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ALTERATIONS IN THE MEDULLARY ENDOCANNABINOID SYSTEM CONTRIBUTE TO AGE-RELATED IMPAIRMENT OF BAROREFLEX SENSITIVITY

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Abstract

As they age, Sprague-Dawley (SD) rats develop elevated systolic blood pressure associated with impaired baroreflex sensitivity (BRS) for control of heart rate. We previously demonstrated in young hypertensive (mRen2)27 rats that impaired BRS is restored by CB₁ cannabinoid receptor blockade in the solitary tract nucleus (NTS), consistent with elevated content of the endocannabinoid 2-arachidonoylglycerol (2-AG) in dorsal medulla relative to normotensive SD rats. There is no effect of CB₁ receptor blockade in young SD rats. We now report in older SD rats that dorsal medullary 2-AG levels are two-fold higher at 70 versus 15 weeks of age (4.22 ± 0.61 vs. 1.93 ± 0.22 ng/mg tissue; $P < 0.05$). Furthermore, relative expression of CB₁ receptor mRNA is significantly lower in aged rats, while CB₂ receptor mRNA is significantly higher. In contrast to young adult SD rats, microinjection of the CB₁ receptor antagonist SR141716A (36 pmol) into the NTS of older SD rats normalized BRS in animals exhibiting impaired baseline BRS (0.56 ± 0.06 baseline vs. 1.06 ± 0.05 ms/mmHg after 60 min; $P < 0.05$). Therefore, this study provides evidence for alterations in the endocannabinoid system within the NTS of older SD rats that contribute to age-related impairment of BRS.

Keywords

aging; endocannabinoid system; baroreflex sensitivity; NTS

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Disclosures

None.

Introduction

Aging is associated with changes in autonomic regulation of blood pressure that may facilitate the progression or maintenance of hypertension and related diseases (1). An imbalance in the autonomic nervous system, typically in which the sympathetic nervous system predominates over the parasympathetic, is a primary cause of increased systolic blood pressure in aged individuals with hypertension (2). Declining baroreflex sensitivity (BRS) for control of heart rate (HR), an index of vagus nerve function and general mortality, is permissive toward reduced heart rate variability, increased sympathetic outflow, and impaired ability to correct subsequent elevations in blood pressure during aging (3). Therefore, identification of central nervous system factors that contribute to age-related reductions in BRS is required to gain a greater understanding of the development of hypertension in the worldwide aging population.

Alterations in the brain endocannabinoid system that accompany age-related cognitive decline (4), cardiovascular diseases or metabolic disorders (5) may contribute to the modulation of baroreflex function during aging. The endocannabinoid system is a central and peripheral cellular signaling system that comprises the endogenous cannabinoids anandamide and 2-arachidonoylglycerol (2-AG), their G protein-coupled CB₁ and CB₂ receptor subtypes, and enzymes regulating their biosynthesis and degradation. Upregulated endocannabinoid tone is reported in hypertension and its metabolic risk factors (6, 7), suggesting a pathogenic or compensatory role for the endocannabinoid system during these negative conditions.

Mounting evidence links upregulated endocannabinoid tone with imbalances in the two active peptides of the renin-angiotensin system (RAS), angiotensin (Ang) II and Ang-(1–7). Ang II exerts a widespread influence over the sympathetic nervous system and impairs BRS during aging (8), and may stimulate production of endocannabinoids through actions at Ang II type 1 (AT₁) receptors (9, 10). We previously demonstrated that blockade of CB₁ receptors within the solitary tract nucleus (NTS) of the dorsomedial medulla, the primary site for termination of baroreceptor afferent neurons, improves the blunted BRS for control of HR in young adult (mRen2)27 rats with Ang II-dependent hypertension, with no effect in age-matched normotensive Sprague-Dawley (SD) rats with normal baroreflex function (11). The impaired BRS in (mRen2)27 rats is also associated with loss of Ang-(1–7) facilitation of the BRS (12), resembling the brain RAS profile of aged SD rats (13). To date there has been no investigation into the role of the NTS endocannabinoid system in impaired BRS associated with aging.

In the present study, we evaluate the effect of the CB₁ receptor antagonist SR141716A on BRS for control of HR when microinjected into the NTS of older SD rats. We also employ mass spectrometry to measure levels of dorsal medullary endocannabinoids, and RT-qPCR to measure mRNA levels of cannabinoid receptors (CB₁ and CB₂), and cannabinoid receptor interacting protein (CRIP)1a (14) in the NTS of younger and older SD rats. Based on evidence for decreased NTS Ang-(1–7) tone in aging (13) and the association between augmented Ang II and endocannabinoid tone in hypertensive rats with impaired BRS (11), we hypothesized a similar trend for endocannabinoid levels in aged rats. The present study

provides functional and biochemical evidence that components of the endocannabinoid system within the NTS change during normal aging and that enhanced CB₁ receptor actions contribute to declining baroreflex function with age.

Methods

Animals

Experiments were performed in 15-, 25-, and 60- to 78-week-old male Hannover SD rats obtained from the Hypertension & Vascular Research Center colony at Wake Forest School of Medicine. Rats were housed two-per-cage in a temperature- and humidity-controlled room that maintained a 12-hour light/dark cycle (lights on at 06:00), with free access to food (standard chow) and water. All procedures were approved by the Institutional Animal Care and Use Committee.

Surgical Procedures and Hemodynamic Measures

As described previously (13, 15), aged SD rats (60- to 69-weeks-old; mean age = 64 ± 1 weeks; $n = 4$) were anesthetized with combination urethane-chloralose (750 and 35 mg/kg, respectively) via intraperitoneal injections with supplemental doses given intravenously as needed (diluted to 3:7 with saline). Polyethylene catheters (Clay Adams) were inserted into the femoral artery and vein, with the venous catheter positioned near the right atrium. Rats were then placed in a stereotaxic frame with the head tilted at a 45° downward angle for surgical exposure of the dorsal medulla, and were provided a mixture of room air and oxygen to breathe. A 30 minute resting and equilibration period was allowed before testing began. Pulsatile arterial pressure (AP) and mean AP (MAP) were recorded by data acquisition software (AcqKnowledge 3.8.1, BIOPAC Systems, Inc., Goleta, CA), and HR was calculated from the AP wave. After obtaining stable measures of MAP and HR, baseline BRS was established by sequential bolus intravenous injection of 3 doses (2, 5 and 10 µg/kg in 50 µL 0.9% NaCl) of phenylephrine (PE), each of 5 second duration and separated by at least 5 minutes, to determine the bradycardic BRS response for increases in AP. BRS for bradycardia was defined as the slope of the relationship between changes in MAP (Δ MAP; mmHg) and the pulse interval (Δ PI; ms) generated from the three doses of PE (mean $R^2 = 0.98$ for all animals) in units of ms/mmHg. Reflex testing was repeated 60 minutes after bilateral microinjection of the CB₁ receptor-selective antagonist SR141716A (obtained from the National Institute on Drug Abuse) into the NTS, and was completed within 15 minutes, with each rat serving as its own control. The dose and timecourse of the study were chosen based on previous experience with SR141716A microinjections in our laboratory (11) in which the effect of the drug on BRS was immediate and sustained over 60 minutes without changing resting MAP or HR. Cardiac chemosensitive vagal fiber activation was assessed at the time of BRS testing by measuring depressor and bradycardic responses to intravenous phenylbiguanide (10 µg/kg in 50 nL 0.9% NaCl) after a 5-second bolus injection.

Indices of spontaneous sympathovagal activity were calculated by spectral analysis of the AP time and frequency domains using software designed for rats (Nevrocard SA-BRS, Medistar, Houston, TX), as previously described (16). Spontaneous BRS was calculated in

the time (Sequence [Seq] Up, Seq Down, and Seq All; in units of milliseconds per mmHg) and frequency (low-frequency [LF] and high-frequency [HF] α indices) domains, determined from a minimum of 10 minutes of AP recordings obtained at baseline and again within 60 minutes of SR141716A microinjection. Time domain analysis was used to calculate differences in HR variability (HRV), an index of cardiac vagal tone, measured as the standard deviation of the beat-to-beat interval (SDRR) in milliseconds, and blood pressure variability (BPV), an index of vascular sympathetic tone, as the standard deviation of the MAP (SDMAP) in mmHg.

NTS Microinjections

SR141716A (36 pmol in a 120 nL volume of 10% DMSO in artificial cerebrospinal fluid vehicle) was bilaterally microinjected into the NTS (0.4 mm rostral, 0.4 mm lateral to the calamus scriptorius [caudal tip of the area postrema], and 0.4 mm below the dorsal surface) using a glass micropipette connected to a hand-held syringe, as reported previously (13). Previous studies in older SD rats from a similar age range in our laboratory revealed NTS microinjection of the vehicle solution has no significant effect on evoked BRS, MAP or HR at any timepoint studied (13). At the end of testing, brains were removed, frozen, and sectioned (30 μ m) for localization of microinjection sites. Data from injections within the medial NTS at the rostrocaudal level -13.3 to -14.0 mm caudal to bregma were used in the analysis.

Mass Spectrometry Detection of Anandamide and 2-AG

Medullary tissue was obtained from separate age groups of naïve SD rats: 15-, 25-, and 67- to 78-weeks-old (mean age of older animals = 70 ± 2 weeks; $n = 4-5$; $P < 0.05$ vs. age of rats used for the microinjection studies). Brains were removed and frozen on dry ice for excision of 3-mm³ dorsal medullary sections. The sections were obtained from 1.5 mm anterior to 1.5 mm posterior to the usual placement of the microinjection pipette, corresponding with the expected injectate spread within the NTS and including portions of the area postrema, dorsal motor nucleus, and nucleus gracilis. Optimal extraction conditions for the tissue were obtained using HPLC-grade acetonitrile and tissue homogenization with 2.3 mm stainless steel beads (BioSpec Products, Bartlesville, OK) that reduced enzymatic degradation and attenuated 2-AG isomerization. Tissue homogenates were fortified with isotopically-labeled internal standards for 2-AG and anandamide 2-[²H₅]2-AG (AG-d5) and [²H₄]anandamide (AEA-d4) on a per-mg basis for sample integrity. The chromatography solvents were Honeywell B&J HPLC-grade ammonium acetate and acetic acid–double distilled (Sigma-Aldrich). Stock solutions of anandamide and 2-AG and their deuterated analogs were freshly prepared and stored as recommended by the manufacturer. Study samples, quality control samples, and standards (targeted analysis) were processed using automated liquid handling to ensure the reproducibility and consistency of the sample preparation method.

An ACQUITY ultra performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA) equipped with an ACQUITY UPLC column was used for the separation of endocannabinoids prior to quantitative analysis by selected reaction monitoring (LC-MS/MS). A triple quadrupole mass spectrometer (API-5000; AB SCIEX, Framingham, MA)

was coupled to the UPLC for quantitative analysis, and operating parameters were optimized for anandamide and 2-AG. Calibration curves spanning the range of quantitation were produced through least-squares linear regression analysis of response (analyte integrated area/internal standard integrated area) versus concentration, and weighted to improve fit, accuracy and precision. Sample content of anandamide or 2-AG is expressed as ng analyte per mg tissue wet weight.

Quantification of CB₁, CB₂, and CRIP1a mRNA

RT-qPCR was used to measure mRNA levels of various effector components of the brain endocannabinoid system, including CB₁ and CB₂ receptor, and CRIP1a, in dorsal medullary tissue from the same 15- and 70-week-old rats in which anandamide and 2-AG were measured. Brains were removed and frozen on dry ice as described above. Isolated RNA from excised tissue was assayed for concentration and stability. As described previously (13), total RNA (1 µg) was reverse transcribed using AMV reverse transcriptase in a 20-µL reaction mixture containing deoxyribonucleotides, random hexamers, and RNase inhibitor in reverse-transcriptase buffer. For RT-qPCR, 2 µL of the resultant cDNA was added to TaqMan Universal PCR Master Mix with the appropriate gene-specific primer/probe set for CB₁ and CB₂ receptors, and CRIP1a (Applied Biosystems), and amplification performed. All reactions were performed in triplicate. 18S ribosomal RNA served as the internal control. Results were quantified as Ct values, in which Ct is the threshold cycle of PCR at which an amplified product is first detected, and was defined as relative gene expression (ratio of target:control).

Analysis of Data

Values are presented as mean ± SEM. Comparisons of changes in BRS from baseline in response to SR141716A in older SD rats were analyzed by Student's paired t-test. Multiple regression analysis was performed on BRS scatterplots to determine slopes of fit lines. One-way ANOVA and post-hoc Tukey multiple comparisons were used to analyze differences in medullary endocannabinoid content between age groups. Differences in relative mRNA expression between young and older rats were analyzed by unpaired t-tests. The criterion for statistical significance was $P < 0.05$. Statistical tests were performed using Prism 5.0 (GraphPad Software, San Diego, CA).

Results

CB₁ Receptor Blockade in the NTS of Aged SD Rats: BRS for Control of HR, MAP, HR, and MAP Responsiveness

Baseline BRS in older SD rats was impaired to a similar level relative to young SD rats, as previously reported (13, 15). Microinjection of SR141716A (36 pmol) into the NTS significantly improved BRS by approximately 89%, from a baseline of 0.56 ± 0.06 ms/mmHg to 1.06 ± 0.05 ms/mmHg ($P < 0.05$; Figure 1A and 1B), in aged SD rats after 60 minutes. Values of MAP (89 ± 8 baseline vs. 84 ± 4 mmHg after 60 minutes) and HR (292 ± 10 baseline vs. 283 ± 13 bpm after 60 minutes) were not significantly altered by SR141716A. In addition, there were no differences in MAP responsiveness to intravenous PE injections during reflex testing by SR141716A (Figure 1C). NTS microinjection of

SR141716A did not alter depressor (-47 ± 3 baseline vs. -47 ± 4 mmHg after 60 minutes) or bradycardic (-114 ± 6 baseline vs. -115 ± 8 bpm after 60 minutes) responses to intravenous phenylbiguanide while assessing cardiac chemosensitive vagal fiber activation.

Influence of CB₁ Receptor Blockade on Indices of Sympathovagal Function

Similar to the evoked baroreflex measurements, NTS microinjection of 36 pmol of SR141716A in older SD rats significantly increased spontaneous BRS (Seq All; $P < 0.05$). Specifically, both sympathetic (Seq Down; $P < 0.05$) and vagal (HFα; $P < 0.05$) indices of spontaneous BRS were enhanced in these animals (Table 1). There was no significant effect of SR141716A on BPV, although HRV trended higher following SR141716A microinjection ($P = 0.07$; Table 1).

Dorsal Medullary 2-AG and Anandamide Content in 15-, 25- and 70-Week-Old SD Rats

Levels of 2-AG were lowest in 15-week-old ($n = 5$) and highest in 70-week-old ($n = 5$) SD rats (1.93 ± 0.22 vs. 4.22 ± 0.61 ng/mg tissue, respectively; $P < 0.001$; Figure 2A). Middle aged SD rats (25 weeks) had intermediate medullary 2-AG content (3.13 ± 0.46 ng/mg tissue; $n = 4$), although not significantly different from either 15- or 70-week-old rats (Figure 2A). In contrast, dorsal medullary anandamide was found in levels 1000-fold lower than 2-AG and there were no significant differences among age groups (Figure 2B).

CB₁, CB₂ and CRIP1a mRNA in Young and Older SD Rats

CB₁ receptor mRNA was approximately 0.45-fold lower in the dorsal medulla of 70-week-old ($n = 6$) relative to 15-week-old ($n = 5$) SD rats ($P < 0.001$; Figure 3A), while medullary CB₂ receptor mRNA was approximately 0.73-fold higher in the older rats compared to young rats ($P < 0.05$; Figure 3B). There was no difference between age groups in relative mRNA levels of CRIP1a (Figure 3C), a CB₁ receptor-associated protein shown to reduce CB₁ receptor signaling (14).

Discussion

In this study we report the following novel observations: (1) blockade of CB₁ receptors in the NTS of aged SD rats improves evoked and spontaneous BRS for control of HR; (2) 2-AG content in the dorsal medulla increases over the lifespan of SD rats; and (3) aging in SD rats is associated with significantly lower CB₁ receptor mRNA expression and significantly higher CB₂ receptor mRNA expression in the dorsal medulla relative to younger animals. Collectively, these results suggest that alterations in the components of the dorsal medullary endocannabinoid system may contribute to age-related decline in baroreflex function.

Progressive autonomic imbalance with blunted sympathetic or parasympathetic reflex control over the heart and vasculature during normal aging is accepted as a primary contributor to elevated blood pressure during aging (17). Several factors within the brain may contribute to the development of age-related autonomic dysfunction, including the RAS (8) and the metabolic hormones insulin and leptin (18). The precise interrelationship among factors involved in impaired baroreflex function during aging remains unclear. However, reported signaling interactions between the endocannabinoid system and the RAS (19),

insulin (20) and leptin (21) may illuminate some of the mechanisms underlying age-related reductions in BRS for control of HR.

The RAS peptides Ang II and Ang-(1–7) have opposite effects on baroreflex modulation, with Ang II in the NTS reducing and Ang-(1–7) facilitating BRS (22), but the provenance of alterations in the components of the RAS associated with aging are unknown. A shift in balance between the content or actions of the counterbalancing Ang peptides occurs during aging that results in predominance of Ang II tone in the NTS, leading to age-related impairment of BRS (8). This is illustrated by previous studies in which AT₁ blockade in the NTS by candesartan improved BRS for control of HR in both young and older SD rats, but *mas* receptor blockade by d-Ala⁷-Ang-(1–7) following candesartan treatment impaired BRS only in young rats (13). The same study also reported that mRNA expression of neprilysin, an endopeptidase that cleaves angiotensinogen to form Ang-(1–7) (23), was lower in dorsal medulla of older rats (13), in line with reports of lower neprilysin activity in forebrain and plasma of older animals (24).

Increased dorsal medullary 2-AG content also occurs in young adult transgenic (mRen2)27 hypertensive rats (11), which feature downregulated NTS Ang-(1–7) tone associated with markedly impaired BRS for control of HR (12) compared to young SD rats and transgenic Ang-deficient ASrAOPEN rats with enhanced baseline BRS (25). Therefore, the dorsal medulla of aged SD rats represents the second *in vivo* setting in which an increased Ang II-to-Ang-(1–7) RAS imbalance is associated with impaired BRS and increased levels of 2-AG. Of note, the relative 2-AG levels parallel the trend in resting conscious SBP and are inversely related to the baseline BRS for control of HR reported across the SD rat lifespan (26). Functional data from the current study support the hypothesis that enhanced NTS endocannabinoid tone contributes to defective baroreflex function in older animals because blockade of NTS CB₁ receptors in aged SD rats significantly improved BRS for control of HR. The BRS values obtained after SR141716A microinjection are similar to published values of baseline BRS in young SD rats (13, 27), indicating that NTS CB₁ receptor blockade normalized impaired BRS in these older animals. These results are consistent with previous observations that CB₁ blockade in the NTS of younger SD rats had no effect on BRS but dose-dependently improved BRS in (mRen2)27 rats (11).

In addition to the RAS, endocannabinoids are known to interact with central insulin (20) and leptin (21) pathways. Insulin resistance in the periphery and central nervous system is a cardinal risk factor for age-related diseases including Alzheimer's dementia, obesity, type II diabetes, and cardiovascular disease (28). Direct CB₁ receptor-mediated inhibition of insulin receptor signaling has been shown in the periphery (29), and endocannabinoids modulate insulin signaling pathways in the brain (20). Defective leptin signal transduction in the brain is associated with aging and age-related obesity (30, 31), and accompanies overactive endocannabinoid signaling independent of obesity or aging (21). Importantly, deficient central leptin receptor signaling during aging directly influences CB₁ receptor mRNA expression in the dorsal medulla (32). Both insulin (33) and leptin (27) decrease BRS for control of HR through actions in the NTS, so it is certainly possible that endocannabinoids may modulate the actions of either hormone to suppress BRS in hypertension or aging. Other factors involved in BRS modulation at the level of the dorsal medulla during aging

and known to interact with the endocannabinoid system include serotonin (34), catecholamines (35), TRPV1 ion channels (36), and the inhibitory neurotransmitter γ -aminobutyric acid (GABA) (37). We cannot discount that endocannabinoid interactions with any of these factors are reflected in our present functional data, especially with GABA because GABA_A receptor-mediated inhibitory responses in neurons become less sensitive to cannabinoids with age (38).

The current study provides evidence that enhanced CB₁ receptor tone in the NTS functionally attenuates BRS in aged SD rats, because microinjection of 36 pmol of SR141716A normalized BRS for control of HR in response to increases in blood pressure. Spectral analysis methods for measuring baroreflex regulation (16) revealed that 36 pmol of SR141716A significantly enhanced spontaneous BRS (Seq All), including both vagal (HF α) and sympathetic (Seq Down) indices of baroreflex regulation, to levels comparable to baseline sBRS in young SD rats (16, 27). In addition, HRV trended higher following SR141716A microinjection. Whereas the PE pressor method measures BRS after evoked changes in AP in an open-loop system, the spontaneous method measures changes in a closed-loop model over a much smaller (beat-to-beat) range, although a highly significant correlation exists between them (16). Collectively, blockade of endocannabinoid actions at NTS CB₁ receptors improved blood pressure regulation in older SD rats as assessed using several indices of BRS and sympathovagal function, consistent with upregulated systemic CB₁ receptor tone during conditions associated with aging including hypertension (6) and metabolic syndrome (5).

Expression of CB₁ receptors in dorsal medullary sites along the baroreflex arc, including in terminals of nodose ganglion vagal afferent neurons (39), the NTS (40), and dorsal motor nucleus (41), has been previously documented in younger rats. Lower CB₁ mRNA in this region in older rats is consistent with higher local tissue 2-AG levels, although we did not previously detect a difference in CB₁ mRNA expression in young (mRen2)27 rats that also exhibited higher medullary 2-AG relative to SD rats (11). Interestingly, there was no difference in CRIP1a mRNA, which has been previously reported to show an opposite regulation pattern as the CB₁ receptor (42). In contrast to receptor mapping studies over lifespan in humans, which find no changes in CB₂ receptor binding distribution with age in healthy individuals (43), we report that dorsal medullary CB₂ mRNA is increased in aged relative to younger SD rats. Expression of CB₂ receptors on brain microglia is upregulated during neuroinflammation (44), which may be the source of elevated CB₂ receptor mRNA in dorsal medulla of older rats detected by our study. The relevance of the CB₂ receptor to autonomic and cardiovascular homeostasis is still emerging, however, and less is known about its role in aging. Although we cannot exclude a role for upregulated CB₂ receptor expression in the modulation of cardiovagal BRS in aged rats, we clearly show a functional role for CB₁ receptors in age-related BRS decline because selective blockade of NTS CB₁ receptors via SR141716A, reported to be over 1000-fold more selective for CB₁ versus CB₂ receptors (45), normalized impaired BRS.

Functional or structural changes in the peripheral vasculature associated with aging likely further contributes to diminished BRS (46). Older SD rats have an attenuated vascular response to intravenous PE, an index of sympathetic activity at vascular α 1-adrenergic

receptors, as well as a lower resting HR compared to younger rats (13). The attenuated pressor response to PE in older rats may elicit inadequate carotid sinus stretch, which in turn may reduce baroreceptor input to the NTS to yield reduced BRS. Lower HR in aging may be associated with altered cardiac pacemaker cell function or density or responsiveness of cardiomyocyte β -adrenergic receptors, both of which would reduce BRS for control of HR (47). The extent to which either of these factors is reflected in animals with impaired baseline BRS in our current study is unknown, although normalized BRS following NTS CB₁ receptor blockade in these animals suggests their contribution can be overcome. A central nervous system component to impaired BRS during aging is consistent with a previous report of rapid desensitization of central baroreflex neurons to sensory input in older animals (48). Finally, unaltered cardiac chemoreflex responses to intravenous phenylbiguanide indicate selectivity of SR141716A actions on BRS pathways because the two responses are mediated by distinct sensory inputs in the NTS (49).

At present, the actions of the endocannabinoid system during aging are understudied relative to its body of literature. Descriptions of how the endocannabinoid system contributes to age-related cardiovascular diseases are lacking. However, evidence we present for upregulated medullary 2-AG production and NTS CB₁ receptor tone in aged SD rats suggests that altered endocannabinoid system components in this region contribute to impaired cardiovagal BRS for control of HR during aging. Future studies will determine if endocannabinoids influence BRS through direct regulation of NTS neurotransmitter pathways or indirectly through interactions with other autonomic regulatory systems.

Conclusions

Baroreflex function declines with age in healthy human populations, which can contribute to the self-perpetuating progression of hypertension target organ damage and other cardiovascular diseases (1). The importance of addressing impaired BRS during treatment for hypertension is now recognized (2), creating the need for research into factors that may influence BRS. Blockade of the endocannabinoid system in conditions associated with upregulated RAS activity, including in hypertension, obesity (50), and during aging (8), with co-administration with a RAS inhibitor or Ang-(1–7) replacement (51) may yield positive synergistic effects in these conditions, with a concomitant positive influence over autonomic reflexes. Observations of an endocannabinoid-mediated contribution to age-related pathologies may advance support for the endocannabinoid system as a therapeutic target in older populations with impaired cardiovagal BRS for control of HR.

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Non-standard abbreviations

BPV	blood pressure variability
HFα	high-frequency alpha
HRV	heart rate variability
LFα	low-frequency alpha
SDMAP	standard deviation of mean arterial pressure
SDRR	standard deviation of beat-to-beat heart rate interval
Seq	sequence

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Figure 1. Effect of NTS Microinjection of SR141716A (36 pmol) in Aged SD Rats on BRS for Control of HR

A, In aged SD rats, SR141716A significantly improved BRS by approximately 89%. B, SR141716A increased the slope of the BRS regression line in rats with impaired baseline BRS (0.56 ± 0.06 ms/mmHg baseline; 1.06 ± 0.05 ms/mmHg 60 minutes after 36 pmol of SR141716A; $P < 0.01$; $R^2 = 0.67$ to 0.87 for pooled data). C, There was not a significant change in MAP responsiveness to intravenous PE 60 minutes after NTS microinjection of SR141716A. * $P < 0.05$ vs. baseline; $n = 4$.

◇ Baseline
 ◆ +SR141716A
 ◇ Baseline
 ◆ +SR141716A

Figure 2. 2-AG and Anandamide Content in the Dorsal Medulla of 15-, 25- and 70-Week-Old SD Rats

A, Mass spectrometry revealed levels of 2-AG were significantly higher in the dorsal medullary tissue of 70- (n = 5) relative to 15-week-old (n = 5) SD rats. B, Anandamide was detected at levels 1000-fold lower than 2-AG in the same tissue samples, and there were no significant differences among age groups. *P < 0.05 vs. 15 weeks.

Figure 3. Expression of CB₁, CB₂ and CRIP1a mRNA in the Dorsal Medulla of 15- and 70-Week-Old SD Rats

A, RT-qPCR determined that relative gene expression of CB₁ receptor was significantly lower in dorsal medullary tissue of 70- (n = 6) versus 15-week-old (n = 5) SD rats. B, Relative gene expression of CB₂ receptor was significantly higher in the same tissue of 70- versus 15-week-old rats. C, There was not a significant difference in relative gene expression of CRIP1a in the dorsal medulla between 15- and 70-week-old rats. *P < 0.05 vs. 15 weeks; ***P < 0.001.

Table 1Influence of NTS CB₁ Receptor Blockade on Indices of Spontaneous BRS and Sympathovagal Tone.

Parameter	Baseline	After SR141716A
LFα	1.36 $\pm$ 0.41	1.96 $\pm$ 0.49
HFα	0.81 $\pm$ 0.30	2.04 $\pm$ 0.62[*]
Seq Up (ms/mmHg)	1.00 $\pm$ 0.32	1.41 $\pm$ 0.52
Seq Down (ms/mmHg)	0.86 $\pm$ 0.41	1.78 $\pm$ 0.28[*]
Seq All (ms/mmHg)	0.94 $\pm$ 0.37	1.68 $\pm$ 0.37[*]
HRV SDRR (ms)	2.77 $\pm$ 0.32	4.83 $\pm$ 0.71 [†]
BPV SDMAP (mmHg)	3.33 $\pm$ 0.38	2.58 $\pm$ 0.43

Values are mean $\pm$ SEM.^{*} P < 0.05 vs. baseline;[†] P = 0.07; n = 4 all groups.