... Phosphate-Buffered Saline

PFA .... Paraformaldehyde

PVN .... Paraventricular Nucleus of the Hypothalamus

RHO70 .... Rhodamine Isothiocyanate-Dextran

SD .... Sprague-Dawley

SD .... Standard Deviation

SEM .... Standard Error of the Mean

INTRODUCTION

Hypertension

Hypertension, the persistent increase in blood pressure, is the foremost, modifiable risk factor for morbidity and mortality contributing to one in ten deaths worldwide, (Danaei et al., 2011; World Health, 2013). This ultimately makes hypertension a global public health problem. In the United States alone, on average one in two adults are hypertensive; this rate increases in adults above the age of 65 as three in four individuals are hypertensive (Muntner et al., 2018; Whelton et al., 2018). As the leading risk factor for chronic kidney disease, stroke, and myocardial infarction, hypertension is clinically diagnosed as blood pressure at or above 130 mmHg systolic or 80 mmHg diastolic (Danaei et al., 2011; World Health, 2013; Whelton et al., 2018). In comparison, normal blood pressure measurements are at or below 120 mmHg systolic and 80 mmHg diastolic (Whelton et al., 2018). The small change between hypertension and normotension enhances the importance that minor increases in blood pressure play a significant role in the pathology of hypertension. Decreases in blood pressure of even 1 mmHg have been shown to ultimately reduce hypertension-related mortality (Group et al., 2015; Hardy et al., 2015).

The Salt Sensitivity of Blood Pressure

Salt sensitivity is an exaggerated pressor response to elevated levels of dietary salt intake (Franco & Oparil, 2006) and is a known predictor for the development of salt-sensitive hypertension. Salt sensitivity, which is involved in sodium retention and hypertension, is a sympathoexcitatory response to an increase in dietary salt consumption

(Bayorh et al., 1998; Dobesova et al., 2002; Brooks et al., 2005, Stocker et al., 2013). Salt resistance is described as the conservation of normotension, characterized by a sympathoinhibitory in response to increased dietary salt intake, maintaining natriuresis (Brooks et al., 2005; Osborn et al., 2008, Kapusta et al., 2013). While the American Heart Association recommends a daily intake of 1.5 g of salt per day, the average American consumes about 3.5 g of salt daily, well in excess of the daily recommendation, making dietary sodium intake a dominant issue for hypertension (Mozaffarian et al., 2014).

The Sympathetic Nervous System as a Regulator for Blood Pressure

The sympathetic nervous system, in parallel with the parasympathetic nervous system, is responsible for the involuntary regulation of physiological homeostasis. Specifically, the sympathetic nervous system is responsible for increasing heart rate, vasoconstriction, and norepinephrine release, as well as indirectly eliciting renin release from the kidney which subsequently stimulates the renin-angiotensin-aldosterone system, amongst other functions, and plays a vital role in the long-term regulation of blood pressure. Through changes in norepinephrine, the sympathetic nervous system increases heart rate, vasoconstriction, and renal sodium reabsorption in the kidney, affecting the heart, the kidney, and central and peripheral vasculature. Increases in sympathetic tone have been observed in adults with increased blood pressure (Narkiewicz et al., 2005); thus sympathetic tone is likely relevant in age-dependent hypertension).

The paraventricular nucleus (PVN) of the hypothalamus plays an essential role in the neurohumoral regulation of blood pressure. As a key cardiovascular control center, the PVN is an integration center within the central nervous system, receiving projections from the kidney and sodium sensing sites like the circumventricular organs in the brain and sending projections to the brain stem and spinal cord to control autonomic output to the periphery (Guyenet, 2006; Ferguson et al., 2008; Stocker et al., 2010, Stocker et 2013). There is ample evidence implicating the PVN in the regulation of hypertension and the salt sensitivity of blood pressure. The PVN is implicated in sympathoinhibition and the maintenance of normotension in animals with salt-resistance, such as the Dahl salt-resistant rat or the young male SD rat (O'Donaughy & Brooks, 2006; Wainford et al., 2015, Moreira et al, 2019). In salt-sensitive animal models, such as the Dahl salt-sensitive rat, the PVN plays a role in sympathoexcitation, and thus the development of hypertension due to increased dietary salt intake (Shi et al., 2007; Bardgett et al., 2014; Holbein & Toney, 2015; Wainford et al., 2015).

The Blood Brain Barrier

The blood brain barrier (BBB) is the highly regulated and selective, semipermeable tissue barrier created by endothelial cells and tight junctions lining the vessel walls within the vasculature of the brain (Abbott et al., 2010). The BBB creates an exclusive barricade between the central nervous system (CNS) and the circulating peripheral blood supply, selectively restricting the passive transportation and paracellular traffic of even extremely small molecules, importantly to prevent toxins, pathogens, and circulating neurotransmitters, and macromolecules from passing into the CNS (Abbott et al., 2010). This is done in collaboration with the collective cells of the neurovascular unit (NVU). The NVU is the combined unit of barrier endothelial cells, pericytes, basement membranes, astrocytes, and neurons and microglia, which are located directly surrounding the vasculature of the central nervous system (Obermeier et al., 2013). The astrocytic endfeet processes create another barrier, termed the glia limitans, by making contact with the endothelial cells from the side of the brain parenchyma in order to prevent more unwanted movement from the periphery to the central nervous system (Abbott et al., 2010; Sofroniew et al., 2010). This combination of protective barriers is key for the brain to maintain proper homeostasis, cognitive function, and immune privilege within the central nervous system (Ueno et al., 2016). Aging plays a significant role in the dysregulation and deterioration of the BBB and further, dysregulation of the BBB has been indicated as a key factor in many age-related neurodegenerative diseases and cognitive impairment (Chung et al., 2013; Ueno et al., 2016; Costea et al., 2019). In studies of Angiotensin II (AngII) hypertension and Dahl salt-sensitive hypertension, blood pressure has more recently been implicated in BBB dysfunction and is associated with increased levels of inflammation in the brain, as well as cognitive decline (Pelisch et al., 2011; Biancardi et al., 2014). Inflammation has been linked to playing a role in BBB permeability (Setiadi et al., 2018). Aging and hypertension are both associated with chronic, low-grade inflammation (Coffman, 2011; Zubcevic et al., 2011; Franceschi et al., 2014). While peripheral markers of inflammation, including T-cells and proinflammatory cytokines, have been shown to be upregulated in hypertension (Guzik et al., 2007; Coffman, 2011), circulating cytokines, as well as increases in markers of neuroinflammation, have been shown to be upregulated in the brain in models of hypertension (Shi et al., 2010; Biancardi et al., 2014; Winklewski et al., 2015).

Microglia and astrocytes, both of which are components of the NVU, while aiding in the maintenance of brain homeostasis, also play a role in inflammation. Microglia are the innate immune cells of the brain and have been implicated in having a role in the maintenance of hypertension (Shi et al., 2010; Biancardi et al., 2014; Moreira et al., 2019). Astrocytes, the most abundant glial cell in the brain, work together with microglia to reestablish physiologic homeostasis in the brain when they are activated. When in a pathological state, they have been implicated in sympathoexcitation, BBB dysfunction in hypertension (Stern et al., 2016; Marins et al., 2017; Setiadi et al., 2017). This suggests the NVU, while playing a role in maintaining the blood brain barrier when in a pathological state, may also be playing a role in the autonomic regulation of blood pressure.

Mechanisms which promote an increase in blood pressure remain incompletely understood, despite decades of research. Due in part to blood pressure being regulated by numerous systems, a complete understanding of the mechanistic underpinnings of the disease remains elusive. We hypothesize in age-dependent hypertension, there is a progressive increase in BBB permeability and neuroinflammation which is exacerbated by high dietary salt intake. Here, we have investigated hypertension in male SD rats as a model of normal aging. We established this model of normal aging, through changes in blood pressure, the development of the salt sensitivity of blood pressure, and alterations in sympathetic tone. Further, we assessed alterations in blood brain barrier permeability and a potential role for inflammation.

METHODS

Animals

Male SD rats aged approximately 3-, 8-, and 16-months-old were acquired from Envigo (Indianapolis, IN, USA) and transported to Boston University School of Medicine to be housed in the Animal Science Center. Rats were housed separately in a 12:12-hour light-dark cycle, temperature (20-26˚C) and humidity-controlled (30-70%) environment Rats were allotted tap water and a randomly assigned standard irradiated rodent diet (Envigo Teklab, WI, Teklab Global Diet #2918, 18% protein, 5% crude fat, 5% fiber, total NaCl content 0.6% [174 mEq Na+/kg]) or experimental high salt diet (Envigo Teklab Diets, WI, TD.03095, 19% protein, 5% crude fat, 3% fiber, total NaCl content 4% [678 mEq Na+/kg]) ad libitum. All animal protocols used were approved by the Institutional Animal Care and Use Committee (IACUC) at Boston University School of Medicine, in agreement with guidelines set by the university and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Every step possible was taken in order to minimize pain and suffering. Euthanasia was performed as per the above guidelines, in accordance with approved IACUC protocols.

Surgical Procedures

Acute Femoral Artery and Femoral Vein Cannulations

Age- and dietary salt intake matched groups (N=6 per group) of male SD rats were anesthetized using sodium methohexital (200 mg/kg intraperitoneally (i.p.), with 10 mg/kg administered intravenously (i.v.) as needed). Cannulation of the femoral artery and vein was performed as previously described by our lab (Carmichael et al., 2016; Walsh et al., 2016, Frame et al., 2019, Frame et al., 2019). In brief, an incision into the left femoral triangle was made and the femoral artery and vein were dissected from the adjacent tissue. Using a cannula made from PE-50 tubing, the cannula was inserted into the femoral vein to allow for administration of i.v. anesthesia, isotonic saline during recovery periods, as well as experimental challenges using pharmacological agents. An additional PE-50 cannula was placed in the left femoral artery to record heart rate and mean arterial pressure (MAP). Cannulas were tied in place using 4-0 silk sutures and the incision was closed around the free ends of the cannulas. Rats were positioned in a Plexiglas rat holder and the cannula for the femoral artery was attached to an external pressure transducer, while the cannula for the femoral vein was attached to an infusion pump. A two-hour recovery period was allowed, during which an i.v. infusion of isotonic saline (20 μL/min) was performed and rats returned to full consciousness, with stable cardiovascular and renal function. After the two-hour recovery period, measurements for heart rate and baseline MAP were recorded constantly over a one-hour period in conscious rats through the femoral artery cannulation using the computer-driven data acquisition software, MP150 and AcqKnowledge 3.8.2 (BIOPAC, CA).

Intravascular Infusion of Dextrans

Age-matched and dietary sodium intake-matched SD rats (N=6 per group) were anesthetized (30 mg/kg ketamine i.p., 3 mg/kg xylazine i.p.) (Gumerlock et al., 1990) and an incision was made into the left carotid triangle. The sternomastoid and sternohyoid muscles were mobilized to expose the carotid sheath. The carotid artery was dissected from surrounding tissues and isolated. A non-occluding, beveled PE-50 catheter was placed into the exposed left common carotid artery and secured (Shi et al., 2008, Biancardi et al., 2014). Rats were infused with fluorescein isothiocyanate-Dextran (FITC10) at 10 kDa (10 mg/mL, 2.86 μL/g, Sigma-Aldrich) and rhodamine B isothiocyanate-Dextran (RHO70) at 70 kDa (10 mg/mL, 2.86 μL/g, Sigma-Aldrich) for 60 seconds (~1 μL/1 s). Fluorescent dextrans were allowed to circulate for 20 minutes before animals were sacrificed via decapitation using a small animal guillotine (Biancardi et al., 2014). A group of 3-month-old rats (N=6) on a normal salt diet were infused with hypertonic mannitol (1.4 mol/L, 2 mL per 200-250 g) 5 minutes before the dextrans infusion as a positive control (Kozler et al., 2003, Shi et al., 2008, Yao et al., 2013, Biancardi et al., 2014). After sacrifice, brain tissue was extracted and post-fixed in 4% paraformaldehyde (PFA) at pH 7.4 at 4˚C for 48-72 hours, then immersed in a 1M phosphate-buffered saline (PBS)/30% sucrose solution for cryoprotection.

In Vivo Studies in Conscious Animals

Assessment of Vascular Sympathetic Tone

In animals that underwent acute femoral artery and vein cannulation procedures, peak depressor response to a pharmacological challenge of a ganglionic blockade (hexamethonium, 30 mg/kg, i.v.) was used to estimate sympathetic tone to the vasculature as previously described (Walsh et al, 2016). Baseline blood pressure was defined as the average blood pressure that occurred during the ten minutes before the bolus of hexamethonium was administered. The lowest blood pressure measured that occurred within five minutes of the administration of hexamethonium, was used to calculate the difference compared with baseline blood pressure.

Euthanasia

Conscious and Unconscious Decapitation

In a sub-group of animals (N=6 per group), conscious decapitation was performed using a small animal guillotine for collection of whole blood for plasma norepinephrine (NE) and renal NE content. As anesthesia suppresses the sympathetic nervous system, sedation was not used in these animals. Kidneys were harvested and renal cortex was dissected from the medulla for renal NE content. Animals subjected to intravascular infusions of fluorescent dextrans were deeply anesthetized using a ketamine/xylazine cocktail (30 mg/kg ketamine i.p., 3 mg/kg xylazine i.p.), and sacrificed via decapitation using a small animal guillotine. Brains were harvested for further evaluation.

Transcardial Perfusion

Sub-groups of age-matched and salt-matched rats (N=6 per group) were deeply anesthetized (3o mg/kg ketamine i.p., 3 mg/kg xylazine i.p.). Rats were transcardially perfused with 200 mL of 1M PBS and 200 mL of 4% PFA. Upon completion, brains were extracted and immersed in 4% PFA for 48-72 hours and stored at 4˚C, then placed in 30% sucrose-PBS solution until sectioning for immunohistochemistry.

Molecular Techniques

Assessment of Plasma Norepinephrine Content

Plasma taken following conscious decapitation was used to assess Plasma NE via ELISA (IB89552, IBL America) according to manufacturer's directions. As our lab has previously described (Kapusta et al. 2012, Wainford et al., 2015, Moreira et al., 2019), whole blood harvested from rodents was centrifuged at 4℃ and plasma was isolated. Plasma NE was extracted in hydrochloric acid and transferred to a microtiter ELISA plate where a dual antibody detection system captured NE. Spectrophotometric quantification was performed against a standard curve of known values.

Assessment of Renal Norepinephrine

Immediately following conscious decapitation, kidneys were removed. Renal artery, vein, ureter, and renal capsule were dissected from the kidney. Renal cortex was dissected from medulla and pelvis, and flash frozen using liquid nitrogen and stored at -80°C until ready for experimentation. Renal cortex was manually homogenized in a buffer as previously described (Frame et al., 2019) and whole protein was isolated. Renal norepinephrine tissue content was assessed using ELISA (IB89537, IBL America) and performed according to manufacturer's directions. In brief, NE was extracted from whole protein in hydrochloric acid, NE was transferred to a microtiter ELISA plate, captured with antibodies, and detected via spectrophotometric quantification against a standard curve of known values.

Immunohistochemistry

Immunohistochemistry was performed as previously described by our laboratory (Frame et al., 2019; Moreira et al., 2019). Brain tissue from perfused rats was post-fixed in 4% PFA followed by 30% sucrose solution before sectioning using a cryostat at 40μm and stored in cryoprotectant until ready for experimentation. Sections containing the PVN were selected using Paxinos & Watson's *The Rat Brain* atlas, between Bregma -1.6 mm and

Bregma -2.16. Sections of the PVN were found posterior to the interventricular foramen but anterior to the CA1 region of the hippocampus (Carmichael et al, 2016; Moreira et al., 2019). Sections were washed in 0.1M PBS to remove cryoprotectant, incubated in 0.3% hydrogen peroxide to clear the tissue of its endogenous peroxidases, and rinsed in PBS to remove peroxide. Sections were then blocked and permeabilized in PBS-diluent (0.01M PBS with 3% normal horse serum and 0.25% Triton X-100) for 2 hours before incubation in primary antibodies against rat OX-42/CD11b/c (1:100, BD Biosciences) for microglia (Shi et al., 2010, Bardgett et al., 2014, Moreira et al, 2019) and against rat GFAP (1:2000, Abcam) for astrocytes (Reiner et al., 2016, Moreira et al., 2019). Then, sections were incubated overnight at 4˚C. Following incubation in primary antibodies, sections were washed in PBS then incubated in secondary antibodies, biotinylated goat anti-mouse IgG (1:100, Vector Laboratories) and biotinylated horse anti-goat (1:100, Vector Laboratories). Sections were washed again in PBS and incubated in avidin and biotin. Sections were developed in 0.04% 3,3'-diaminobenzidene and 0.04% nickel ammonium sulfate in 0.1M PBS, mounted on gelatin-subbed slides and dehydrated overnight. Coverslips were secured using Permount mounting medium.

Vascular Integrity of the Blood Brain Barrier Analysis

Following intravascular infusions of fluorescent dextrans, and unconscious decapitation, brains were harvested and post-fixed in 4% PFA for 3 days, before being moved to 0.1M PBS/30% sucrose for cryoprotection. Brains were sectioned at 40 μm using a cryostat to obtain PVN tissue between Bregma -1.6 mm and Bregma -2.16 (Moreira et al., 2019) and placed into three serial vials of cryoprotectant. One serially sectioned vial of hypothalamic tissue was rinsed in 0.01M PBS to remove cryoprotection and mounted on gelatin-subbed slides using Prolong Diamond Antifade mountant with DAPI (Invitrogen) to delineate nuclei.

Analytical Techniques

Microscopy

Sections processed via fluorescence and immunohistochemistry were imaged on a Keyence BZ-9000 Fluorescence Microscope. The microscope was used in both brightfield and fluorescence to visualize appropriate tissues. Images were captured at 10X, 20X, and 40X magnifications. Dichroic filters for Texas Red at 585nm, GFP at 495nm, and DAPI at 400nm were used.

Analysis of Astrocytes

Right and left side images of the PVN were taken at 20X magnification and blinded for analysis. Images were converted to RGB Color using ImageJ. Total astrocytic density of the PVN was quantified by selecting all astrocytes in the PVN, including perivascular astrocytes, in pixels and dividing it by the total area of the PVN in pixels in order to obtain the total percentage of astrocytic tissue density (Pekny et al., 2014, Moreira et al., 2019). Interstitial astrocytic density was quantified in a similar manner, however when selecting astrocytes in the PVN, all perivascular astrocytes were excluded to give interstitial astrocytic tissue density.

Morphologic Analysis of Microglia

Images of the left and right side of the PVN at 40X magnification were used to assess morphology of microglia and blinded for analysis. As previously described (Shi et al., 2010; Moreira et al., 2019), sections were graded on immunoreactivity to OX42/CD11b/c antibody on a scale from one to four, one representing faint staining with low reactivity, two representing mild reactivity and staining, three representing moderate reactivity and staining, and four representing high reactivity and heavy staining. A randomly selected 200µm by 200µm box was used for analysis. Number of microglia within the box, soma size in microns, number of processes, and process length were measured (Shi et al., 2010, Bardgett et al., 2014, Moreira et al., 2019). Microglia were deemed activated, displaying heterotrophic or amoeboid morphology, if a majority of the process lengths were less than that of the diameter of the soma.

Sholl Analysis of Microglia

Images from both the right and left side of level 2 of the PVN at 40X magnification were taken as described above. Images were blinded for analysis and opened in Adobe CC Photoshop and overlaid with a 3x3 grid. Ten microglia from each animal were systematically selected at random, making for 120 microglia analyzed per group. One microglia from each box created by the grid was selected and cropped into its own image, with the tenth microglia randomly selected from any grid box. Each new image was opened using ImageJ/FIJI (National Institute of Health) and converted to an 8-bit. "Image → Adjust → Threshold" command was used in order to separate each microglia from the existing background, and then subjected to Sholl analysis. Concentric rings were place 1 µm apart beginning at the center of the cell and radiating towards the branches. T

The number of intersections at each concentric ring for a distance of 25µm was used to analyze branching complexity and process length in order to characterize morphology of each individual microglia (Go et al., 2019, Moreira et al., 2019).

Statistical Analysis

All data are presented as mean ± SEM, unless otherwise noted. Using a one-way repeated measures analysis of variance (ANOVA), the cardiovascular parameters of blood pressure and estimated sympathetic tone were compared with the average age group control. Post-hoc analysis was performed using Bonferroni test. The difference between each age group and treatment group were further assessed using a two-way repeated measures ANOVA. For astrocytic tissue density analysis, one-way repeated measures ANOVA was performed with a Bonferroni post-hoc test and individual Student's t-tests to assess the difference of age for each treatment group. Data regarding microglia are presented at mean ± standard deviation (SD). One-way repeated measures ANOVA was used to compare differences between age groups with respect to treatment for microglial morphology with a post-hoc Bonferroni test and individual Student's t-test. For Sholl analysis of microglia, the number of intersections for each ring was plotted as group means. One-way ANOVA was used to compare overall differences in trend lines across all ages for each salt diet with a Bonferroni post-hoc test. Using Prism 8 (GraphPad Software, CA), all statistical analysis was carried out with p-value of less than 0.05 being used to define statistical significance in all studies.

RESULTS

Mean Arterial Pressure

Male SD rats on a lifelong NS diet experienced age-dependent increases in MAP ([mmHg] 3-month-old 124± 2; 8-month-old 135 ± 4; 16-month-old 149 ± 3, $p < 0.05$; Figure 1). Aged, 16-month-old male SD rats experienced an exacerbation of MAP when fed a 21-day HS diet and thus, developed of the salt sensitivity of blood pressure ([mmHg] 3-month-old 126 ± 3; 8-month-old 143 ± 5; 16-month-old 169 ± 1, $p < 0.05$; Figure 1). These findings demonstrate male SD rats develop hypertension with age; when fed a HS diet, aged rats develop the salt sensitivity of blood pressure.

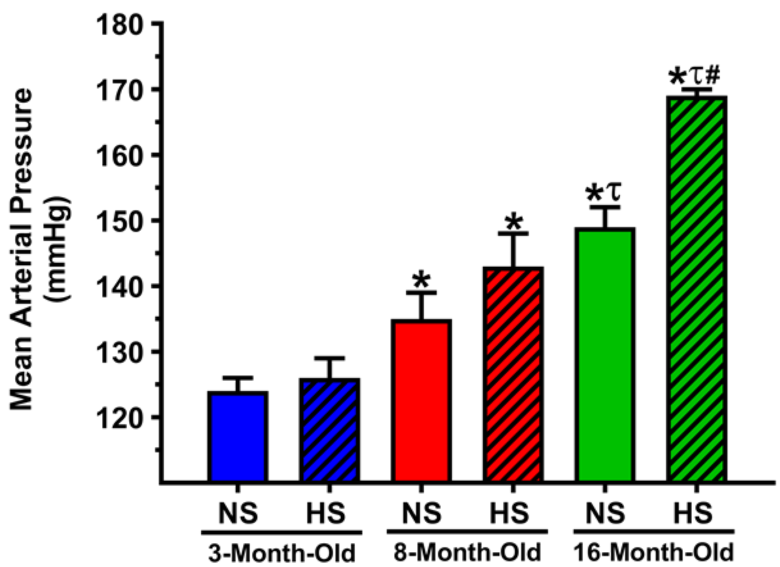

Figure 1: The Role of Aging and Dietary Salt Intake on Blood Pressure

Mean arterial pressure (MAP; mmHg) was measured in conscious male Sprague-Dawley rats aged 3-, 8-, and 16-months after a 21-day normal salt (NS; 0.6% NaCl) or high salt (HS; 4% NaCl) diet; N=6 per group. *p < 0.05 vs the respective 3-month-old group, τp < 0.05 vs respective 8-month-old group, #p < 0.05 vs respective normal salt group

Alterations in the Sympathetic Nervous System

Sympathetic Tone to the Vasculature

Sympathetic tone to the vasculature was estimated using ganglionic blockade in order to assess peak changes in mean arterial pressure as a depressor response. Male SD rats on a lifelong NS diet showed a significant increase in peak depressor response to ganglionic blockade with age (Peak ΔMAP; [mmHg] 3-month-old -33 ± 4; 8-month-old -60 ± 3; 16-month-old -60 ± 3, $p < 0.05$; Figure 2) When fed a HS diet, a comparably significant increase in depressor response to a ganglionic blockade with age occurred in male SD rats (Peak ΔMAP; [mmHg] 3-month-old -24 ± 3; 8-month-old -65 ± 6; 16-month-old -67 ± 7, $p < 0.05$; Figure 2). These results suggest with age regardless of salt intake, there is a consistent elevation in sympathetic tone to the vasculature at both 8- and 16-months-old.

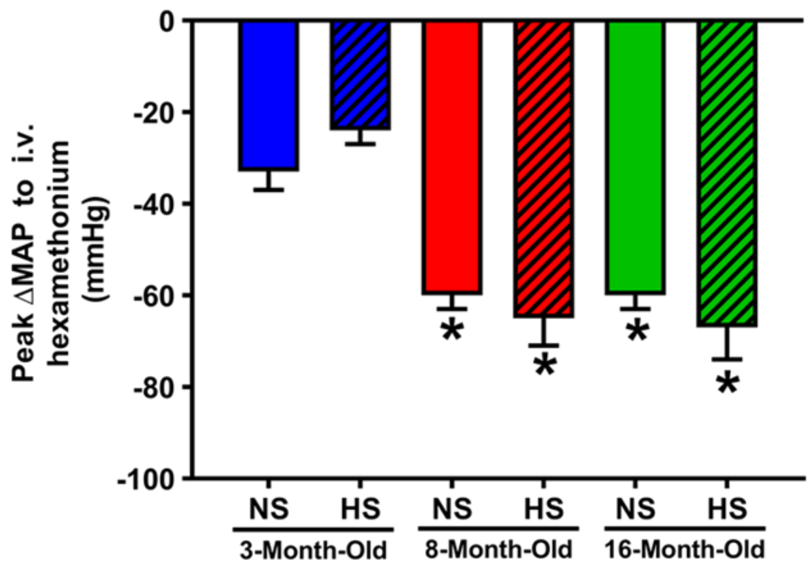

Figure 2: The Role of Aging and Dietary Salt Intake on Sympathetic Tone to the Vasculature

Estimated peak depressor response expressed as change in mean arterial pressure (Δ MAP, mmHg) in response to a ganglionic blockade using an intravenous (i.v.) bolus of hexamethonium (30 mg/kg, i.v.) was estimated in conscious male Sprague-Dawley rats aged 3-, 8-, and 16-months. Measurements occurred after a 21-day normal salt (NS; 0.6% NaCl) or high salt (HS; 4% NaCl) diet; N=6 per group. *p < 0.05 vs the respective 3-month-old group

Plasma Norepinephrine

Global sympathetic tone was estimated via plasma NE content. Male SD rats fed a lifelong NS diet showed a significant elevation in plasma NE with age ([nmol/L] 3-month-old 41 ± 4; 8-month-old 48 ± 4; 16-month-old 56 ± 8, $p < 0.05$; Figure 3). Male SD rats fed a 21-day HS diet suppressed plasma NE in young, 3-month-old rodents, however this ability to suppress NE was lost with age as evidenced by a significant increase in plasma NE with age ([nmol/L] 3-month-old 28 ± 4; 8-month-old 44 ± 5; 16-month-old 42 ± 6, $p < 0.05$; Figure 3). In aging male rats, the ability to suppress global sympathetic tone decreases, compared to young rats on a HS diet.

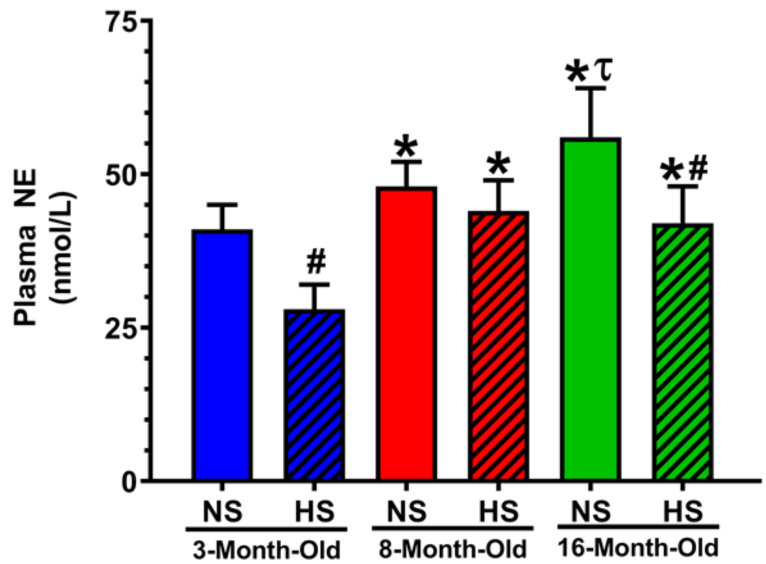

Figure 3: The Role of Aging and Dietary Salt Intake on Global Sympathetic Tone

Plasma norepinephrine (NE) concentration (nmol/L), an estimate of global sympathetic tone, was assessed in separate groups of male Sprague-Dawley rats aged 3-, 8-, and 16-months maintained on a 21-day diet of normal salt (NS; 0.6% NaCl) or high salt (HS; 4% NaCl) diet. N=6 per group; *p < 0.05 vs the respective 3-month-old group, τp < 0.05 vs respective 8-month-old group, #p < 0.05 vs respective normal salt group

Renal Norepinephrine

Sympathetic tone to the kidney was estimated as renal NE content. Male SD rats on a NS diet showed an increase in renal NE content with age ([pg/mg] 3-month-old 612 ± 36; 8-month-old 835 ± 48; 16-month-old 974 ± 39, $p < 0.05$; Figure 4). Rats aged 16-months-old SD rats on a 21-day HS diet showed an increase in renal NE content ([pg/mg] 3-month-old 368 ± 32; 8-month-old 722 ± 48; 16-month-old 1019 ± 134, $p < 0.05$; Figure 4). These results demonstrate young and middle aged rats maintain the ability to suppress sympathetic tone to the kidney, while aged rats develop age-dependent salt sensitivity of blood pressure suggested by the loss of ability to suppress renal sympathetic tone.

Overall, aging male SD rats exhibit impaired suppression of sympathetic tone during an increase in dietary sodium intake.

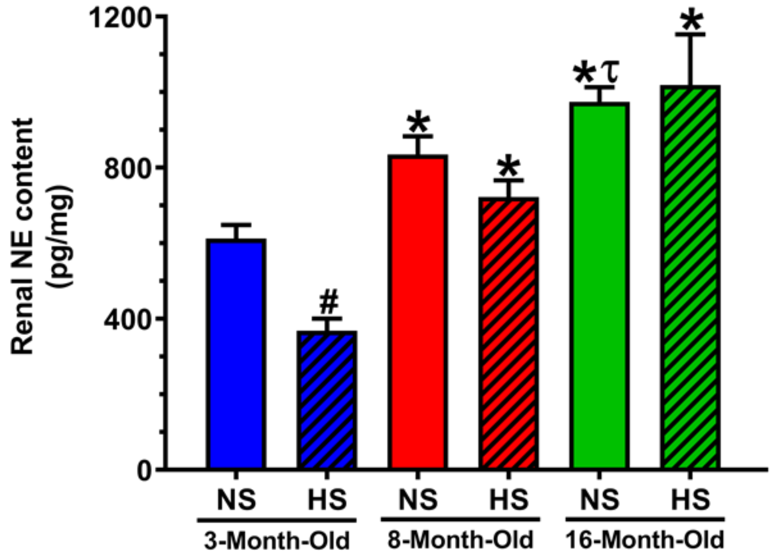

Figure 4: The Role of Aging and Dietary Salt Intake on Sympathetic Tone to the Kidney

Renal norepinephrine (NE) content (pg/mg), an estimate of sympathetic tone to the kidney, was assessed in separate groups of male Sprague-Dawley rats aged 3-, 8-, and 16-months on a 21-day diet of normal salt (NS; 0.6% NaCl) or high salt (HS; 4% NaCl) diet. N=6 per group; *$p < 0.05$ vs the respective 3-month-old group, τ$p < 0.05$ vs respective 8-month-old group, #$p < 0.05$ vs respective normal salt group

Vascular Integrity of the Blood Brain Barrier

Rats that underwent an intravascular infusion of fluorescently conjugated dextrans were assessed for extravasation of dextran-conjugated fluorescein isothiocyanate (FITC10) into the surrounding brain parenchyma of the PVN. On qualitative appearance, rodents at 3-months-old, regardless of salt diet, did not show evidence of extravasation of FITC10 into the parenchyma of the PVN (Figure 5). However, rats aged 8-months-old and 16-months-old showed a progressive increase in the visual intensity of the extravasation of dextrans regardless dietary sodium intake (Figure 5). These results demonstrate there was a loss of vascular integrity at the BBB with age. Mannitol, a drug which has hyperosmotic properties, was used as a positive control in young rats on a NS diet to assess blood brain barrier permeability. Extravasation of FITC10 was visualized in mannitol-treated rodents, confirming that FITC10 leaks at the BBB (Figure 6).

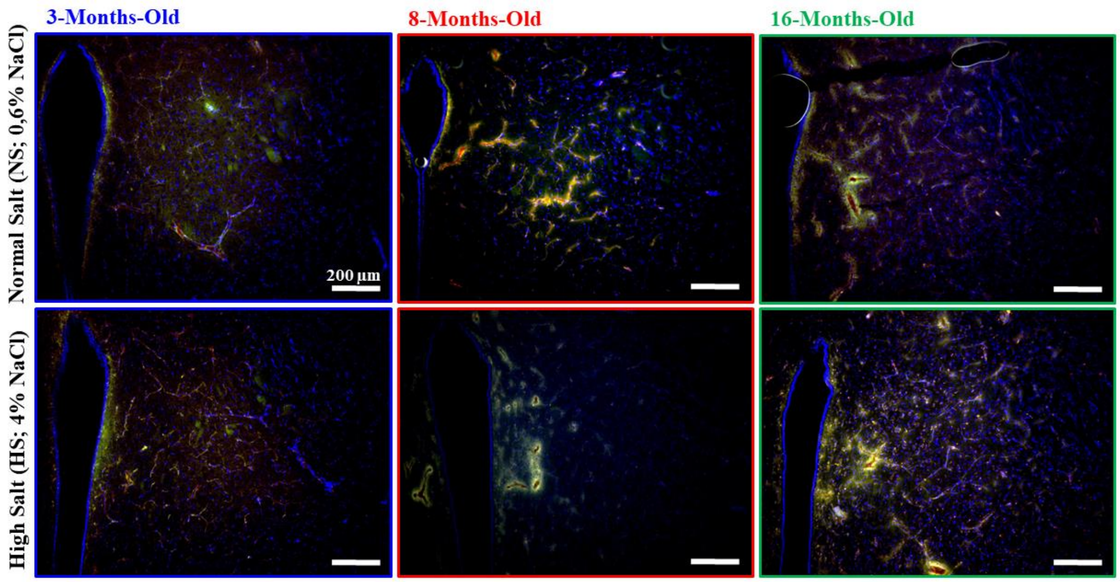

Figure 5: The Role of Aging and Dietary Salt Intake on the Vascular Integrity of the Blood Brain Barrier

The vascular integrity of the blood brain barrier in the PVN was assessed via intravascular co-infusion of dextran-conjugated fluorescein isothiocyanate (FITC10; green; 10 kDa) and dextran-conjugated rhodamine B isothiocyanate (RHO70; red; 70 kDa) and was assessed qualitatively in separate groups of male Sprague-Dawley rats aged 3-, 8-, and 16-months on a 21-day diet of normal salt (NS; 0.6% NaCl) or high salt (HS; 4% NaCl). Images taken at 10X magnification, Scale bar = 200 µm; N=6 per group

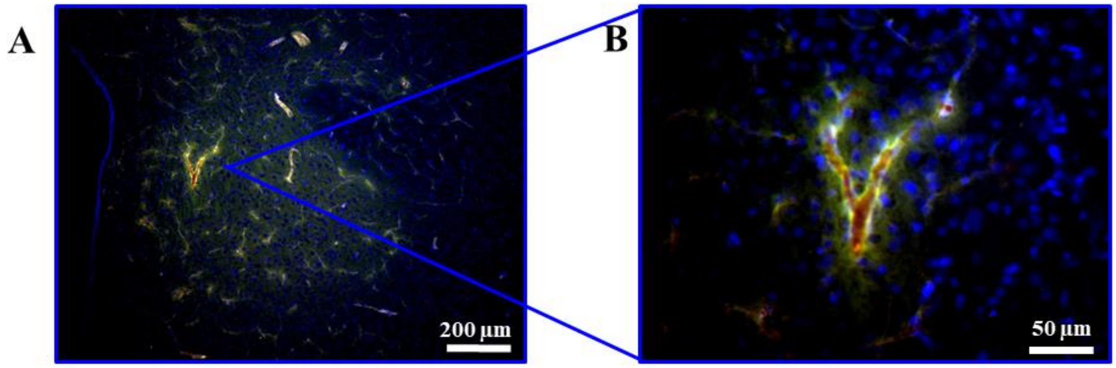

Figure 6A-B: The Impact of Hyperosmotic Mannitol on the Blood Brain Barrier

The vascular integrity of the blood brain barrier in the PVN after an infusion of hyperosmotic mannitol was assessed via in combination with intravascular co-infusion of dextran-conjugated fluorescein isothiocyanate (FITC10; green; 10 kDa) and dextran-conjugated rhodamine B isothiocyanate (RHO70; red; 70 kDa) qualitatively in a group of male Sprague-Dawley rats aged 3-months-old on a normal salt (NS; 0.6% NaCl). (6A) Image taken at 10X magnification, Scale bar = 200 µm; (6B) Image taken at 40X magnification, Scale bar = 50 µm; N=6 per group

Astrocyte Density

Immunohistochemistry for glial fibrillary acidic protein (GFAP), a known marker for astrocytes, was completed across ages and salt diets.

Interstitial Astrocytes

Astrocyte activation was assessed as astrocytic tissue density in the PVN. Activation of interstitial astrocytes, which excluded perivascular astrocytes, or astrocytes who's end feet are not in contact with a blood vessel, was assessed. Perivascular exclusion was performed to assess astrocytes not actively playing a role in the BBB. In young rodents, there was a significant increase in interstitial astrocytic density in rats fed a HS diet (% interstitial density; NS 39.50 ± 1.40%; HS 49.88 ± 1.46%; $p < 0.05$; Figure 7A,B). This same phenomenon was seen in middle aged rats (% interstitial density; NS 41.14 ± 0.63; HS 51.61 ± 1.23; $p < 0.05$; Figure 7A,B). Interestingly, this increase in astrocytic density was lost in aged rats (% interstitial density; NS 42.54 ± 1.24; HS 44.12 ± 1.31, $p > 0.05$; Figure 7A,B).

Total Astrocytes

Total astrocyte density, which included perivascular astrocytes, was assessed in the PVN. In young rats, there was a significant increase in total astrocytic density in rats fed a high salt diet with age (% total density; NS 41.58 ± 1.73%; HS 54.93 ± 1.69%; $p < 0.05$; Figure 7A,C). A high salt diet evoked the same response in middle aged rats (% total density; NS 43.65 ± 0.94; HS 58.28 ± 1.81; $p < 0.05$; Figure 7A,C). This increase in astrocytic density was lost in aged rats (% total density; NS 47.18 ± 1.09; HS 50.66 ± 1.45, $p > 0.05$; Figure 7A,C).

Collectively, these data suggest that dietary sodium intake evokes an increase in astrocytic tissue density in young and middle aged rodents, regardless of relation to the vascular supply. In aged rats, there is a loss of this activation regardless of vascular supply.

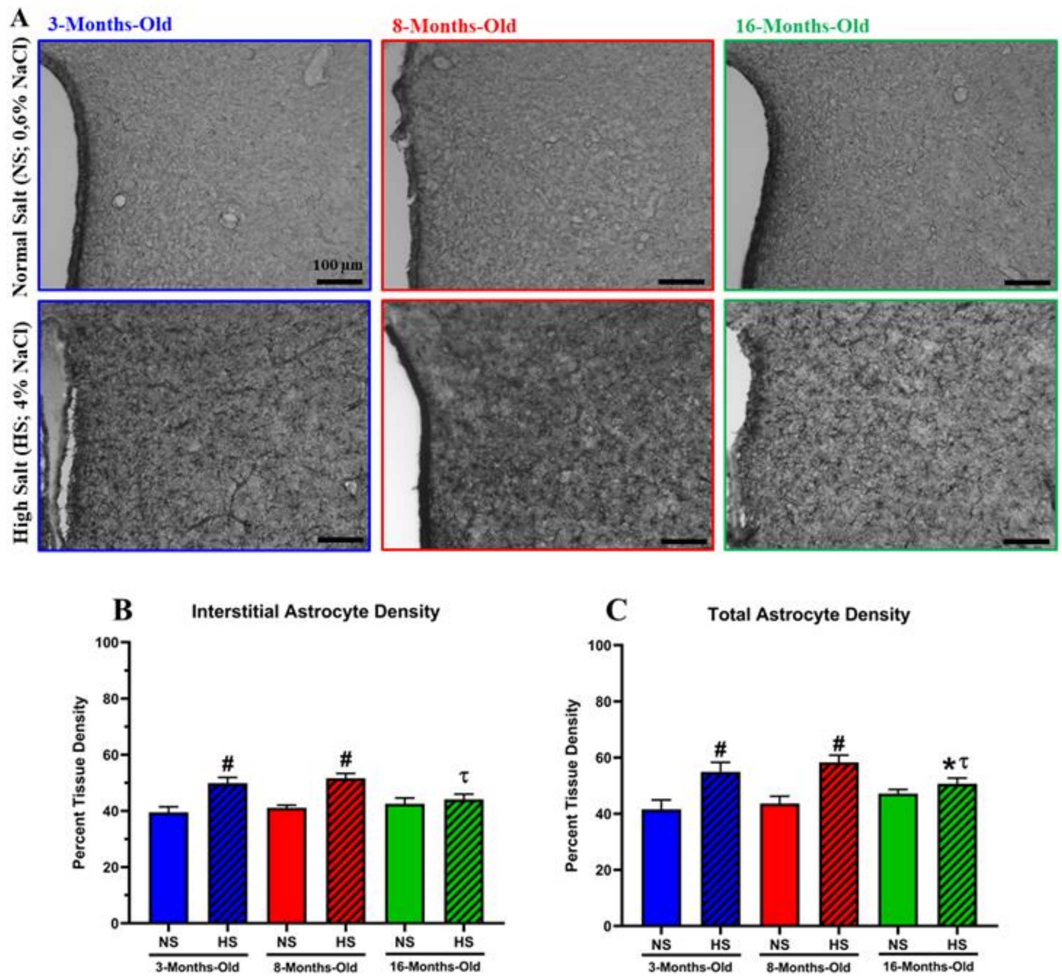

Figure 7A-C: The Role of Aging and Dietary Salt Intake on Astrocytic Density

Interstitial astrocytic density (perivascular exclusion) and total astrocytic density (total pixels of astrocytes in PVN vs total PVN area) in the PVN was assessed in male Sprague-Dawley rats aged 3-, 8-, and 16-months. Measurements occurred after a 21-day normal salt (NS; 0.6% NaCl) or high salt (HS; 4% NaCl) diet. Images taken at 20X magnification, Scale bar = 100 µm; N=6 per group, $\tau p < 0.05$ vs respective 8-month-old group, $\#p < 0.05$ vs respective normal salt group

Microglia Analysis

Immunohistochemistry for OX42 (CD11b/c), a marker of microglia, was performed in all ages and salt diets to assess microglial activation and branching complexity. All microglia morphology and Sholl analysis data are presented as mean ± SD.

Morphological Analysis

Morphological assessment was performed in the PVN to assess changes in number of active microglia, total number of microglia, and the proportion of microglia active in all groups. On a NS diet, there was no change in the number of active microglia (active microglia; 3-month-old 7.58 ± 4.23; 8-month-old 8.50 ± 5.74; 16-month-old 9.42 ± 4.29, $p > 0.05$; Figure 8A,B) or the total number of microglia (total microglia; 3-month-old 27.67 ± 3.20; 8-month-old 27.92 ± 4.10; 16-month-old 29.08 ± 3.65, $p > 0.05$; Figure 8A,C) with age. There was no change in the percent of active microglia with age (% active microglia; 3-month-old 27.03 ± 13.88%; 8-month-old 29.53 ± 17.62%; 16-month-old 32.06 ± 12.34%, $p > 0.05$; Figure 8A,D). On a HS diet, there was a significant increase in the amount of active microglia from 3-months-old to 8-months-old with a decrease at 16-months-old (active microglia; 3-month-old 5.83 ± 4.93; 8-month-old 12.00 ± 4.91; $p < 0.05$; Figure 8A,B), but not in the total number of microglia (total microglia; 3-month-old 26.00 ± 2.26; 8-month-old 30.00 ± 4.86; 16-month-old 26.92 ± 3.15, $p > 0.05$; Figure 8A,C). There was a significant increase in percent of active microglia from 3-months-old to 8-months-old with a decrease at 16-months-old (% active microglia; 3-month-old 22.44 ± 18.97%; 8-month-old 40.33 ± 14.29%; $p < 0.05$; Figure 8A,D).

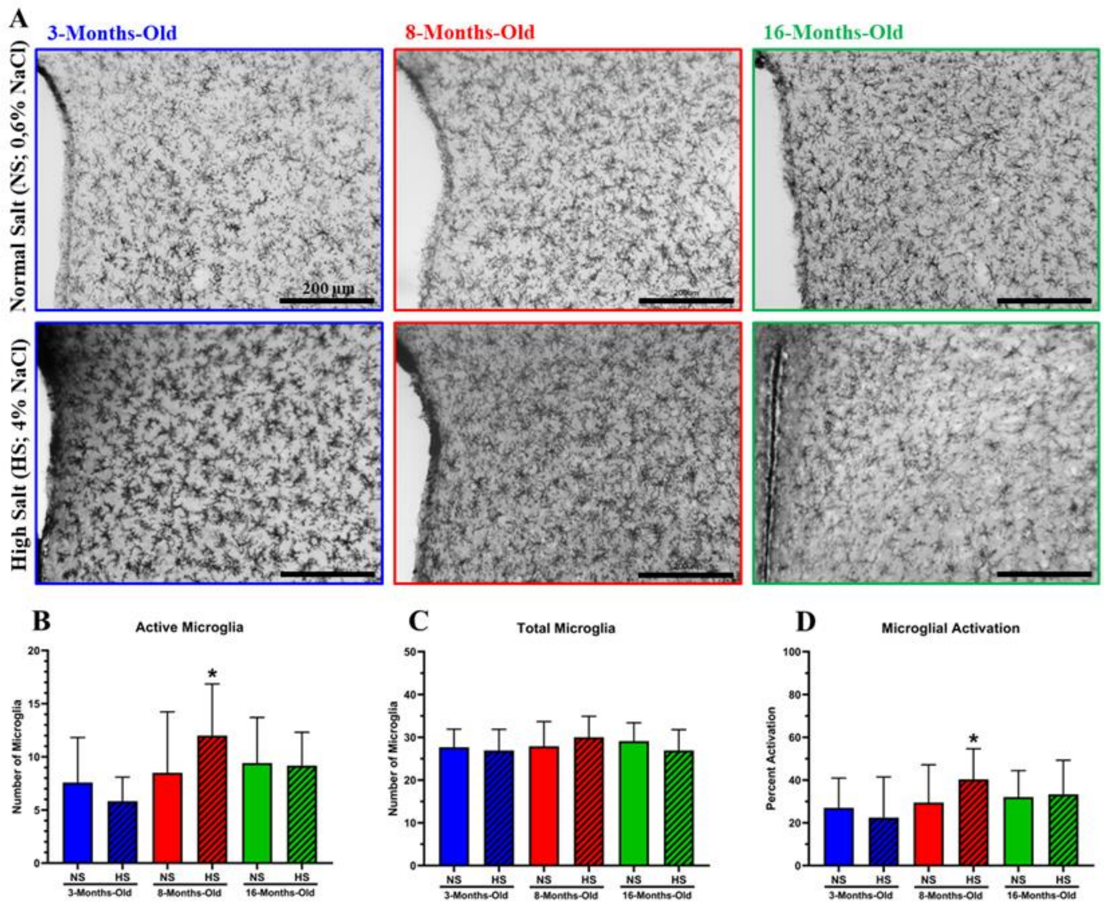

Figure 8A-D: The Role of Aging and Dietary Salt Intake on Microglial Morphology

Microglial activation based on cell morphology in the PVN was assessed using soma size, process length, and number of processes in order to determine the number of active microglial cells in male Sprague-Dawley rats aged 3-, 8-, and 16-months. Measurements occurred after a 21-day normal salt (NS; 0.6% NaCl) or high salt (HS; 4% NaCl) diet. (7A) Photomicrographs of PVN microglia at Level 2. Data are presented as mean ± SD. Images taken at 20X magnification, Scale bar = 200 µm; (7B) The average number of active microglia cell in each age group and salt diet. (7C) The average total number of microglia in the PVN in each age group and salt diet (7D) Percent of active microglial cells is expressed. N=6 per group; *p < 0.05 vs the respective 3-month-old group

Sholl Analysis

Microglia in the PVN were assessed via Sholl analysis and morphology. Sholl analysis of microglia did not detect any significant differences in the number of intersections per micron from the center of the soma regardless of age or salt intake (Figure

9A-C). In turn, there was no significant change in branching complexity in microglia with age or with salt.

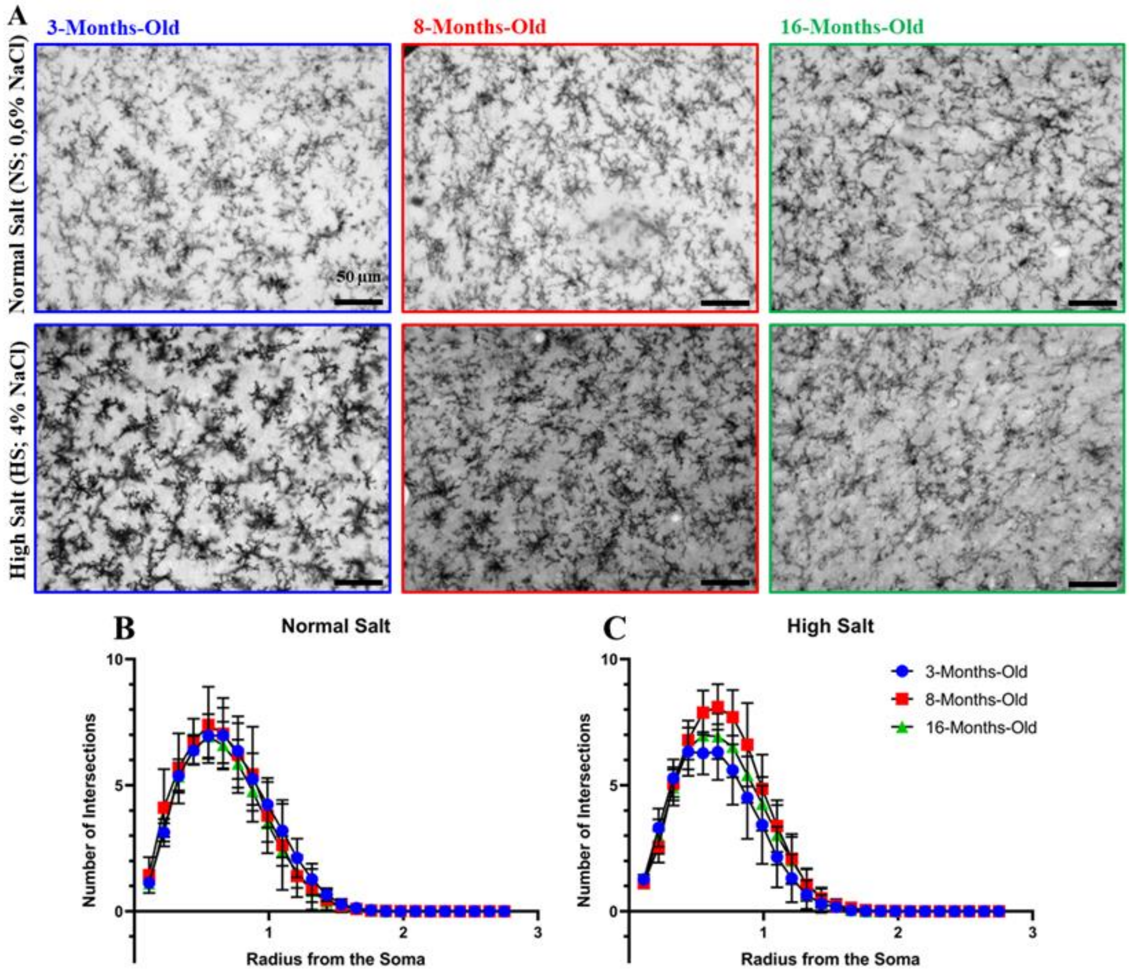

Figure 9A-C: The Impact of Aging and Dietary Salt Intake on Microglial Complexity

Microglial activation in the PVN was assessed via Sholl analysis (number of intersections per concentric ring in ascending microns from the some) in male Sprague-Dawley rats aged 3-, 8-, and 16-months. Measurements occurred after a 21-day normal salt (NS; 0.6% NaCl) or high salt (HS; 4% NaCl) diet. (8A) Photomicrographs of Level 2 of the PVN. Error bars represent standard deviation. Images taken at 40X magnification, Scale bar = 50 µm. (8B) Sholl analysis of microglia in rats fed a normal salt diet; not significant (8C) Sholl analysis of microglia in rats fed a high salt diet; not significant. N=6 per group

DISCUSSION

We hypothesized that aging and increased dietary sodium would evoke increases in mean arterial pressure, and impair sympathetic tone, ultimately increases blood brain barrier permeability and evokes a neuroinflammatory responses in the PVN. Our results showed in male SD rats as a model of normal aging, (1) an increase in mean arterial pressure (MAP) which is exacerbated with an increase in dietary sodium intake with age, (2) an alteration in estimated sympathetic tone with age and dietary sodium intake, (3) a loss in vascular integrity of the blood brain barrier with age regardless of salt intake, (4) changes in neuroinflammation as a result of dietary sodium intake. Overall, we accept our hypothesis that age-dependent hypertension evokes elevations in sympathetic tone and alterations in the vascular integrity of the blood brain barrier. However, the role of neuroinflammation remains unclear in age-dependent hypertension.

Increases in Blood Pressure and Salt Sensitivity Are Age-Dependent

Hypertension effects the aging population at an alarmingly high rate of almost 75% of the population over the age of 65 showing clinically relevant elevated blood pressure (Muntner et al., 2018, Whelton et al., 2018). As aging is a leading risk factor for hypertension, it is peculiar that studies of both humans and animal models are limited. While in human models of aging, selecting the proper control group is often of heated debate; in animal models, separating aging as the main factor responsible for driving increases in blood pressure has been a challenge (Coleman et al., 1977; Owen & Heywood, 1986; Beckett et al., 2008; Buford, 2016). Aging, which has been linked to increased blood pressure, has also been linked to the development of the salt sensitivity of blood pressure in both human and animal models of age-dependent hypertension, as well as elevated levels of sympathetic tone (Luft et al., 1991; Whelton et al., 2018). Regardless, age-dependent hypertension and the salt sensitivity of blood pressure have been difficult to model in animals (Coleman et al., 1977; Bilusic et al., 2008; Leong et al., 2015) and thus, very few studies investigating age-dependent hypertension and the salt sensitivity of blood pressure in rodent models have been performed.

Our lab has modeled salt-sensitivity of blood pressure genetically using Gai_2 protein-dependent model and Dahl salt-sensitive model, as well as experimentally using SD rats with a NE infusion (Walsh et al., 2016; Frame et al., 2019; Moreira et al., 2019). SD rats have been previously used by our lab and others to demonstrate salt-resistance in young SD rats (Kapusta et al., 2013; Walsh et al., 2016; Frame et al., 2019). However, our lab has shown young SD rats develop salt-sensitivity as a result of an infusion of subcutaneous norepinephrine (Walsh et al., 2016, Frame et al., 2019). As elevations in sympathetic tone have been noted in age-dependent hypertension in humans (Narkiewicz et al., 2005), we used the SD rats to model normal aging in rodents. In order to model normal aging and the development of salt-sensitive hypertension, we assessed parameters in 3-month-old, as young, 8-month-old, as middle aged, and 16-month-old, as aged, SD rats, as SD rats over 20-months-old have been shown to have renal dysfunction (Owen & Heywood, 1986), which contributes to increased blood pressure.

To assess changes in blood pressure and alterations in salt-sensitivity, age-matched SD rats were placed on a NS or HS diet for 21-days. Rats that were maintained on a NS diet exhibit age-related increases in MAP, thus developing hypertension with age. Rats fed a HS diet, dietary sodium had no impact on blood pressure in 3-month-old and 8-month-old rats. However, 16-month-old rats showed robust increases in blood pressure, thus they exhibit the development of salt-sensitive hypertension.

Sympathetic Tone is Impaired with Age

Salt-sensitive hypertension is linked with increased sympathetic tone in young rats (Walsh et al., 2016; Frame et al., 2019; Moreira et al., 2019). Aging has been implicated in increased elevations in sympathetic tone and changes in renal sodium handling, and is linked with the increased incidence of salt-sensitive blood pressure in both aging humans and aging animal models (Luft et al., 1991; Esler et al., 2002; Whelton et al., 2018). In cases of salt-sensitivity, increased dietary sodium has been known to elicit sympathoexcitation and hypertension, while in cases of salt-resistance, increases dietary sodium are linked to maintained sympathoinhibition and normotension (Dobesova et al., 2002; Brooks et al., 2005; Osborn et al., 2008; Stocker et al, 2013).

Sympathetic tone was estimated using tissue or plasma NE content, and response to a ganglionic blockage. These means of assessment provide indirect measurements sympathetic nervous system activity. Nerve recording techniques, such as renal nerve recording, have been performed by some groups to provide a more direct assessment of sympathetic activity, but because not every nerve bundle can be recorded, they are still indirect measures of nerve activity. Thus, estimates of vascular, global, and renal sympathetic tone were performed in aging SD rats. Rodents on a NS diet exhibited age-related increases in depressor response, plasma NE, and renal NE in association with age-related increases in blood pressure. Blood pressure and renal NE progressively increased with age, while estimates of global sympathetic tone and sympathetic tone to the vasculature increased at 8-months-old, without further progression at 16-months-old.

In young rats, a HS diet evoked the suppression of vascular, global, and renal sympathetic tone and had no effect on blood pressure. This compares with our previous findings that 3-month-old SD rats maintain the ability to sympathoinhibit as they have maintained their salt-resistance (Walsh et al., 2016). However, in 8-month-old rats, HS did not increase age-related changes in blood pressure, and the ability to suppress renal, vascular, and global sympathetic tone is reduced. Rats aged 16-months fed a HS diet develop salt-sensitive hypertension and show sodium evoked increases in renal NE content levels. While levels of plasma NE and vascular tone are similar to that of 8-month-old rats, at 16-months-old, these rat's ability to suppress renal NE is lost. Thus, age-related hypertension and the salt-sensitivity of blood pressure are associated with a loss in sympathoinhibition. Further studies must be carried out to confirm levels of sympathoinhibition and sympathoexcitation in this model of normal aging in rodents.

Integrity of the Blood Brain Barrier is Lost with Age

In order to maintain immune privilege and homeostasis in the brain, the BBB plays an integral role providing a protective barrier within the vasculature. While the neurovascular unit as a whole is responsible for maintaining this barrier, in age and pathological disease the BBB has been known to be breached by peripheral cells with the potential to wreak havoc on the otherwise privileged central nervous system (Abbot et al., 2010; Chung et al., 2013; Ueno et al., 2016; Costea et al., 2019).

While other groups have described BBB dysfunction as a result of hypertension in both ANGII models and models using spontaneously hypertensive rats, as well as Dahl salt-sensitive hypertension (Mayhan et al., 1989; Ueno et al., 2004; Vital et al., 2010; Pelisch et al., 2011; Biancardi et al., 2014), dysfunction of the BBB has yet to been described in age-dependent hypertension. While this is likely due to the lack of available models of age-related hypertension (Coleman et al., 1977; Bilusic et al., 2008; Leong et al., 2015), the integrity of the BBB has been studied in numerous models of normal aging, Parkinson's Disease, and dementia, amongst others (Chung et al., 2013; Gray & Woulfe, 2015; Ueno et al., 2016; Erdo et al., 2017; Costea et al., 2019).

To our knowledge, we are the first to report a leak in the BBB of rodents with age-dependent hypertension. Regardless of dietary salt intake, FITC10 extravasated from the microvasculature of the brain into the brain parenchyma of the PVN in middle aged rodents. Evidence of this extravasation progressively increased in aged rodents. As both 8-month-old and 16-month-old rodents were hypertensive even on a normal salt diet, these findings were consistent with other studies of hypertension and its role in blood brain barrier dysfunction (Mayhan et al., 1989; Ueno et al., 2004; Vital et al., 2010; Pelisch et al., 2011; Biancardi et al., 2014): hypertensive rodents showed increased levels of BBB dysregulation compared to their normotensive counterparts. This suggests increases in blood pressure play a role in the dysregulation of the vascular integrity of the BBB.

While other studies regarding hypertension investigated alterations components of the BBB, they have also examined peripheral infiltration through the dysregulated BBB, as well as in cardiovascular control centers other than the PVN in the brain (Biancardi et al., 2014). While ANGII has been known to gain access to the brain (Biancardi et al., 2014), peripheral immune cells have also taken advantage of this weakness and infiltrate the CNS proper (Sofroniew 2014; Winklewski et al., 2015; Setiadi et al, 2018).

In order to fully understand the mechanistic underpinnings at play, further investigation into alterations in barrier components, ultrastructural analysis of the brain vasculature using electron microscopy, as well as peripheral infiltration into the brain parenchyma should be performed. Further quantitative analysis of our findings should also be completed. With a proper quantification of FITC10 leakage, we will be able to better understand the severity of vascular integrity loss due to aging and dietary sodium intake. A complete understanding of the mechanisms behind BBB dysfunction dysregulation in age-dependent hypertension remains at large.

Neuroinflammation is Increased by High Dietary Sodium

The central immune processes contribute to the propagation and maintenance of hypertension in a number of models (Shi et al., 2010; Shi et al., 2011; Jiang et al., 2018; Moreira et al., 2019). This raises the concern that the brain's immune system and thus the maintenance of homeostasis could also be playing a role in age-dependent hypertension, as well as its associated role in BBB dysregulation. As the NVU is actively maintained by microglia and astrocytes, we sought to address a potential role of astrocytes and microglia in age-dependent hypertension.

Astrocytes, regulators of CNS homeostasis and inflammation, as well as microglia, the innate immune cells of the brain, both react when a pathological stimulus is identified (Shi et al., 2010; Pekny et al., 2014). Hypertension and aging, amongst other disease pathologies have been noted in increasing excitatory responses in astrocytes (Stern et al, 2016; Marins et al., 2017; Setaidi et al., 2017; Palmer & Ousman, 2018). Increased dietary sodium reportedly triggers the activation of microglia and the release of proinflammatory cytokines (Summy & Long et al., 2008; Moreira et al., 2019). In contrast, density of astrocytes has been shown to decrease with increased levels of sodium (Gankam Kengne et al., 2011; Schmidt-Pogoda et al., 2018). Interestingly, we found high dietary sodium elicits a heighted response from astrocytes in young and middle aged rodents. However, we observe a drop-off in this response in aged rodents, with levels returning to that of a rodent on a NS diet. Increased levels of dietary sodium also played a role in the activation of microglia. On a HS diet, there was an increase in reactivity, but not the total number of microglia in middle aged rodents. However, this increase in activation was not maintained with age, as the response in activation returned to that of a normal salt animal. Typically, in cases of inflammatory response, microglia become reactive, proliferate and recruit other microglia. As no change was observed in the total number of microglia in these rodents, it is difficult to conclude if the increase in the number of active microglia was an instance of inflammatory response. Variability in chromogen staining may have played a role in the inconclusiveness of these findings.

While we speculated that neuroinflammation might play a role in barrier dysfunction, our current findings do not allow us to make substantial inferences connecting the two pathologies. In future studies, quantifying FITC10 extravasation and its localization with neurons and glial cells may provide more insight into the mechanistic role that microglia and astrocytes may play in the maintenance and dysregulation of the BBB.

Limitations and Future Directions

The role of sex has been implicated in hypertension. In young individuals, the incidence of hypertension is elevated in men compared to women, however these statistics reverse as women reach menopause (Muntner et al., 2018; Whelton et al., 2018). Our current studies were conducted using only male SD rats. Further investigations of blood pressure and sympathetic tone, as well as vascular integrity of the BBB should be completed in female animals in order to elucidate a potential role for sex.

A hallmark of normal aging is subtle loss in cognitive ability. Future studies should investigate alterations in animal behavior in all age-groups in order to extrapolate on changes in cognition with age and increased blood pressure.

Further analysis of the BBB and the neurovascular unit as a whole should be carried out. Changes in barrier protein expression, component composition, and microbleeds have all been indicated with both aging and hypertension separately. Here we have shown there is a breakdown in BBB function with increased pressure and age, further studies should be performed in order to assess where and how this breakdown in integrity is occurring. We anticipate alterations in tight junction proteins, basement membrane thickness, total number of pericytes, and astrocytic endfeet with age.

Conclusions

In these studies, we found aged SD rats become hypertensive and develop the salt sensitivity of blood pressure. These findings are complimented by increased alterations in global, renal, and vascular sympathetic tone. Aging and increased blood pressure evokes increased permeability in the blood brain barrier, while a high salt diet triggers a protective inflammatory response in the brain that is lost with age. Taken together, this supports our global hypothesis that in age-dependent hypertension, there is an increase in permeability of the blood brain barrier. However, while dietary sodium evokes an inflammatory response centrally, it does not do so in aging animals.

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CURRICULUM VITAE

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