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Unprovoked atrial tachyarrhythmias in aging spontaneously hypertensive rats: the role of the autonomic nervous system

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¹University of Medicine and Pharmacy of Targu Mures, Romania; ²Unité de Neurocardiologie, Université Lyon 1, F-69008, Lyon, France; ³Hospices Civils de Lyon, Hôpital Louis Pradel, Service de Rythmologie, Lyon, France; and ⁴Institut de Médecine Légale de Lyon, France

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Scridon A, Gallet C, Arisha MM, Oréa V, Chapuis B, Li N, Tabib A, Christé G, Barrès C, Julien C, Chevalier P. Unprovoked atrial tachyarrhythmias in aging spontaneously hypertensive rats: the role of the autonomic nervous system. *Am J Physiol Heart Circ Physiol* 303: H386–H392, 2012. First published June 1, 2012; doi:10.1152/ajpheart.00004.2012.—Experimental models of unprovoked atrial tachyarrhythmias (AT) in conscious, ambulatory animals are lacking. We hypothesized that the aging, spontaneously hypertensive rat (SHR) may provide such a model. Baseline ECG recordings were acquired with radiotelemetry in eight young (14-wk-old) and eight aging (55-wk-old) SHRs and in two groups of four age-matched Wistar-Kyoto (WKY) rats. Quantification of AT and heart rate variability (HRV) analysis were performed based on 24-h ECG recordings in unrestrained rats. All animals were submitted to an emotional stress protocol (air-jet). In SHRs, carbamylcholine injections were also performed. Spontaneous AT episodes were observed in all eight aging SHRs (median, 91.5; range, 4–444 episodes/24 h), but not in young SHRs or WKY rats. HRV analysis demonstrated significantly decreased low frequency components in aging SHRs compared with age-matched WKY rats ($P < 0.01$) and decreased low/high frequency ratios in both young ($P < 0.01$) and aging ($P = 0.01$) SHRs compared with normotensive controls. In aging SHRs, emotional stress significantly reduced the number of arrhythmic events, whereas carbamylcholine triggered AT and significantly increased atrial electrical instability. This study reports the occurrence of unprovoked episodes of atrial arrhythmia in hypertensive rats, and their increased incidence with aging. Our results suggest that autonomic imbalance with relative vagal hyperactivity may be responsible for the increased atrial arrhythmogenicity observed in this model. We also provide evidence that, in this model, the sympatho-vagal imbalance preceded the occurrence of arrhythmia. These results indicate that aging SHRs may provide valuable insight into the understanding of atrial arrhythmias.

spontaneous atrial arrhythmias; experimental model; autonomic imbalance

ATRIAL FIBRILLATION (AF) AFFECTS ~1% of the general population and up to 8% of subjects over the age of 80 (9). Incomplete understanding of the pathophysiological mechanisms of arrhythmia explains the relative failure of available antiarrhythmic strategies to effectively treat atrial electrical instability (3, 21).

Numerous animal models have been developed to explore the mechanisms that initiate and maintain atrial tachyarrhythmias (AT), including AF, and to evaluate different therapeutic strategies (6, 12, 30). Yet, none of these models manage to faithfully reproduce the human clinical condition. Experimen-

tal models of spontaneous AT in conscious, ambulatory animals are still lacking. Previous studies have reported “spontaneous AT or AF” in animals. However, in those models, arrhythmia occurred only after applying programmed stimulation protocols (6). A number of essential features for AT occurrence, such as structural, electrical, or autonomic remodeling, could have been artificially induced in these models. Therefore, in our opinion, “spontaneous” is not the proper term for describing the type of arrhythmias reported in those articles; we believe that the term “spontaneous” should be reserved for situations that have not used artificial means to trigger the arrhythmia.

The purpose of the present study was to develop a model that could reproducibly exhibit spontaneous AT in rats and to describe its phenotypic features. Because aging (1) and arterial hypertension (1, 7) are the most frequent risk factors associated with atrial arrhythmogenicity, we hypothesized that aging rats of the spontaneously hypertensive rat (SHR) strain might provide a reliable model of spontaneous atrial arrhythmia. Accordingly, we aimed to assess, in baseline conditions, the occurrence of spontaneous AT and several other markers of increased atrial arrhythmogenicity (i.e., atrial premature contractions, couplets, and bigeminisms) in young and aging SHRs. These parameters were compared with those observed in normotensive controls. For this study, we used 24-h continuous ECG recordings, acquired with radiotelemetry.

Autonomic imbalance has been implicated as a potential facilitator in the occurrence and maintenance of AT in humans and various animal models. Several factors, such as heterogeneous autonomic innervation of the atria (19) or a complex and dynamic inter-relationship between the two branches of the autonomic nervous system (26, 31), may be involved in arrhythmia occurrence. Accordingly, the presence and the pattern of an autonomic imbalance were searched using heart rate (HR) variability (HRV) analysis and two experimental protocols that targeted the autonomic nervous system (i.e., emotional stress and carbamylcholine administration).

MATERIALS AND METHODS

Animals. Male SHRs and normotensive Wistar-Kyoto (WKY) rats were purchased from Elevage Janvier (Le Genest Saint Isle, France).

All animals were housed in a climate-controlled room (21° to 22°C), with a 12-h:12-h light/dark cycle, in an accredited animal facility. The rats were housed individually in polycarbonate cages and fed standard rat pellets and tap water ad libitum.

All experiments were performed in compliance with the French Ministry of Agriculture guidelines for animal experimentation and adhered to the American Physiological Society Guiding Principles in

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the Care and Use of Vertebrate Animals in Research and Training, and they were approved by the local Animal Ethics Committee.

Transmitter implantation procedure. Radiotelemetry ECG transmitters (TA11 CA-F40; Data Sciences International, St. Paul, MN) were implanted under isoflurane anesthesia. Each animal received subcutaneous prophylactic injections of penicillin G (50,000 IU) and ketoprofen (2 mg/kg). The body of each transmitter was placed in a dorsal, subcutaneous pocket, and the two ECG leads were tunneled subcutaneously and secured in a lead II configuration. After the implantation procedure, animals were allowed at least 1 wk of recovery before recording.

ECG recording. For baseline quantification of atrial arrhythmic events, 24-h continuous ECG monitoring was performed on unrestrained, conscious rats. Recordings were conducted on eight 55-wk-old SHR (471 \pm 5 g), four age-matched WKY rats (576 \pm 11 g), eight young (14-wk-old) SHR (342 \pm 3 g), and four young (14-wk-old) WKY rats (397 \pm 5 g). ECG signal capture was accomplished with receivers (RPC-1; Data Sciences International) placed under each experimental cage. Telemetry ECGs were converted to analog signal (Analog ECG Output Adapter R08; Data Sciences International) and routed to a personal computer equipped with a signal acquisition card (NI PCIe-6251; National Instruments, Austin, TX). An acquisition program developed in our laboratory using LabVIEW 2009 software (National Instruments) allowed the signal to be continuously recorded with a 2,000-Hz sampling frequency and stored on hard disk.

ECG analysis. ECG data were analyzed using a program recently developed in our laboratory using LabVIEW 2010 software (National Instruments) to automatically detect R waves and measure RR intervals, as previously described (23). All ECG tracings were visually assessed by two independent cardiologists. In the vast majority of time ECG quality allowed easy recognition of all atrial arrhythmic events. Infrequently, intense animal somatomotor activity was associated with altered ECG signal and artifacts; this impaired proper assessment of the atrial electrical activity. Therefore, all artifactual periods were discarded before analysis.

AT was defined as rapid, irregular, supraventricular rhythm [irregular ventricular response with narrow QRS complexes, exceeding by at least 50 beats/min the HR measured during sinus rhythm], of at least 3 beats. The diagnosis also required that P waves were absent or replaced by atrial waves with morphology significantly different compared with sinus rhythm P waves. Premature atrial contractions (PACs) were defined as early atrial activations, with morphology different compared with sinus rhythm P waves. Atrial couplets were defined as a pair of atrial premature activations in a row, whereas the regular succession of a normal sinus beat and an atrial premature activation was defined as atrial bigeminy.

The total numbers of AT episodes, PACs, atrial couplets, and bigeminis were quantified on the 24-h recording. The duration of an AT episode was measured from the first arrhythmic beat of the episode (top of the R wave) to the first sinus rhythm beat that followed the episode.

To test for day-to-day reproducibility, four aging SHR underwent 48-h continuous recording, instead of the standard 24-h recording. Data used for between-group comparisons corresponded to the first 24-h period. To test for medium-term reproducibility, comparisons were also performed, for all eight aging SHR, between the 24-h recording used in between-group comparisons and a second recording, performed 1 wk earlier.

HRV analysis. All arrhythmic events and compensatory pauses were excluded manually before analysis. The mean HR was automatically calculated over 24-h intervals. The HRV during sinus rhythm was assessed in the 24-h recordings for each animal.

HRV was assessed by analyzing beat-to-beat variations in RR intervals in the time and frequency domains, as previously described (2). For the time domain analysis, SD of normal RR intervals, root-mean-square of successive RR-interval differences, and percent-

age of adjacent RR intervals that differed by >5 ms (pNN5) were calculated. For the frequency domain analysis, time series were resampled at 20 Hz using a cubic spline, then spectral power in the low-frequency (LF) band (0.3–0.6 Hz), high-frequency (HF) band (0.6–2.5 Hz), and the LF-to-HF ratio were estimated on 2,048-point (102.4 s) segments windowed by the Hanning function and overlapping by 50% using a fast Fourier transform.

Additionally, to identify potential significant changes in autonomic tone preceding arrhythmic events, we analyzed several intervals of intense arrhythmic activity (frequent PACs and AT episodes) and the 5-min periods preceding these intervals. The resulting frequency-domain parameter values were compared with baseline values.

Emotional stress protocol in conscious rats. Because of its ability to induce pure sympathetic stimulation, while lowering the sensitivity of the cardiac baroreceptor reflex (4, 14), a mild emotional stress protocol elicited by blowing a jet of air into the cage for 20 min was applied to each of the following animals: at the age of 16 wk for young SHR and WKY rats, 47 wk for aging WKY rats, and 64 wk for six aging SHR. Air was delivered through a tube (6 mm internal diameter) inserted in a hole at the bottom of the cage, as previously described (33). The pressure in the relief valve of the air tank was set at 1 bar. The stress test was applied when rats were in a quiet resting state.

A baseline recording was acquired for each animal before the onset of the protocol. Arrhythmic events and HRV analyses were assessed over 10-min intervals. The stress-related values were compared with baseline values.

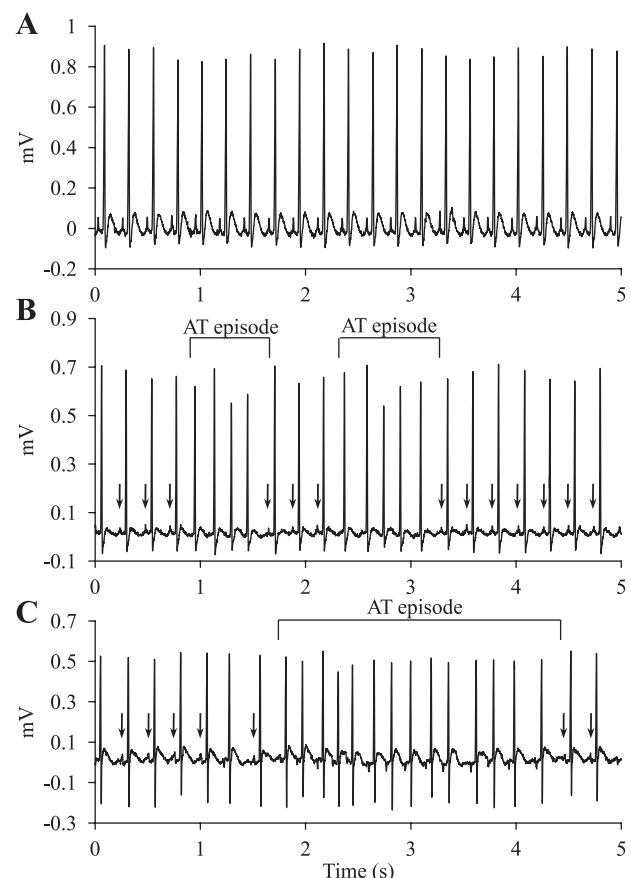


Fig. 1. ECG tracings in 55-wk-old spontaneously hypertensive rats (SHRs) showing sinus rhythm (A); 2 atrial tachyarrhythmias (AT) episodes suggesting a diagnosis of atrial fibrillation (rapid, irregular rhythm, with persistence of narrow QRS complexes, in the absence of visible P waves) (B); and AT episode suggesting a diagnosis of multifocal AT (rapid, irregular rhythm, with persistence of narrow QRS complexes and wide, inverted atrial waves, with continually changing morphology) (C). B and C, arrows: sinus rhythm beats.

Subcutaneous injection of carbamylcholine in conscious rats. A baseline recording was obtained for three aging SHR at the age of 60 wk and for all eight young SHR at the age of 16 wk. A 0.4 mg/kg dose of carbamylcholine was then injected subcutaneously. A 10-min period initiated 5 min after carbamylcholine injection was analyzed, and the number of arrhythmic events was compared with that recorded during the 10-min preinjection period. None of the WKY rats underwent this protocol.

Invasive blood pressure measurement. To evaluate the degree of hypertension, intra-arterial blood pressure was measured in conscious, unrestrained rats, as previously described (13). The catheterization procedure was performed in five SHR at the age of 64 ± 2 wk and in all four WKY rats at the age of 68 wk. One rat in the aging SHR group died during arterial catheterization. None of the young SHR or WKY rats underwent this procedure.

Statistics. Data are expressed as means \pm SE or median and range, as appropriate. Nonparametric ANOVA (Kruskal-Wallis test) was used for multiple comparisons. Between-group comparisons were performed using the Mann-Whitney *U* test using Holm's method for multiple comparisons. Differences within the same group were tested for significance with the Wilcoxon signed-rank test. Nonparametric repeated-measures ANOVA (Friedman test) were used for repeatability data. Frequency domain parameters were also analyzed by a nonparametric (using a rank transform) two-way ANOVA, factoring for the effects of both blood pressure status (normotensive vs. hypertensive) and age (young vs. aging). Medium-term reproducibility in the number of AT episodes was analyzed using Spearman correlation. To assess day-to-day reproducibility, the mean percent variation of the number of AT episodes was calculated.

A *P* value of less than 0.05 was considered statistically significant. Statistical analyses were undertaken using GraphPad Prism software (GraphPad Software; San Diego, CA).

RESULTS

Atrial arrhythmic activity at baseline. Figure 1 depicts typical ECG tracings recorded in 55-wk-old SHR.

The number of tachyarrhythmic episodes in aging SHR was significantly greater than that in young SHR ($P < 0.001$), whereas no episodes were recorded in normotensive rats, regardless of their age (Fig. 2A). Two of the eight young SHR

presented a single episode of AT, while all eight aging SHR had tachyarrhythmic episodes (median, 91.5; range, 4–444 episodes/24 h). Similar differences were observed in the number of PACs (Fig. 2B), atrial couplets (Fig. 2C), and bigemini (Fig. 2D).

In aging SHR, over 24 h, the median total duration of episodes was 60 s/24 h (range, 2–372 s). The median duration of tachyarrhythmic episodes was 0.71 s (range, 0.53–1.63 s). In this group, episode duration ranged from 0.43 s to 10.90 s; in the two young SHR that presented AT, the episode durations were 0.4 s and 0.52 s.

In aging SHR, the RR intervals during AT episodes were $69 \pm 1\%$ shorter than the RR intervals during sinus rhythm ($P < 0.001$). The ECG aspect was consistent with the diagnosis of AF (rapid, irregular, supraventricular rhythm, in the absence of visible P waves) in 30.6% of cases. In the remaining cases, the ECG morphology suggested a diagnosis of atrial tachycardia (rapid, irregular, supraventricular rhythm, with atrial waves with significantly different morphology compared with sinus rhythm P waves).

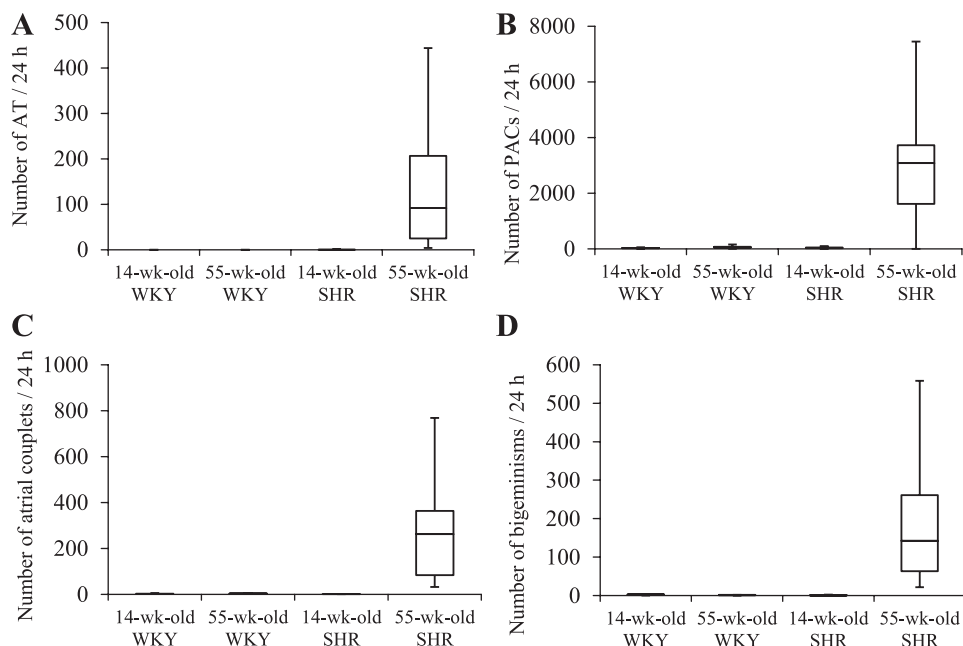
Aging SHR showed high day-to-day (mean percent variation of the number of AT episodes $18.1\% \pm 4.7\%$) and medium-term ($P = 0.01$) reproducibility of the number of arrhythmic episodes (Fig. 3, A and B).

Occasionally, aging SHR showed isolated, monomorphic, premature ventricular contractions.

Assessment of the autonomic tone. The 24-h ECG monitoring indicated that aging SHR were more bradycardic (278 ± 4 beats/min) than age-matched WKY rats (335 ± 2 beats/min, $P = 0.01$), young SHR (326 ± 6 beats/min, $P < 0.01$), or young WKY rats (355 ± 5 beats/min, $P < 0.01$).

HRV analysis demonstrated significantly decreased LF components in aging SHR compared with age-matched WKY rats, and decreased LF-to-HF ratios in both young and aging SHR compared with age-matched WKY rats (Table 1). A two-factor ANOVA demonstrated that LF components were significantly affected by blood pressure status ($P = 0.002$), but they were

Fig. 2. Number of supraventricular arrhythmic events: episodes of AT (A); premature atrial contractions (PACs; B); atrial couplets (C); and atrial bigemini (D), during 24-h ECG recordings in 14-wk-old ($n = 4$) and 55-wk-old ($n = 4$) Wistar-Kyoto (WKY) rats, and in 14-wk-old ($n = 8$) and 55-wk-old ($n = 8$) SHR. Data are expressed as median and interquartile range (IQR). The ends of the whiskers represent the lowest value within 1.5 IQR of the first quartile and the highest value within 1.5 IQR of the third quartile.



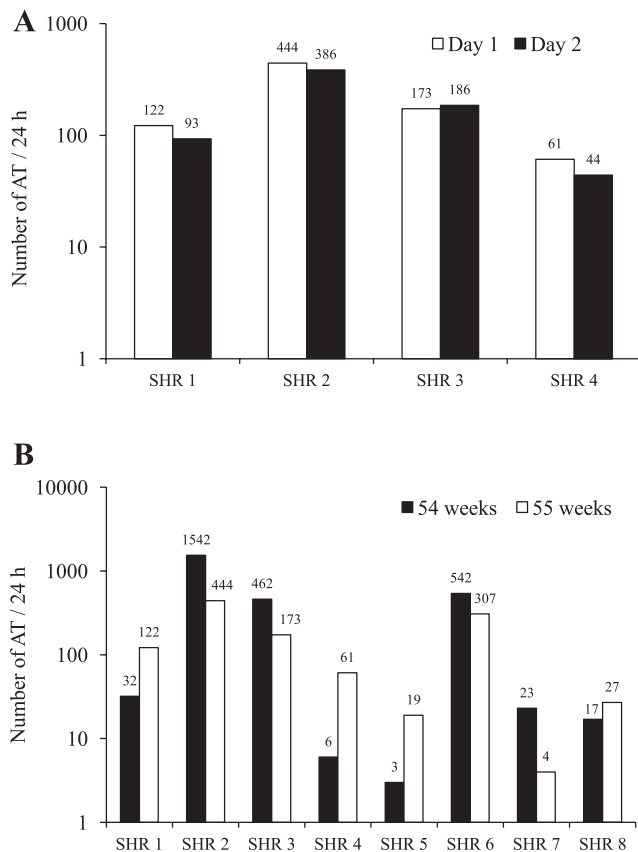


Fig. 3. Day-to-day (A) and medium-term (B) reproducibility of AT episodes in individual aging SHRs. A: comparison between the total number of arrhythmic episodes during the first (day 1) and second (day 2) 24-h period in 4 individual, 55-wk-old SHRs that underwent 48-h continuous ECG monitoring. The mean percent variation in the number of AT episodes was $18.1\% \pm 4.7\%$. B: comparison between the total number of arrhythmic episodes in 8 individual SHRs at 54 and 55 wk of age, respectively (Spearman correlation coefficient, 0.83; $P = 0.01$). Results are shown in log scale.

not significantly affected by age ($P > 0.05$), and there was no interaction between blood pressure status and age ($P > 0.05$). The same analysis demonstrated that the LF-to-HF ratio was significantly affected by both age and blood pressure status (both $P < 0.05$); again, we found no interaction between age and blood pressure status ($P > 0.05$).

In aging SHRs, recordings performed 1 wk apart showed significant reproducibility of all HRV parameters (all $P > 0.05$).

In aging SHRs, the HRV analysis showed no significant changes in LF, HF, or LF-to-HF components in the 5-min intervals that preceded the onset of periods of intense arrhythmic activity compared with baseline values (all $P > 0.05$). Most of the arrhythmic episodes occurred during periods of intense arrhythmic activity (frequent PACs), but in 28% of cases AT episodes occurred after sinus bradycardia or sinus pauses.

The effect of stress on atrial arrhythmogenicity. In the six aging SHRs, a median of 0.5 (range, 0–5) tachyarrhythmic episodes was observed during the 10-min interval preceding the onset of the stress protocol, whereas no episode was recorded during stress exposure. Similarly, the number of PACs decreased from a median of 13.5 (range, 1–33) before stress to a median of 1 (range, 0–14) during stress exposure ($P = 0.04$).

No AT or PACs were present in WKY rats, regardless of their age, before or after the onset of the stress protocol. No AT and few PACs were observed in young SHRs, before or during stress exposure.

The emotional stress induced an increase in HR; HR increased in young WKY rats from 288 ± 9 to 313 ± 18 beats/min, $P = 0.25$; in aging WKY rats from 315 ± 10 to 341 ± 12 beats/min, $P = 0.38$; in young SHRs from 282 ± 6 to 412 ± 10 beats/min, $P = 0.007$; and in aging SHRs from 273 ± 10 to 363 ± 9 beats/min, $P = 0.03$. Since only SHRs presented spontaneous episodes of AT, HRV analysis before and during stress was only performed in these groups. In aging SHRs, the HRV analysis showed significant increases in the LF-to-HF ratio during stress compared with prestress values (Table 2). Comparable results were obtained in young SHRs: LF increased during stress from $0.60 \pm 0.14 \text{ ms}^2$ to $1.11 \pm 0.23 \text{ ms}^2$ ($P = 0.007$), HF decreased from $5.62 \pm 0.10 \text{ ms}^2$ to $3.19 \pm 0.39 \text{ ms}^2$ ($P = 0.03$), and the LF-to-HF ratio increased from 0.11 ± 0.01 to 0.35 ± 0.05 ($P = 0.007$).

Carbamylcholine administration and atrial arrhythmias. Before carbamylcholine administration, aging SHRs presented a median of 15 PACs (range 6–29), and no episodes of AT were recorded. In the 10-min interval following carbamylcholine injection, a median of 70 PACs (range 40–271) and a

Table 1. Baseline heart rate variability parameters in young and aging groups of WKY rats and SHR

Parameter	WKY rats, weeks		SHRs, weeks		P Value
	14	55	14	55	
n	4	4	8	8	
Time domain					
SDNN, ms	28.33 ± 1.21	25.14 ± 1.82	$21.42 \pm 0.85^*$	$27.09 \pm 3.16^\dagger$	0.01
RMSSD, ms	4.05 ± 0.45	4.85 ± 0.54	3.58 ± 0.32	3.80 ± 0.32	0.28
pNN5, %	0.17 ± 0.03	0.26 ± 0.04	0.14 ± 0.03	0.16 ± 0.03	0.21
Frequency domain					
LF, ms^2	1.57 ± 0.35	1.96 ± 0.31	1.00 ± 0.17	$0.86 \pm 0.16^*$	0.03
HF, ms^2	5.99 ± 1.33	7.70 ± 1.56	5.53 ± 1.11	6.13 ± 1.37	0.62
LF/HF	0.26 ± 0.01	0.27 ± 0.04	$0.19 \pm 0.01^*$	$0.14 \pm 0.01^{*\dagger}$	0.001

Values are means \pm SE. Recordings were characterized in the time domain: SDNN, SD of normal RR intervals; RMSSD, root-mean-square of successive RR-interval differences; pNN5, percentage of adjacent RR intervals that differed by >5 ms. Recordings were characterized in the frequency domain: LF and HF, low-frequency (0.3–0.6 Hz) and high-frequency (0.6–2.5 Hz) signals, respectively; LF/HF, the ratio of low to high frequency components. P values refer to comparisons between groups based on multiple comparisons ANOVA. $^*P < 0.05$ for spontaneously hypertensive rats (SHRs) vs. age-matched Wistar-Kyoto (WKY) rats; $^\dagger P < 0.05$ for 55-wk-old SHRs vs. 14-wk-old SHRs.

Table 2. Heart rate variability parameters before and during stress in aging SHR

Parameter	Stress		P Value
	Before	During	
Time domain			
RMSSD, ms	3.58 ± 0.66	2.22 ± 0.53	0.01
pNN5, %	0.14 ± 0.05	0.04 ± 0.02	0.06
Frequency domain			
LF, ms ²	0.67 ± 0.15	1.70 ± 0.69	0.20
HF, ms ²	4.33 ± 1.35	2.57 ± 0.86	0.27
LF/HF	0.19 ± 0.03	0.56 ± 0.09	0.01

Values are means ± SE; *n* = 6 aging SHRs. Recordings were characterized in the time and frequency domains. *P* values refer to comparisons between conditions based on the Wilcoxon signed-rank test.

median of 3 (range 1–29) episodes of AT were observed. The AT episodes that occurred after carbamylcholine administration had a median duration of 1.89 s (range 0.90–10.13 s), significantly longer than the duration of AT episodes in the 24-h baseline recordings (*P* < 0.001).

No AT and few PACs were observed in young SHRs, before or after carbamylcholine administration.

In aging SHRs, the high electrical instability observed after carbamylcholine administration, with an elevated number of PACs and AT episodes and very few periods of stable sinus rhythm, did not allow HRV analysis to be properly performed.

Blood pressure measurement. Systolic, diastolic, and mean blood pressure, and pulse pressure values in aging SHRs and WKY rats are presented in Table 3. As expected, SHRs had significantly higher systolic, diastolic, and mean blood pressure than WKY rats (all *P* = 0.02). Similarly, pulse pressure was significantly higher in SHRs than in normotensive controls (*P* = 0.03).

DISCUSSION

The main findings of the present study were 1) all aging SHRs developed spontaneous, unsustained AT, whereas young SHRs and both young and aging WKY rats did not; 2) in aging SHRs, the arrhythmic burden showed high day-to-day and medium-term reproducibility; 3) in aging SHRs, sympatho-vagal imbalance, with reduced sympathetic tone, was observed; 4) in aging SHRs, sympathetic activation by emotional stress removed all AT and significantly reduced the number of PACs, whereas parasympathetic stimulation with carbamylcholine had marked pro-arrhythmic effects; and 5) sympatho-vagal imbalance was already present in young SHRs and preceded the occurrence of arrhythmia.

To the best of our knowledge, this is the first study to report the occurrence of spontaneous AT in animals, in the absence of any artificial means that could have favored the onset of arrhythmia. The short duration of AT episodes in our model was due to characteristic anatomical features of small animals. Reentrant arrhythmias have generally been considered to be restricted to large animals, because the generation and maintenance of reentrant arrhythmias require a minimum tissue mass (>100–200 mm³) (10). Consequently, to date, reentrant arrhythmias are often considered impossible in small rodents, since the myocardial mass of rats and mice is <35 mm³, too small to sustain multiple reentrant wavelets. Accordingly, in SHRs, most episodes of AT spontaneously reversed to sinus

rhythm, usually after a small number of arrhythmic beats; this confirmed that the role of a critical myocardial mass cannot be underestimated.

Despite this relative limitation, our model closely resembled the human presentation of arrhythmia. The spontaneous occurrence of AT in aging, hypertensive subjects corresponded to clinical observations that linked AT with aging and high blood pressure (1, 16, 17). In the Manitoba study, the prevalence of hypertension among patients with AF was 53% and the estimated risk of AF was 1.42 times higher in hypertensive than in normotensive subjects (17). The Framingham Heart Study reported a 1.9-fold higher risk of AF in hypertensive than in normotensive individuals (15). Frequent PACs have also been reported in hypertensive patients and in elder subjects (28). The inter-individual variability in the number of AT episodes in the 24-h recordings further supported the close resemblance between this model and the human presentation of AT.

The autonomic nervous system. It was previously proposed that sympatho-vagal imbalance could facilitate atrial arrhythmias. A number of clinical and experimental studies have provided evidence that the autonomic nervous system plays a crucial role in the pathophysiology of AF. Coumel (8) described different types of AF resulting from increased vagal or adrenergic tone. A number of studies have amassed evidence for an enhanced vagal tone in AT (5, 11, 29, 32). In a study by Patterson et al. (24), application of acetylcholine shortened the action potential duration in pulmonary veins and induced early-after-depolarizations, whereas simultaneous infusion of acetylcholine and norepinephrine triggered tachycardia-pause rapid firing in the same preparations. Those authors also demonstrated that combined parasympathetic and sympathetic nerve stimulation triggered firing in canine pulmonary veins (25). Chen and colleagues (6) used extrinsic and intrinsic cardiac nerve activity recordings in ambulatory dogs and applied intermittent rapid left atrial pacing to induce paroxysmal AF or AT. Their results suggested that intrinsic cardiac nerve activation, alone or associated with extrinsic cardiac nerve activation, could invariably trigger paroxysmal AT. Along the same lines, Tan et al. (27) reported that simultaneous sympatho-vagal discharges were common triggers for spontaneous atrial arrhythmias, and cryoablation of extrinsic sympatho-vagal nerves eliminated these episodes; those results suggested a causal relationship between the discharges and the occurrence of arrhythmia (27).

In the present study, HF components, an index of parasympathetic modulation, did not differ between aging SHRs, which presented numerous episodes of AT, and age-matched WKY rats, which did not experience AT. However, LF components

Table 3. Blood pressure values in aging WKY rats and aging SHRs

Variables	WKY rats	SHRs	P Value
<i>n</i>	4	5	
Blood pressure, mmHg			
Systolic	135 ± 2	236 ± 7	0.02
Diastolic	90 ± 0.4	171 ± 8	0.02
Mean	107 ± 0.5	199 ± 8	0.02
Pulse pressure, mmHg	46 ± 2	65 ± 5	0.03

Values are means ± SE. *P* values refer to comparisons between groups based on the Mann-Whitney U test.

were significantly reduced in aging SHR rats compared with those in WKY rats, and the LF-to-HF ratio was significantly smaller in aging SHR rats than in WKY rats. Given that the LF-to-HF ratio is an index of parasympathetic and sympathetic interactions in the rat heart (18), our results highlighted a significant sympatho-vagal imbalance in animals that experienced AT. The reduction of sympathetic tone, and therefore, the relative vagal hyperactivity, appeared to facilitate the occurrence of arrhythmias. In our model, reduced sympathetic tone appeared to result from persistent hypertension, but both aging and high blood pressure appeared to affect the sympatho-vagal balance.

Acute changes in autonomic tone that preceded the onset of AT episodes were difficult to assess, due to the fact that the AT episodes occurred during periods of intense arrhythmic activity (frequent PACs). Consequently, most of the HRV analysis was actually performed on intervals immediately preceding periods of frequent PACs rather than actual AT episodes. This could have influenced our results. Therefore, although frequency-domain parameters showed no acute changes before the onset of arrhythmic episodes, these results should be interpreted with caution; the role of the autonomic nervous system should not be oversimplified based on these results.

Emotional stress restored the autonomic balance by increasing sympathetic tone and had marked antiarrhythmic effects. Indeed, in aging SHR rats, the LF-to-HF ratio increased during stress compared with the baseline value, despite unchanged LF values. However, given the reduction in total HR variance in aging SHR rats during stress (decreased root-mean-square of successive RR-interval differences and pNN5), it is not surprising that the absolute values of LF did not significantly change.

In aging SHR rats, carbamylcholine had marked pro-arrhythmic effects. This was shown by the increased number of PACs and AT episodes and the significantly longer duration of AT episodes following carbamylcholine administration compared with baseline recordings. Unfortunately, HRV analysis after carbamylcholine administration could not be achieved, due to the increased number of arrhythmic events. However, based on the well-known effects of the stress protocol (pure sympathetic stimulation), the known parasympathetic effect of carbamylcholine, and the opposite effects induced by stress and carbamylcholine on atrial arrhythmogenicity, it appears that accentuating the autonomic imbalance by further increasing vagal tone enhances even more atrial arrhythmogenicity in this setting.

As suggested by the absence of AT episodes in young SHR rats, despite the decreased LF-to-HF ratio, the presence of an autonomic imbalance did not appear to be sufficient for inducing AT. Indeed, LF-to-HF ratio was altered more in aging SHR rats than in young SHR rats; this suggested that autonomic imbalance became more severe with advancing age. However, other features, such as anatomical remodeling with extensive atrial fibrosis, abnormal distribution and/or density of autonomic nerves within the atria, or molecular abnormalities, may also act as arrhythmia facilitators in this model. The role of atrial fibrosis in atrial arrhythmogenicity was previously reported in senescent normotensive rats (22).

Potential limitations. Based solely on the results from a surface ECG recording, a junctional origin of arrhythmias cannot be excluded. However, rapid ectopic activity arising from the atrio-ventricular node or His bundle has only been reported in special circumstances, including recent cardiac surgery or acute myocardial infarction, but not in patients with

hypertension alone, and, to the best of our knowledge, has never been reported in rats. Additionally, the presence of relatively long PQ intervals during arrhythmic episodes that presented atrial waves and the continual changes in the morphology of atrial waves during those episodes strongly support the diagnosis of multifocal AT and not of a tachycardia originating from a single subsidiary pacemaker.

The protocols used in this study provide only indirect evidence of autonomic imbalance in SHR rats that presented atrial arrhythmias. Additional functional studies are required to quantify the extent of abnormal sympatho-vagal imbalance and its precise role in atrial arrhythmogenicity.

Conclusions

This study reports the occurrence of unprovoked episodes of atrial arrhythmia in hypertensive rats, and their increased incidence with aging. Our results suggest that autonomic imbalance with relative vagal hyperactivity may be responsible for the increased atrial arrhythmogenicity observed in this model. We also provide evidence that, in this model, the sympatho-vagal imbalance preceded the occurrence of arrhythmia. This work represents a potentially useful, new experimental model of atrial arrhythmias that may facilitate studies of pathophysiological mechanisms involved in atrial arrhythmias, including AF. Additional functional, anatomic-histological, and molecular studies in this setting will be of interest.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.S., C.J., and P.C. conception and design of research; A.S., V.O., N.L., and A.T. performed experiments; A.S., C.G., M.M.A., V.O., B.C., N.L., and A.T. analyzed data; A.S., C.G., B.C., A.T., G.C., C.B., C.J., and P.C. interpreted results of experiments; A.S. and B.C. prepared figures; A.S. drafted manuscript; A.S., C.G., M.M.A., V.O., B.C., N.L., A.T., G.C., C.B., C.J., and P.C. approved final version of manuscript; C.G., M.M.A., V.O., B.C., N.L., A.T., G.C., C.B., C.J., and P.C. edited and revised manuscript.

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