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Low glial angiotensinogen improves body habitus, diastolic function, and exercise tolerance in aging male rats

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Abstract

Objectives—Long-term systemic blockade of the renin–angiotensin system (RAS) with either an angiotensin (Ang) II type 1 receptor antagonist or an angiotensin-converting enzyme inhibitor attenuates age-related cardiac remodeling and oxidative damage, and improves myocardial relaxation. However, the role of the brain RAS in mediating the development of diastolic dysfunction during aging is not known. We hypothesized that low brain RAS protects against the development of age-related diastolic dysfunction and left ventricular remodeling.

Methods—Sixty-week-old transgenic male ASrAOGEN rats (n =9), with normal circulating Ang II and functionally low brain Ang II, because of a GFAP promoter-linked angiotensinogen antisense targeted to glia, and age-matched and sex-matched Hannover Sprague–Dawley (SD; n= 9) rats, with normal levels of both circulating and brain Ang II, underwent echocardiograms to evaluate cardiac structure and function. Postmortem hearts were further compared for histological, molecular, and biochemical changes consistent with cardiac aging.

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Conflicts of interest
There are no conflicts of interest.

Results—ASrAOGEN rats showed preserved systolic and diastolic function at mid-life and this was associated with a lower, more favorable ratio of the phospholamban–SERCA2 ratio, reduced incidence of histological changes in the left ventricle, and increased cardiac Ang-(1–7) when compared with the in-vivo functional, and ex-vivo structural and biochemical indices from age-matched SD rats. Moreover, ASrAOGEN rats had lower percent body fat and a superior exercise tolerance when compared with SD rats of the same age.

Conclusion—Our data indicate that the central RAS plays a role in the maintenance of diastolic function and exercise tolerance in mid-life and this may be related to effects on body habitus.

Keywords

brain; cardiac aging; diastolic dysfunction; renin–angiotensin system; SERCA2; tissue Doppler

Introduction

Age-related cardiac structural changes lead to reductions in left ventricular (LV) relaxation and compliance, increases LV end-diastolic pressure, exercise intolerance, and frequently, diastolic heart failure. Stimuli for cardiac remodeling associated with aging also include hypertension [1], increases in body weight gain [2], insulin resistance [3], and renal dysfunction [4]. The peripheral and cardiac renin–angiotensin system (RAS), alone or as a consequence of these comorbidities, may further contribute toward the senescent cardiac phenotype by augmenting oxidative pathways and enhancing extracellular matrix formation [5–7]. In hearts from senescent rodents, elevated cardiac angiotensin (Ang) II levels associate with LV fibrosis [8] and late-life treatment with angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin II receptor (AT1R) blockers attenuates age-related collagen deposition and oxidative damage [9]. Long-term, low-dose treatment with ACE-I improves cardiac sarcoplasmic reticulum (SR) calcium-regulatory protein expression in the same aged Brown Norway × Fisher 344 rodent model [9]. Although there are reports of increases in angiotensinogen, AT1R, and AT2R expression, in myocardial tissue from senescent rats independent of circulating RAS [10,11], it is not entirely clear whether the benefits of systemically administered RAS blockade represent a local cardiac tissue and/or a centrally mediated mechanism.

Indeed, orally administered ACE-I and angiotensin receptor blockers can cross the blood–brain barrier [12–14], and experimental evidence shows that central application of various RAS inhibitors attenuates the pressor responses to centrally administered Ang II [15,16]. All components of the RAS have also been identified in the brain [17–19]. Various transgenic animal models suggest that long-term regulation of the cardiovascular system, in states of both disease and good health, involves correlated actions of the RAS and the sympathetic nervous system [20–24]. Taken together with recent clinical data showing that sympathetic overactivity and baroreflex impairment contribute toward hypertension-related diastolic dysfunction [25], it is reasonable to expect that the central RAS may also play an important role in cardiac aging.

In this study, we hypothesized that low brain RAS protects against the development of diastolic dysfunction and LV remodeling at mid-life. Previous reports indicate that the young (ASrAOGEN) rat has normal circulating Ang II and functionally low brain Ang II because of a GFAP promoter-linked angiotensinogen antisense targeted to astrocytes relative to the Hannover Sprague–Dawley (SD). The control SD rats show normal levels of both circulating and brain Ang II [24,26,27] and undergo age-related elevations of arterial pressure and decreases in autonomic and metabolic dysfunction, whereas ASrAOGEN rats are protected from such age-related changes [22]. Therefore, we compared the cardiac

function, structure, and cardiac angiotensin peptides in a group of middle-aged rats with opposing degrees of brain RAS activation.

Methods

Middle-aged male rats from the two lines [Hannover SD (n=9) or ASrAOGEN (n=9)] were obtained from the colonies maintained in the Hypertension and Vascular Research Center at Wake Forest University. The Hannover SD rats are the parent line for the transgenic ASrAOGEN rat. All animals were bred and exposed to the same paired housing conditions (12 : 12 light : dark cycle) and provided food and water *ad libitum*. The two strains underwent echocardiograms at 60 weeks of age and postmortem hearts acquired immediately following sacrifice by rapid guillotine decapitation (~62 weeks of age) were used for histological and biochemical analyses. Animal care was provided in accordance with the Laboratory Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 2011), and all experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Wake Forest School of Medicine. Accordingly, all possible steps were taken to avoid animal suffering at each stage of the experiment.

Echocardiography

For the echocardiogram, rats were anesthetized with an isoflurane (1.5%)/oxygen mixture by a nose cone during spontaneous ventilation. Using a Philips 5500 echocardiograph (Philips Medical Systems, Andover, Massachusetts, USA) and a 12MHz phased array probe, animals were imaged in a shallow left lateral decubitus position while lying on a warmed mat, with electrocardiographic adhesive electrodes applied to the paws [8,28]. The measurements were carried out using an off-line analysis system (Xcelera 3.1; Koninklijke Philips Electronics, Amsterdam, the Netherlands) by an experimentally blinded observer. LV M-mode images were obtained in the two-dimensional short-axis view, close to the papillary muscles. Diastolic posterior wall thickness (PWTed) and LV end-diastolic dimension (LVEDD) and LV end-systolic dimension (LVESD) were measured using the leading-edge method of the American Society for Echocardiography [29]. The percentage of LV fractional shortening (%FS), an index of the global systolic function, was calculated as [(LVEDD−LVESD)/LVEDD] × 100. The relative wall thickness was calculated as: 2 × PWT/LVEDD. LV mass was calculated using a standard cube formula, which assumes a spherical LV geometry to the formula: LV mass = 1.04 × [(LVEDD+PWT+AWT)^3 −LVEDD], where 1.04 is the specific gravity of muscle. Transmitral flow measurements of early filling velocities (E_{max}) and early deceleration time ($E_{dectime}$) were obtained using pulsed-Doppler, with the sample volume placed at the tips of mitral leaflets from an apical four-chamber orientation. Because of the relatively high heart rates and fusion of the early and late Doppler profiles, only the early transmitral filling velocity was measured. Pulsed-Doppler tissue imaging to assess septal mitral annular descent (e') was also obtained from the four-chamber view. The ratio of early transmitral filling-to-mitral annular descent, or E/e', was used as an index of filling pressure. All measured and calculated systolic and diastolic indices are represented as the average of at least five consecutive cardiac cycles to minimize beat-to-beat variability.

Blood pressure

Systolic blood pressure was measured in conscious rats between 55 and 60 weeks of age using an automated tail-cuff system (Narco Bio Systems, Houston, Texas, USA). This noninvasive blood pressure monitoring technique utilizes a methodology similar to the peripheral blood flow occlusion technique used in humans. In brief, an electric pulse transducer located distally from the cuff converts force applied to the active surface of the transducer from the tail blood pressure pulse into an electrical signal. The tail cuff inflates to

a pressure greater than the systolic arterial pressure, at which time the blood pressure pulse is nonexistent. As the cuff pressure is released and tail blood pressure exceeds cuff pressure, blood begins to flow through the artery and the returning pulse is detected by the transducer. This corresponds to the systolic arterial pressure. To assist with arterial vasodilation, rats were placed in a 39°C warming box for 10 min before the determination of blood pressure. At least five determinations were carried out in each animal and averaged for a single determination for the session. Rats were not trained to the tail-cuff procedure but were trained to handling by the same investigators who performed the tail-cuff procedures.

Histopathology

Formalin-fixed, postmortem hearts obtained from a separate 60-week-old cohort following sacrifice were transversely sectioned in the mid-ventricular region and processed to paraffin blocks. One routine section from each block was stained with hematoxylin and eosin and evaluated for histopathologic changes, including interstitial fibrosis, myofiber necrosis, intramural coronary artery degeneration, and mononuclear infiltrates, by an investigator at Experimental Pathology Laboratories (Charlottesville, Virginia, USA) who was masked to rat strain. Incidence rates (%) were used to describe representative age-related degenerative lesions in each strain.

Immunoblotting

SR membranes and LV tissue homogenates were prepared as described previously [28]. Briefly, samples were separated by SDS-PAGE and transferred onto polyvinylidene fluoride membranes. Immunoblots were probed for the key calcium-regulatory proteins, anti-sarcoplasmic endoplasmic reticulum calcium ATPase 2 (SERCA2) (1 : 1000 dilution; Abcam, Cambridge, Massachusetts, USA), and anti-phospholamban (PLB) (1 : 5000 dilution; Abcam). To normalize the variability of protein loading, the antibody to glyceraldehyde-3-phosphate dehydrogenase (1 : 5000 dilution; Cell Signaling Technology, Danvers, Massachusetts, USA) or β-actin (1 : 2000; Cell Signaling Technology) was probed onto the stripped membranes corresponding to the aforementioned proteins (Western Blot Recycling Kit; Alpha Diagnostic International Inc., San Antonio, Texas, USA). The bands were scanned and digitized (MCDI Image Analysis Software; Imaging Research Inc., St Catherines, Ontario, Canada). Each band was normalized to its own loading control protein and expressed in arbitrary units. The PLB-to-SERCA2 ratio was used as a measure of SERCA2 inhibition.

Biochemical analysis

At sacrifice, LV tissues were rapidly collected and snap-frozen in liquid N_2 and stored at −80°C for later assay. Angiotensin peptides were extracted from the tissue samples using C18 Sep-Pak Columns (Waters, Milford, Massachusetts, USA), and the eluate was analyzed by radioimmunoassay for Ang I, Ang II, and Ang-(1–7) as described previously [30].

Body composition analysis

In a separate group of 55-week-old rats representing each group (n=3/group), global body composition was measured with Hologic Discovery (Delphi A; Hologic Inc., Waltham, Massachusetts, USA), a whole-body dual-energy X-ray absorptiometry (DXA) scanner, with software adaptions (software version 3.3 APEX, Delphi A; Hologic Inc.) for small animal scanning [31]. The densitometer was calibrated before rodent scans using a small animal step phantom supplied by the manufacturer. Rats were anesthetized before measurements with an intramuscular injection of a solution containing a mixture of ketamine (60 mg/kg) and xylazine (5 mg/kg). After anesthesia, rats were positioned ventrally on a platform. Forelimbs were positioned about 75° from the base of the neck and hind limbs were

externally rotated, with hip, knee, and ankle articulations in 90° flexion. Rat whole-body measurements required 2–3 min for completion. The percent coefficients of variation at our center are 0.54% for bone mineral content, 0.58% for bone mineral density, 0.40% for fat-free mass, and 1.66% for fat mass [31].

Exercise tolerance test

Exercise tolerance tests were conducted in another group of 55-week-old SD and ASrAOGEN rats (n=5/group) to further differentiate functional disparities between strains. Indeed, exercise intolerance is often the first clinical manifestation of diastolic dysfunction [32]. Before testing, animals were acclimated to the one-lane rodent treadmill (Scientific Instruments, Stoelting, Woodale, Illinois, USA) by walking at a speed of 20 cm/s, 10 min/day, for 2 weeks. After acclimatization, 55-week-old rats underwent exercise tolerance testing that involved walking at 20 cm/s for 3min, followed by 2 cm/s increases in speed every 2 min until exhaustion. Time to exhaustion (s) was determined when the rat sat at the lower end of the treadmill, near a shock bar, for more than 5 s.

Statistical analyses

Data shown are expressed as mean ± SEM. Unpaired t-tests were used to compare each genotype at their respective ages. All analyses were carried out using GraphPad Prism (version 5; Graphpad Inc., La Jolla, California, USA). P value less than 0.05 was used for significance.

Results

Physical characteristics

Age, systolic blood pressure, and body weights are shown in Table 1. As reported previously [33,34], the ASrAOGEN had a 37–45% lower body weight and a 10% lower systolic blood pressure when compared with SD rats of the same age. Moreover, % fat, the total fat-to-lean body mass ratio, and the % bone mineral density were lower in 55-week-old ASrAOGENs when compared with their age-matched controls (Table 1).

Left ventricular structure and function

Echocardiographic analysis showed that LV chambers and posterior wall dimensions were smaller in ASrAOGENs when compared with SD rats of the same age (Table 2). Although the relative wall thicknesses were not overtly different between groups, calculation of LV mass using echocardiographic parameters showed decreased LV mass in ASrAOGEN rats. Systolic function, as measured by FS, was not significantly different between groups (P=0.06). As reported previously [22,27,35], the mean heart rate of ASrAOGENs, obtained during echocardiograms performed under anesthesia, was almost 10% higher than the SDs heart rate.

Assessments of diastolic function using transmitral and tissue Doppler measures are shown in Fig. 1. Early transmitral flow velocities were lower (ASrAOGEN: 74 ± 4 cm/s vs. SD: 90 ± 5 cm/s; P=0.01) and deceleration times were longer in ASrAOGENs (ASrAOGEN: 0.042 ± 0.001 s vs. SD: 0.034 ± 0.002 s; P<0.01). Corresponding to the favorable early deceleration time in ASrAOGEN rats, the echocardiographic index of the LV filling pressure, or E/e', was 42% lower when compared with age-matched SD rats (P<0.05). Mitral annular velocity, or the tissue Doppler index of myocardial relaxation, was also 17% higher in the transgenic rats with low brain angiotensinogen vs. SD rats, but this did not achieve statistical significance (P=0.118).

Immunoblotting

Consistent with the modestly improved functional profile of the middle-aged ASrAOGEN, the hearts from these rats showed a lower PLB-to-SERCA2 ratio compared with the control SD rats. Given that PLB inhibits the action of the SR calcium-regulatory pump [36], or SERCA2, our data indicate that intracellular calcium reuptake is superior in the ASrAOGEN strain (Fig. 2).

Left ventricular histology

LV histopathology of postmortem hearts from ~60-week-old animals was assessed by hematoxylin and eosin staining (Fig. 3). Although the severity of interstitial fibrosis was considered modest for both groups, it was present in 30% more of the SD than ASrAOGEN specimens at this age. Myofiber necrosis and coronary artery degeneration were identified in almost all of the specimens from SD hearts, but none from ASrAOGEN hearts. A low grade of mononuclear infiltrates was also identified and this occurred in ASrAOGEN and SD hearts at an incidence rate of 40 and 50%, respectively.

Biochemical analyses

The results indicating the potential contribution of the cardiac angiotensins to the subtle differences in diastolic function and LV structure between groups (ASrAOGEN hearts, n=5; SD hearts, n=6) show that cardiac levels of the antiproliferative peptide Ang-(1–7) were significantly higher in ASrAOGEN rats (17.7 ± 3.0 pg/mg tissue; P<0.02) when compared with SD rats (11.8 ± 2.5 pg/mg tissue), and this was associated with a significant increase in Ang-(1–7)/Ang I, or the peptide ratio representing neprilysin (ASrAOGEN: 6.3 ± 1.2 vs. SD: 1.5 ± 0.2; P< 0.01) as opposed to Ang-(1–7)/Ang II, or the peptide ratio representing ACE2 (ASrAOGEN: 4.9 ± 0.2 vs. SD: 4.5 ± 0.5). Cardiac levels of Ang I (ASrAOGEN: 17.7 ± 3.0 pg/mg vs. SD: 11.8 ± 2.5 pg/mg protein; P=0.160) and Ang II (ASrAO GEN: 6.3 ± 1.3 pg/mg vs. SD: 3.8 ± 0.7 pg/mg tissue; P= 0.098) tended to be higher in ASrAOGENs when compared with age-matched SDs.

Exercise tolerance

To further differentiate the functional differences between SD and ASrAOGEN rats, exercise capacity testing was performed in a group of age-matched rats at 55 weeks of age (n=5/group). Time to exhaustion was 26% shorter in SD rats compared with ASrAOGEN rats (Fig. 1).

Discussion

Our study shows that ~60-week-old ASrAOGENs rats, with low brain levels of angiotensinogen, and Ang I and evidence of functional loss of Ang II actions (relative to the control SD parent strain [26,27,37], are protected against early cardiac aging when compared with age-matched SD controls. Specifically, ASrAOGENs showed preserved systolic and diastolic function, and this was further associated with a reduced incidence of cardiac fibrosis, a more favorable SR cardiac calcium-regulatory protein profile, and a higher level of cardiac Ang-(1–7) when compared with the in-vivo functional, and ex-vivo structural, and biochemical indices from SD rats obtained at parallel time points. In conjunction with the conserved cardiac phenotype of the mid-aged ASrAOGENs, these rats also showed a healthier body weight, or lower body weight and % body fat, and superior exercise tolerance when compared with age-matched SDs. Although the bone density of ASrAOGENs was lower than that observed in SDs, the clinical relevance of this observation is not clear, given that both positive and negative relationships between body mass and bone mineral density have been reported in epidemiologic and clinical studies [38]. Nonetheless,

the potential role of a low glial RAS contributing toward the low bone mass phenotype of the ASrAOGEN is doubtful as bone remodeling and, specifically, osteoclastogenesis is related to an activated RAS or SNS [39] and in conscious ASrAOGENs, resting sympathetic activity is presumed to be reduced as evidenced by a slightly lower mean arterial pressure (MAP) and heart rate, with higher baroreceptor sensitivity when compared with age-matched SD rats [21,22,33].

In recent years, the RAS has been proposed as a potential target to limit diastolic dysfunction and cardiac remodeling because of aging. We, and others, have shown previously, in senescent rodent models, that increases in cardiac Ang II are associated with cardiac collagen deposition and impairments in diastolic function [8]. In addition, we found that ACE inhibition or AT1R blockade limits the oxidative damage that contributes toward age-associated LV remodeling and lusitropic dysfunction [9,40]. Studies using aged mice also show that enalapril administered from weaning until 24 months of age limits the development of myocardial sclerosis, attenuates age-related reductions in myocardial mitochondrial number, and increases survival [41]. Clinically, and in support of a RAS-mediated mechanism of age-related LVEDD, ACE-I or AT1R blockers are considered first-line therapy for the management of preclinical heart failure (stages A and B) because of hypertension or diabetes [42], two disease processes known for accelerated cardiovascular aging [43–45]. Moreover, in a small randomized, double-blind study involving elderly hypertensive patients with mild diastolic dysfunction, Little *et al.* [46] showed that 6 months of AT1R blockade led to reductions in exercise-induced increases in systolic blood pressure and improvements in exercise tolerance and quality of life, whereas treatment with the non-RAS-regulated antihypertensive, hydrochlorothiazide, affected exercise-induced increases in blood pressure alone. Taken together, these studies hint at a common causal mechanism in the pathogenesis of diastolic dysfunction, namely, an activated tissue RAS.

Our data in the ~60-week-old ASrAOGENs are consistent with findings from younger animal models of ischemic heart disease that show that inactivation, or low brain RAS, may also protect against LV remodeling and cardiac dysfunction. Specifically, blockade of AT1Rs in the brain of rats subjected to coronary ligation reversed the sympathetic hyperactivity associated with heart failure [47]. Chronic AT1R blockade or attenuation of brain ouabain-like activity (which also contributes toward sympathetic hyperactivity associated with heart failure) retarded the development of LV dilation and dysfunction in rats after myocardial infarction [48,49]. Similarly, transgenic rats with low glial angiotensinogen (ASrAOGEN) are protected from sympathetically induced LV dysfunction and remodeling after myocardial infarction [24] and have an increased lifespan compared with SD rats [21]. Similarly, Araujo *et al.* [50] reported that intracerebral injections of the ACE inhibitor captopril in rats after an experimental myocardial infarction reduced LV dilatation and improved LV filling. In contrast, selective overexpression of brain ACE2, the counter-regulatory RAS enzyme that breaks down Ang II into its antagonistic product Ang-(1–7), attenuated sympathetic activity and improved baroreflex function in mice with congestive heart failure [51]. Overall, these data imply that the brain RAS, through the modulation of peripheral sympathetic nerve activity, plays a major role in ventricular remodeling and myocardial dysfunction associated with heart failure [21], and this might also explain the subtle cardiac differences between ASrAOGENs and SDs observed in our study.

In the present study, ASrAOGENs showed a more favorable diastolic functional and structural cardiac phenotype than age-matched rats from the parent SD strain. Specifically, the increased early deceleration time and reduced E/e', or filling pressure, in the ASrAOGENs vs. SDs was associated with a lower PLB-to-SERCA2 ratio and a reduced prevalence of cardiac fibrosis and coronary artery degeneration. Although it is not yet clear

how a low brain RAS, independent of circulating angiotensin peptides [24,26,37,52], defends against a presumed age-related reduction in SERCA2 expression, maintenance of calcium uptake into the SR could explain the improved Doppler profile of the diastolic function in this strain. As FS was not overtly different between groups, it means that systolic function, *per se*, did not play a major role in the relative enhancement in diastolic function in ASrAOGENs. Indeed, evidence from various experimental and human models shows that SERCA2 downregulation or an increase in the PLB-to-SERCA2 ratio might contribute toward diastolic heart failure [53,54]. We have also reported a similar relationship between SERCA2 expression and the tissue Doppler index of myocardial relaxation, or e', in various rodent models mimicking normal cardiac aging [8,28] and that chronic ACE inhibition improves cardiac SR calcium-regulatory protein expression and myocardial relaxation [9]. In addition to a low glial angiotensinogen–cardiac calcium-regulatory connection that could contribute toward the maintenance of diastolic function among ASrAOGENs, further studies will be required to determine the mechanistic link between low brain angiotensinogen and attenuated age-related myocardial remodeling, ventricular stiffness, and elevated filling pressures observed in middle-aged transgenic rats.

Alternatively, or in addition to the beneficial effects of a low brain RAS, enhanced local cardiac Ang-(1–7) production could contribute directly toward a delay in cardiac aging. Although cardiac levels of Ang I and Ang II were not significantly different between groups, there were clear trends for increases in ASrAOGENs, providing the substrate for a relative increase in the production of Ang-(1–7) in ASrAOGENs. Ang-(1–7), together with its receptor mas, and the enzyme ACE2 represent a cardioprotective axis within the RAS system that balance the ACE/Ang II/and AT1R's adverse effects [52]. Experimental studies show that increases in Ang-(1–7) or ACE2, through overexpression or exogenous administration, prevent the cardiac fibrosis associated with Ang II [55] and improve cardiac function after myocardial infarction [56]. Moreover, recently, we found that chronic inhibition of ACE2 increases LV remodeling in transgenic hypertensive rats, independent of circulating RAS [30]. Nonetheless, the current study suggests that neprilysin, as opposed to ACE2, might be the preferential enzyme responsible for the elevation in Ang-(1–7) at this age in the ASrAOGENs hearts as the ratio representing neprilysin, or Ang-(1–7)/Ang I, was increased but not the ratio representing ACE2, or Ang-(1–7)/Ang II. Indeed, studies involving enzyme metabolism would be required to confirm a neprilysin vs. ACE2 enzyme-regulatory preference, as well as to exclude other potential sources [e.g. increased substrate availability such as (i.e. more cardiac angiotensiogen or Ang I), less Ang-(1–7) metabolism, or less chymase), leading to an increase in cardiac Ang-(1–7). In fact, in Wistar and SD rats, lower levels of neprilysin mRNA or activity have been reported in other tissues [57,58]. Interestingly, neprilysin has been shown to be an Ang-(1–7)-forming enzyme in both the circulation and the cardiovascular tissue [59]. In the aged Brown Norway×Fisher 344 rat, increases in cardiac Ang-(1–7) and the ratio Ang-(1–7)/Ang I are believed to represent a compensatory mechanism regulating 'healthy' cardiac aging [28]. Although it is not known how low brain angiotensinogen and/or alterations in central sympathetic activity could influence the local cardiac RAS milieu, targeting therapy to increase Ang-(1–7) could slow cardiac aging and the development of diastolic dysfunction. In fact, long-term treatment with an AT1R antagonist in Fischer 344 rats was associated with an increase in neprilysin and ACE2 mRNA in brain medulla, suggesting a shift to the Ang-(1–7) axis [60]. Ang-(1–7), when administered systemically, protects against cardiac injury in severe forms of hypertension and some of the cardioprotective effects of ACE inhibitors may involve Ang-(1–7) [61,62]. In Lewis rats, decreases in arterial pressure because of the continuous administration of either an ACE-I (lisinopril) or an AT1R antagonist (losartan) increased cardiac ACE2 gene expression and cardiac ACE2 activity, whereas none of the treatments had an effect on cardiac neprilysin mRNA. Losartan but not lisinopril administration was accompanied by increases in cardiac Ang II and Ang-(1–7) content [63]. These data indicate

that the regulation of factors influencing the expression and activity of Ang-(1–7) is tissue dependent.

Progressive weight gain is one of the hallmarks of the aging process and obesity has been linked to the development of diastolic dysfunction. Obese individuals [64–67] and experimental animal models of human obesity [68,69] show increases in LV mass that are coupled to reductions in LV filling dynamics. Moreover, the LV hypertrophy that accompanies obesity often develops independent of hemodynamic load [70,71], pointing to a metabolic cause for these cardiac consequences [72,73]. Undoubtedly, the increase in body weight and percent body fat of the ~60-week-old SDs relative to the ASrAOGENs may be a factor in the increased heart size and the decreased LV compliance of this group, as indicated by the lower deceleration time and the higher filling pressure, or E/e'. Both overweight and obesity have been independently associated with impaired diastolic function in individuals at a high cardiovascular risk [74]. Furthermore, given that Wong *et al.* [65] showed that BMI was a significant predictor of diastolic dysfunction among community dwellers, even after adjusting for age, MAP, and LV mass index, the differences in diastolic function observed between rat strains in the present study might also be because of dissimilarities in body habitus rather than blood pressure and/or heart size. Indeed, accumulation of abnormal metabolites such as triglycerides and extracellular matrix cross-linking by advanced glycation end products could also lead to stiffening of the aged heart and to obesity-related diastolic dysfunction [45,75], particularly given clinical reports of a relative independence of diastolic impairment from LV geometry in obesity [71].

Furthermore, and in support of a metabolic hypothesis to accelerated cardiac aging, the insulin resistance component of the metabolic syndrome has been linked to LV remodeling and diastolic functional stiffness [73]. Although we did not directly measure intramyocardial lipids [68,69] or metabolic parameters as a cause for the alterations in cardiac structure and function in the present study, our previous data show that ASrAOGENs remain lean and insulin sensitive during the aging process, whereas SD rats gain excess weight and develop insulin and leptin resistance during their lifespan [34] similar to mice treated long term with RAS blockers [76]. Future studies will determine whether consumption of a hypercaloric diet offsets the benefits that a low glial angiotensinogen has on maintaining a lean body habitus, favorable fitness level, cardiometabolic profile, and cardiovascular phenotype to middle age and beyond. Similarly, the administration of a high-fat diet to ASrAOGENs could provide additional insights into the complex relationships between fat mass, the mechanical stresses imposed on bone, and subsequent bone density and composition [77].

Limitations

The present study has a few limitations that deserve further discussion. Echocardiograms were carried out under anesthesia, which could have had differential effects on autonomic tone and cardiac function between strains [78]. Indeed, the effects of anesthesia on resting heart rate have reported lower, similar, or higher values in anesthetized ASrAOGEN rats when compared with the parent strain Sprague–Dawley rat depending on the type and the dose of anesthetics [27,35,79–81]. However, the inclusion of tissue Doppler facilitates the assessment of diastolic function as it is less affected by loading conditions and heart rate than conventional Doppler [82–84]. Second, neither a weight-matched nor a low blood pressure control group was included, which does not negate an indirect effect of a RAS–body habitus interaction or life-time lower MAP, either of which could influence the cardiac phenotype. Third, without the inclusion of a ‘young’ group, it is not entirely clear whether the echocardiographic differences observed between groups are a reflection of aging. Importantly, isolated heart preparations from 5-month-old male ASrAOGENs and Hanover

SDs showed similar heart-weight- to-body-weight ratios and basal hemodynamics, including LV pressure, $+dP/dt_{max}$, and $-dP/dt_{max}$ [85].

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Clinical perspective

Cardiac aging is a complex multifactorial process unlikely to be because of a singular cause but rather a combination of alterations in neuroendocrine and inflammatory signaling that contribute toward functional decline and damage to cardiac macromolecules. Although normal cardiovascular aging cannot be prevented, individualized interventions in middle-aged individuals that are focused on counteracting obesity, sedentarism, insulin resistance, and high blood pressure can help slow down ‘pathological aging’. Certainly, higher cardiac Ang-(1–7) in the ASrAOGEN rats relative to age-matched SD rats is consistent with the beneficial cardiac effects observed with the long-term administration of Ang-(1–7) in a number of studies. Herein, using transgenic rats with a targeted disruption of glial angiotensinogen, we conclude that the protection against mid-life increases in body weight gain, blood pressure, LV stiffness, and reductions in exercise tolerance directly or indirectly follows from this disruption. In this respect, the aging ASrAOGEN rat provides a valuable experimental tool for elucidating the potential role of manipulating components of the central RAS in attenuating cardiac aging and, specifically, diastolic dysfunction. A final point not addressed in this discussion and actually shown by the measures of blood pressure is that ASrAOGEN rats do not show the characteristic increase in systolic blood pressure associated with the aging process [33]. This may be a critical factor in the expression of the changes found in the heart as the increased blood pressure amplitude with age (higher systolic blood pressure and lower diastolic blood pressure) is now linked to changes in vascular distensibility or stiffness (e.g. aortic input impedance and LV performance) or what is commonly described as ventricular–vascular coupling [86].

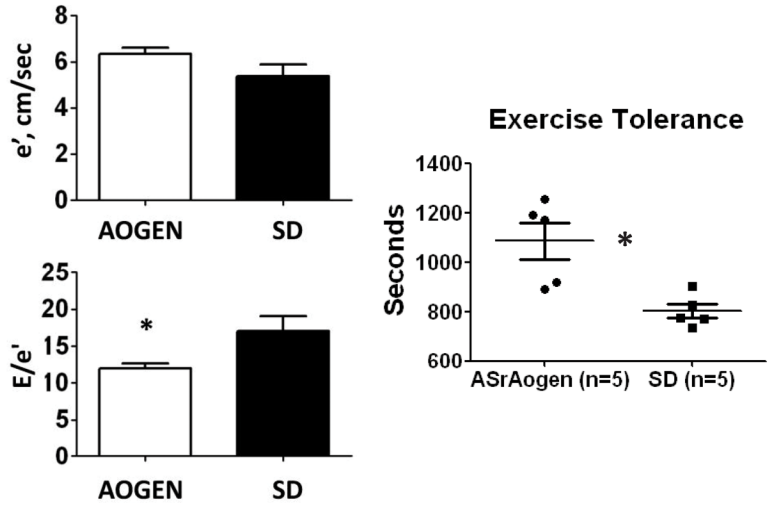

Fig. 1.

The tissue Doppler measure of diastolic function, mitral annular velocity (e'), was not different between groups whereas the Doppler surrogate to left ventricular filling pressure, early transmitral filling-to-mitral annular velocity (E/e'), was significantly lower in ASrAOGENs (n=9) when compared with Sprague–Dawley (SD) rats (n=9). Time to exhaustion by treadmill exercise capacity testing was longer in ASrAOGENs (n=5) vs. SDs (n=5). *P<0.05 vs. SD.

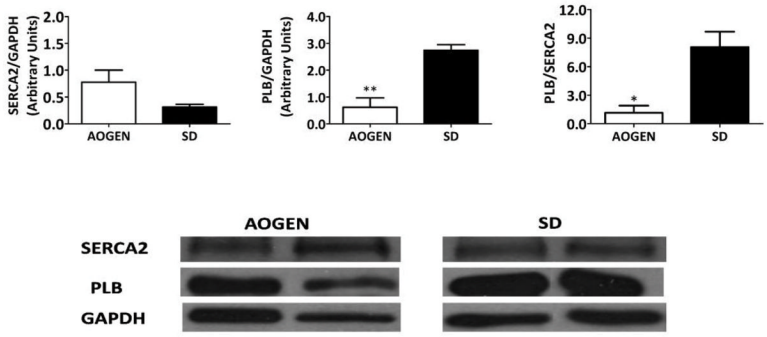

Fig. 2.

Protein concentrations of (a) sarcoendoplasmic Ca^{2+} adenosine triphosphatase [anti-sarcoplasmic endoplasmic reticulum calcium ATPase 2 (SERCA2)] and (b) dephosphorylated phospholamban (PLB) normalized to the respective glyceraldehyde-3-phosphate dehydrogenase (GAPDH) loading controls. (c) The PLB/SERCA2 ratio was lower in the ASrAOGEN rats (n=5) when compared with age-matched Sprague–Dawley (SD) rats (n=5). Given that dephosphorylated PLB blocks SERCA2, the lower PLB/SERCA2 ratio of the ASrAOGEN rats indicates enhanced SERCA2 functioning. Values are means ± SEM. *P<0.05; **P<0.01 vs. SD. (d) Representative immunoblots of SERCA2, PLB, and GAPDH from each group are shown.

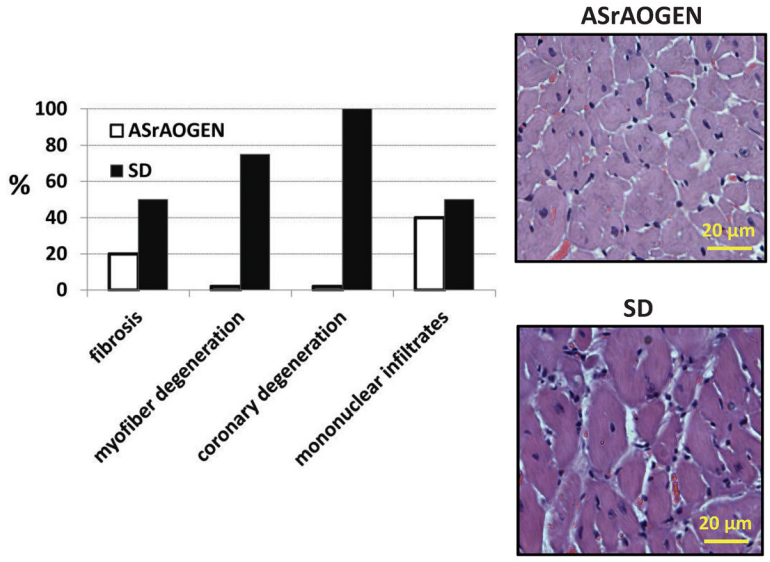

Fig. 3.

(a) Incidence rates (%) of histological findings, which include fibrosis, myofiber degeneration, coronary degeneration, and mononuclear infiltrates, were the highest among Sprague–Dawley (SD) rats (n =6) vs. age-matched ASrAOGENs (n=6). (b) Representative hematoxylin and eosin staining of hearts from each group.

Table 1

Physical characteristics

	ASrAOGEN (*n*=9)	SD (*n*=9)
Age (weeks)	61 ± 2	62 ± 1
Body weight (gm)	339 ± 9 *	617 ± 14
Systolic BP (mmHg)	106 ± 2 †	117 ± 3
Lean body mass[a] (gm)	353 ± 10 *	654 ± 32
Body fat[a] (%)	14.8 ± 2.2 *	27.3 ± 0.3
Fat : lean ratio[a]	0.18 ± 0.03 *	0.38 ± 0.1
Bone mineral density[a] (%)	0.196 ± 0.002	0.234 ± 0.004

Values represent mean ± SEM.

SD, Sprague–Dawley, BP, blood pressure (conscious).

[a]Three 60-week-old animals/group for body composition analyses by DXA (dualenergy X-ray absorptiometry).

*$P < 0.001$;

†$P < 0.05$ vs. SD.

Table 2

Echocardiographic indices of cardiac structure and function

	ASrAOGEN (*n*=9)	SD (*n*=9)
Heart rate (beats/min)	359 ± 3 *	324 ± 11
LVESD (cm)	0.511 ± 0.02 *	0.637 ± 0.03
LVEDD (cm)	0.799 ± 0.03 *	0.931 ± 0.02
FS (%)	37 ± 1	32 ± 2
PWT (cm)	0.180 ± 0.004 *	0.207 ± 0007
RWT (cm)	0.456 ± 0023	0.454 ± 0021
LV mass (gm)	1.01 ± 004 †	1.60 ± 008

Values are mean ± SEM.

FS, fractional shortening; LV, left ventricular; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; PWT, posterior wall thickness at end diastole; RWT, relative wall thickness; SD, Sprague–Dawley.

* $P < 0.01$;

† $P < 0.001$ vs. SD.