Aging and baroreflex control of RSNA and heart rate in rats

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Irigoyen, M. C., E. D. Moreira, A. Werner, F. Ida, M. D. Pi res, I. A. Cestari, and E. M. Krieger. Aging and baroreflex control of RSNA and heart rate in rats. Am J Physiol Regulatory Integrative Comp Physiol 279: R1865–R1871, 2000.—Aging is associated with altered autonomic control of cardiovascular function, but baroreflex function in animal models of aging remains controversial. In this study, pressor and depressor agent-induced reflex bradycardia and tachycardia were attenuated in conscious old (24 mo) rats [57 and 59% of responses in young (10 wk) Wistar rats, respectively]. The intrinsic heart rate (HR, 339 ± 5 vs. 410 ± 10 beats/min) was reduced in aged animals, but no intergroup differences in resting mean arterial blood pressure (MAP, 112 ± 3 vs. 113 ± 5 mmHg) or HR (344 ± 9 vs. 347 ± 9 beats/min) existed between old and young rats, respectively. The aged group also exhibited a depressed (49%) parasympathetic contribution to the resting HR value (vagal effect) but preserved sympathetic function after intravenous methylatropine and propranolol. An implantable electrode revealed tonic renal sympathetic nerve activity (RSNA) was similar between groups. However, old rats showed impaired baroreflex control of HR and RSNA after intravenous nitroprusside (−0.63 ± 0.18 vs. −1.84 ± 0.4 bars·cycle⁻¹·mmHg⁻¹·s⁻¹). Therefore, aging in rats is associated with 1) preserved baseline MAP, HR, and RSNA, 2) impaired baroreflex control of HR and RSNA, and 3) altered autonomic control of resting HR.

METHODS

Experimental animals. Young adult (10 wk old; weight 200–250 g; n = 10) and old (age 24 mo; weight 400–450 g; n = 10) Wistar rats were obtained from the animal care unit of the University of São Paulo School of Medicine. All of the experiments followed the Guide for the Care and Use of Laboratory Animals [DHEW Publication No. (NIH) 85–23, Revised 1985, Bethesda, MD] and the guidelines of the Animal Welfare Act.

Instrumentation. Arterial and venous cannulas were implanted into normal young and old rats 1 day before cardiovascular and nerve monitoring, and all animals were treated with a single injection of penicillin G benzathine (benzathcin 60,000 U). While the rat was under ether anesthesia, polyethylene-tipped Tygon cannulas filled with heparin in normal saline (500 U/ml) were inserted into the abdominal aorta and inferior vena cava through the left femoral artery and

AGING, DEFINED AS CHANGES occurring between adulthood and old age (7), is associated with a variety of alterations in cardiovascular function, glucose homeostasis, and autonomic reflexes in humans (25) and rats (8, 27, 37). Studies on westernized human populations have shown an age-related increase in the systolic (12) and, to some extent, diastolic blood pressure (2). However, studies of small and isolated communities (19) have suggested that factors other than aging are involved in this phenomenon. A tendency for the mean arterial

sympathetic activity; cardiovascular function; autonomic control; vagal effect

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vein, respectively. The ends of the cannulas were tunneled subcutaneously and exteriorized at the top of the skull. On the day of the experiment, a thin bipolar platinum electrode was placed around a branch of the left renal nerve and insulated with silicone rubber (Wacker SiI-Gel 604) while the rat was under ether anesthesia. Measurements were performed 4–6 h after completion of surgery to allow the rat time to recover from the anesthesia. During the experiments, each rat was maintained in the cage in which it had been housed since the previous day (25 × 15 × 10-cm Plexiglas cages with a grid floor). The electrode cable and the arterial cannula were attached to special extensions during the recording period, allowing the rat complete freedom of movement within the cage.

Cardiovascular and nerve monitoring. The arterial pressure (AP) was recorded in conscious rats by connecting the arterial cannula to a pressure transducer (Statham P23 Db, Hato Rey, PR) and a pressure amplifier (model 8805C, Hewlett Packard). The signal from the nerve electrode was recorded after being amplified (Tektronix 5A22N differential amplifier) and filtered (band pass filter, 100 Hz to 2 kHz). Both the AP and the original neurogram were monitored with a storage oscilloscope (Tektronix 5111) and stored on a tape recorder (Hewlett Packard, model 7754A) during a control period of 3 min. The RSNA and HR were recorded under control conditions and after changes in AP induced to test baroreflex sensitivity. Further processing was performed using a data-acquisition system assembled on a personal microcomputer equipped with an analog-to-digital converter board (10 bits, CAD 10/26 Lynx). An electronic circuit was built for preprocessing the neurogram before digital conversion. This circuit allows subtraction of a desired voltage from the input signal, amplification, full-wave rectification, and integration with an analog output provided for oscilloscope monitoring after each stage. At the end of the experiment, a dose of phenylephrine was administered to produce a sudden and marked increase in MAP, which reduced neural activity to a minimum considered to be the baseline bioelectrical or near-noise signal. This near-noise signal was then manually subtracted from the original neurogram with the use of a high-resolution potentiometer, followed by amplification with a variable gain. Integration was performed in a voltage reset mode in which the capacitor is allowed to discharge every time the voltage level equals 10 V, yielding a bar-like signal of constant amplitude. The amplitude, number, and duration of bursts determine the energy content of the neurogram. Changes in any of these parameters will be proportionally reflected in the number of bars at the output of the integrator. AP and integrated sympathetic activity were digitized (120 Hz).

Systolic and diastolic AP, MAP, HR, and RSNA were determined on a beat-to-beat basis using in-house developed software; additional processing was performed with commercial software. HR was determined to be one over the interval between two successive peaks of the pressure wave. The RSNA was expressed as the number of bars per cardiac cycle (bars/cycle) (20, 32). To compare different groups of rats, RSNA values were expressed as bars per cycle or as a percentage of the maximal (100%) and minimal (0%) nerve activity during 1,000 cardiac cycles, as described by Lundin et al. (28). Normalization was necessary to account for the varying intensity of the recorded signal, given its multifiber nature. Briefly, values of maximal and minimal nerve activity (100% and 0%) were determined from the 3% of the recorded cardiac cycles that showed the highest and the lowest activity levels.

Spontaneous and reflex baroreceptor testing. The spontaneous resting relationship between the AP and RSNA was quantified by averaging RSNA values obtained during the recording period that corresponded to each pressure class (of 2 mmHg) from higher to lower systolic pressure values, as described elsewhere (20, 32). Briefly, the recorded 1,000 cardiac cycles were sorted so that systolic AP values were distributed from the highest to the lowest in intervals (classes) of 2 mmHg. To be considered as a class, each 2-mmHg interval had to contain at least 10 systolic AP values. The corresponding RSNA values obtained for each systolic AP within a class were averaged and are illustrated in Fig. 1A. The correlation between the AP and RSNA was expressed by fitting a regression line through the points relating the average RSNA values and increasing systolic pressure values for each class (Fig. 1B). The reflex control of RSNA and HR was evaluated by examining at least three pressure responses (3–40 mmHg) to phenylephrine (0.25–4 μg/ml) and sodium nitroprusside (6–25 μg/ml) injections. The peak increase or decrease in MAP after each injected dose of phen-

Fig. 1. A: relationship between spontaneous systolic blood pressure (SBP) and renal sympathetic nerve activity (RSNA) in one young rat, showing an inverse correlation between the average RSNA (%) and corresponding systolic class (2-mmHg interval). The mean SBP was similar in old (137 ± 3 mmHg) and young (132 ± 3 mmHg) rats. B: the slope (b) of the linear regression line for the young (dotted line) and old (solid line) groups of rats significantly differs between groups. Inset: the number of cardiac cycles at each level of normalized RSNA shows a similar distribution among young and old rats. ∆, Change.
yephine or nitropressure was correlated with the peak reflex change in RSNA or HR. Averaged values obtained during a fixed time interval (1 s) were used to quantify reflex changes. Baroreflex sensitivity was analyzed by the regression line obtained by best-fit points relating changes in RSNA (bars/cycle) or HR (beats/min) and MAP (mmHg). The basal nerve activity was obtained by averaging the RSNA values determined during the first 40 cycles immediately before drug injection.

Pharmacological blockade. Both vagal and sympathetic tone (33) were studied in another set of rats (6 animals each) with injections of methylamphetamine (3 mg/kg iv, Sigma) and propranolol (4 mg/kg iv, Sigma) at a maximal volume of 0.2 ml per injection. The catheters were implanted 24 h before the experiment. On the first day of the study, resting HR was recorded in the quiet, unrestrained rat kept in its own cage. Methylamphetamine was injected immediately after the resting HR was recorded. Because the HR response to methylamphetamine peaks within 10–15 min (33), this time interval was standardized before the measurement of HR. Propranolol was injected 15 min after the methamphetamine injection, and again the response was measured after 10–15 min. To obtain the reverse sequence of blockade, propranolol was administered before the application of methamphetamine on the second day of the experiment. Efficacy of the blockade induced by propranolol and methylamphetamine was confirmed by the elimination of reflex changes in HR produced by phenylephrine and sodium nitropressure administration.

The intrinsic HR (IHR) was evaluated after simultaneous blockade by propranolol and methylamphetamine on each day of the experiment; the IHR was expressed as the mean ± SE of the values obtained during the two experimental sequences. The averaged values of IHR were used for statistical comparisons. The vagal effect was calculated as the difference between the maximum HR after methamphetamine injection and the control HR. The sympathetic effect was evaluated as the difference between the control HR and minimum HR after propranolol injection. The vagal tone was calculated as the difference between the IHR and the HR after propranolol injection. The sympathetic tone was determined as the difference between the HR after methamphetamine injection and the IHR.

Statistical analysis. Data were reported as means ± SE. Statistical analysis of differences between old and young groups were performed using the unpaired Student's t-test. The baroreflex sensitivity was evaluated by regression line analysis, and the slope was tested using the t-test for unpaired data. P < 0.05 was considered significant.

RESULTS

Baseline values did not differ significantly between the old and young adult rats for MAP (112 ± 3 and 113 ± 5 mmHg, respectively), HR (344 ± 9 and 347 ± 9 beats/min, respectively), or RSNA (11 ± 4 and 15 ± 4 bars/cycle, respectively).

Spontaneous and reflex changes in baroreceptor control. The relationship between spontaneous changes in systolic pressure and RSNA in normal young rats, as well as the variability observed in RSNA values corresponding to the different classes of systolic pressure, is illustrated in Fig. 1A. Interestingly, the inverse correlation between systolic pressure (2 mmHg for each class) and average RSNA observed in young rats was 50% reduced in old rats (−0.35 ± 0.1 and −0.71 ± 0.12 bars·cycle·mmHg⁻¹, respectively; Fig. 1B). The mean baseline RSNA values were unchanged in old rats, as was the pattern of distribution of normalized RSNA, as shown in the inset in Fig. 1B. A percentage greater than 60% of cardiac cycles showed RSNA within 0–10% range in young and old rats.

Administration of nitropressure and phenylephrine demonstrated that old rats had impaired sensitivity for baroreflex control of HR. The baroreflex bradycardia elicited by increasing the MAP was significantly attenuated in old rats (−1.45 ± 0.2 beats·min⁻¹·mmHg⁻¹·s⁻¹) compared with young rats (−2.51 ± 0.16 beats·min⁻¹·mmHg⁻¹·s⁻¹; P < 0.05). When the MAP was lowered, HR responses were depressed in aged (−1.70 ± 0.3 beats·min⁻¹·mmHg⁻¹·s⁻¹) compared with young (−2.90 ± 0.4 beats·min⁻¹·mmHg⁻¹·s⁻¹, Fig. 2A) rats.

The RSNA response to an increase in blood pressure was similar in aged rats (−0.60 ± 0.19 bars·cycle⁻¹·mmHg⁻¹·s⁻¹) and young rats (−0.70 ± 0.22 bars·cycle⁻¹·mmHg⁻¹·s⁻¹, Fig. 2B). However, changes in RSNA associated with a decrease in MAP were attenuated in old (−0.63 ± 0.18 bars·cycle⁻¹·mmHg⁻¹·s⁻¹) compared with young (−1.84 ± 0.4 bars·cycle⁻¹·mmHg⁻¹·s⁻¹) rats.

Vagal and sympathetic function. The basal HR was similar for the old and young rats (344 ± 9 vs. 347 ± 9 beats/min). However, the IHR after methamphetamine and propranolol blockade was lower in the old rats (339 ± 5 vs. 410 ± 10 beats/min; Fig. 3A). The vagal effect (Fig. 3B) evaluated by methamphetamine injection was greater in young rats compared with old rats (180 ± 10 and 88 ± 8 beats/min, respectively). Propranolol injection caused a small but insignificant decrease in HR in both groups (49 ± 7 vs. 30 ± 8 beats/min in young rats, P = 0.104), suggesting no changes in the sympathetic effect. There was no difference in vagal tone between old (84 ± 12 beats/min) and young rats (92 ± 10 beats/min) or in sympathetic tone (110 ± 13 vs. 128 ± 18 beats/min, respectively).

DISCUSSION

The results of the present study support previous observations related to aging and the autonomic control of AP and extend those observations in important ways. First, although we did not find differences in baseline values for MAP, HR, and RSNA between old and young rats, we did show a reduction in arterial baroreflex control of HR and a depression in the IHR in old rats. Second, we demonstrated significant age-related impairment of RSNA responses to unloading of the baroreceptors.

In the present experiments, APs recorded continuously over 1 h did not differ between old and young rats. Although studies of Western populations have shown an age-related increase in systolic (12) and diastolic (2) blood pressure, little or no age-related increase in blood pressure has been found in populations with a tribal lifestyle (16). Whether genetic and environmental factors, or simply a shorter average longevity in such populations, explain this difference
with respect to westernized populations is unclear. However, increased blood pressure has been associated with psychosocial and dietary changes, including increased use of salt or alcohol (16, 23). In rats maintained under laboratory conditions, both an increase (39) and no change (13, 31, 37) in blood pressure have been found. These differences may be attributable to differences in the rat strain, age (in months), or type of anesthetic used.

Similar controversies exist concerning data about HR, with reports of no change in HR (35, 37) as well as decreased HR in aged rats (14) and humans (11). In the present study, we found no difference in resting HR between old and young rats, despite the reduction in vagal function (vagal effect) detected in the former. Because HR was normal, a compensatory decrease in sympathetic activity would be expected. However, no difference in the sympathetic function was observed between young and aged rats when pharmacological blockade was performed. Therefore, it may be appropriate to consider changes in pacemaker cells. Indeed, the reduction in the IHR of old rats in the present study is in agreement with data obtained in isolated spontaneously beating hearts, showing lower rates in aged rats (14). In humans, the IHR also decreases with age (21). Therefore, a decreased IHR associated with a reduced vagal effect could explain why the resting HR was normal in aged rats.

Fig. 2. A: baroreflex control of heart rate (ΔHR) in young (dashed line) and old (solid line) rats. The slopes for the HR during increases and decreases in the mean arterial pressure (ΔMAP) were significantly reduced in old rats compared with young rats. B: only the slope for ΔRSNA during a decrease in MAP was significantly reduced in old rats compared with young rats. The values of MAP (112 ± 3 and 113 ± 5 mmHg), HR [344 ± 9 and 347 ± 9 beats/min (bpm)], and RSNA (11 ± 4 and 15 ± 4 bars/cycle) were similar in old and young rats, respectively.

Fig. 3. A: graphs showing no differences in sympathetic (ST) and vagal (VT) tonus between young and old rats. The intrinsic HR (IHR) was lower in old rats. B: the increase in HR (from 347 ± 9 to 527 ± 11 beats/min) after methylatropine injection (vagal effect (VE)) was higher in young than in old rats. *Significant difference between young and old rats (P < 0.05).
The absolute values of nerve activity in multiple-unit recordings are influenced by the proximity of the electrodes to the active fibers, the number of active fibers, and other recording conditions. Multifiber nerve activity is often normalized by expressing averaged activity as a percent change from control or as a percentage of maximum. Different procedures for data normalization (17) have been reported since the first recording was performed (22). Care was taken in the present study to validate comparisons of RSNA between age groups. To properly normalize the data, a zero activity reference is needed. Errors made in determining the zero reference level will result in errors in averaged nerve activity, and thus care must be exercised in its determination (17). The maximum activity, on the other hand, may be determined as the higher spontaneous activity recorded during one or more cardiac cycles. We used the data-normalization procedure previously described (20, 28). The baseline noise of the postfiltered signal was eliminated, and only activity exceeding the noise level was integrated to quantify the RSNA, thus avoiding errors of summation that may occur with other methods (17). Under these conditions and knowing the technical limitations, we found no differences in tonic RSNA levels between old and young rats. In contrast, it has been reported that muscle sympathetic nerve activity is positively correlated with age in humans (38) and that blood pressure and plasma norepinephrine levels are increased in the elderly (29). It also has been repeatedly shown that normotensive elderly subjects have modestly elevated plasma norepinephrine concentration (9) at rest. Elevated plasma norepinephrine concentrations may reflect (as discussed in Ref. 12) 1) an increase in tonic sympathetic nerve discharge, 2) increased quantal transmitter release per impulse, or 3) reduced transmitter reuptake at the nerve junctions. Any of these possibilities will enhance the adrenergic effect on heart and vessels. However, the lower release of epinephrine from the adrenal medulla in elderly subjects (12) is not consistent with an overall increase in the resting sympathetic activity with aging. A similar level of sympathetic control of the resting HR was observed in young and old rats in this study. The complex interactions of the sympathetic and parasympathetic systems may have contributed to this effect. It has been demonstrated that different levels of vagal activity are related to different levels of sympathetic activation (4). Our finding that the RSNA is similar in old and young rats is consistent with unaltered values of norepinephrine spillover in the urine found in aged individuals (24). The finding that sympathetic activity is augmented in skeletal muscle vascular beds (36) does not necessarily imply that tonic sympathetic nerve activity is generally elevated in elderly subjects.

The present study is in agreement with previous observations (23, 25, 38) that baroreflex control of HR is depressed in aged individuals. Baroreflex control of cardiac-sympathetic efferent nerve activity has been reported to be depressed in aged animals (7, 13, 15). However, impaired baroreflex control of cardiac vagal neurons appears to be the major determinant of changes in HR control with age (7, 37, 38). Reduced vagal function on the HR control was found in the present study. Greater bradycardia was found in old rats compared with young rats during electrical stimulation of the vagus nerve and acetylcholine injection, suggesting impairment of central neurons of the baroreflex arc (24).

In old rats, the baroreflex control of RSNA was normal during loading of the baroreceptors but reduced during unloading, despite the fact that the baseline RSNA was similar in both groups. Hajduczok et al. (15) have reported that no correlation exists between the decrease in reflex activity and baseline activity in aging. Both blunted (35) and maintained (25) baroreflex function with aging have been reported in rats. In anesthetized animals administered phenylephrine and nitroprusside, Tanabe and Bunag (35) observed blunted baroreflex control of HR and splanchnic sympathetic nerve activity in 9-mo-old compared with 2-mo-old Sprague-Dawley rats. Kurosawa et al. (25) also found similar reflex attenuation of adrenal sympathetic nerve activity with phenylephrine in 4- vs. 26-mo-old Wistar rats. Tanabe and Bunag (35) and Kurosawa et al. (25) recorded the sympathetic activity of different regions, so these results may reflect regional selectivity in age-related changes in sympathetic output. Furthermore, the use of anesthetics may have affected the results (1). In conscious old rats, we consistently observed significant impairment of baroreflex control of RSNA in response to decreased MAP. In addition, the reduced spontaneous changes in RSNA (inversely related to spontaneous changes in MAP) may reflect impaired baroreflex control, even within the normal range of MAP variation. This may contribute to the hypotension that occurs in elderly human subjects when they assume an upright position (3).

Our data demonstrate impaired baroreflex control of HR and RSNA in aged rats. The normal bradycardia produced in old rats (10), with electric stimulation of the vagal nerve as well as acetylcholine injection, supports the hypothesis that the impaired cardiac-vagal baroreflex control in aged rats does not reflect dysfunction in the efferent branch of the reflex arc. Therefore, changes in the sensory branch of the baroreflex arc should be considered. Previous studies from our laboratory have shown a normal pressure-discharge relationship when all pressure values from threshold to saturation are considered, but a slightly decreased gain sensitivity in response to −10 mmHg (18). Although these data may explain changes in baroreflex control within the physiological range of AP variation (±10 mmHg), the impairment of the reflex responses observed with large (±40 mmHg) AP changes (25, 37) may indicate impairment of the central components of the baroreflex.

In conclusion, changes in baroreflex control of HR and RSNA are likely related to alterations in peripheral baroreceptors and/or central neurons of the reflex pathways. These changes reflect physiological adaptations associated with the aging process and may influ-
ence the effectiveness of blood pressure control in different physiological and pathophysiological conditions. The latter is exemplified by the increased variability in AP (34) as well as myocardial infarction (11) that have been observed in situations in which basal AP values have remained unchanged. Moreover, the reduced RSNA reflex response to unloading of the baroreceptors in aged rats may contribute to the impairment of the peripheral vascular responsiveness regulation observed during hypovolemia in aging (5).

Perspectives

The age-related changes in baroreflex control of HR and RSNA are likely to have important implications in cardiovascular regulation. They may contribute to decreases in the HR variability as well as to the reduced effectiveness of sympathetic nervous system-mediated reflex responses observed in older individuals during postural maneuvers (12, 26, 34).

In the present study, we have also shown that the IHR was reduced while the resting HR was similar in aged compared with young rats, probably due to the reduced vagal function in aged rats. Indeed, changes in mechanisms that regulate HR may be related to age-reduced HR increases to orthostatic stress or to acute increments in AP (26, 34, 37), preserving the cardiac output by avoiding reduction of the diastolic filling time (12, 26).

In previous studies, we have shown that exercise training does not improve baroreflex control of HR in old rats (6). The different responses observed in aged individuals probably represent long-term physiological adaptations to the limitations associated with the aging process, maintaining efficient cardiovascular function.

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