Alterations in the Secretion of Atrial Natriuretic Factor in Atria From Aged Rats

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We measured plasma atrial natriuretic factor levels and atrial natriuretic factor secretion by isolated left atria from aging rats to determine the secretory response to stretch and adrenergic stimulation. Systolic arterial pressure and right atrial pressure were measured in vivo. Twenty-four hours later, atria were removed and studied in vitro in a perfusion system. After removal, stabilization at 0.7 g tension, and equilibration for 65 minutes, atria were stretched by increasing external tension for 20 minutes. After reequilibration atria were perfused with phenylephrine, $10^{-7}$ M, for an additional 30 minutes. Right atrial pressure was not different between young (3 months) and aged (16–24 months) rats. Aged rats had higher plasma atrial natriuretic factor levels (52±8 versus 21±6 pmol/l; $p<0.05$) than young rats. Basal atrial natriuretic factor secretory rate in vitro was greater in atria from aged rats than young rats (875±35 versus 402±22 pg/min; $p<0.05$). Atria from aged rats had an increased response to phenylephrine compared with young rats (1,687±143 versus 788±113 pg/min; $p<0.05$) when means were adjusted for basal secretory rate. The secretory response to stretch was less than that of young rats (673 ±37 versus 773±27 pg/min), although this difference was not significant ($p=0.07$). Atrial natriuretic factor secretion in response to adrenergic stimulation is increased with aging, and these secretory responses may contribute to increased plasma levels that occur during aging. In contrast to increased adrenergic responses, atrial natriuretic factor secretion after external stretch is not increased in aging rats. (Hypertension 1992;20:85–88)

**KEY WORDS** • atrial natriuretic peptides • age factors • phenylephrine • rat studies

Plasma levels of atrial natriuretic factor (ANF) are elevated in healthy elderly people compared with young individuals, but the factors responsible for these increased levels have not been determined. Previous data have suggested that direct alterations in atrial dimension play an important role in the regulation of ANF secretion. A number of clinical conditions such as essential hypertension, congestive cardiomyopathy, chronic renal failure, rapid volume expansion, and tachycardia syndromes are associated with increases in both atrial pressure and ANF secretion. This suggests that direct atrial stretch can stimulate ANF secretion. In addition, secretion of ANF is greatly increased by adrenergic stimulation in vitro. To evaluate changes in atria that occur with aging and their possible contributions to altered ANF secretion, we measured changes in ANF secretion in atria from aging rats to determine the secretory response to stretch and adrenergic stimulation.

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Supported by a fellowship support grant from the Hartford Foundation, by grant HL-18575 from the National Heart, Lung, and Blood Institute, and by support from the American Heart Association and its Indiana Affiliate, Inc. J.D. performed this work during the tenure of a Clinician-Scientist Award from the American Heart Association.

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Received October 8, 1990; accepted in revised form February 28, 1992.

**Methods**

Sixteen 3-month-old rats (Fisher 344 line, Harlan Sprague Dawley Inc., Indianapolis, Ind.) weighing 266±10 g, five 16-month-old rats (Fisher 344 line, Harlan Sprague-Dawley Inc., National Institute of Aging contract colony obtained from Dr. Bennett Cohen) weighing 461 ±22 g, and five 24-month-old rats (Fisher 344 line, Harlan Sprague Dawley Inc., National Institute of Aging contract colony) weighing 405 ±14 g were studied. They were placed on a diet consisting of low sodium rat chow (ICN Biomedicals, Costa Mesa, Calif.) and given 1% saline in place of drinking water 5 days before study. One day before the in vitro experiment, systolic arterial pressure was measured via the tail-cuff method using a programmed electrosphygmomanometer (model PE300, Narco BioSystems, Austin, Tex.). Right atrial pressure was then measured by a fluid-filled catheter placed into the atrium via the external jugular vein while the animal was anesthetized with sodium pentobarbital. A Gould Statham physiological pressure transducer (model P23ID, Vigo-Spectramed, Oxnard, Calif.) was used for these measurements. Pentobarbital dosage was 50 mg/kg for young rats and 35 mg/kg for aged rats. On the day of the in vitro experiment, rats were decapitated, and trunk blood was collected in tubes containing ethylenediaminetetraacetic acid–sodium. Plasma was stored at −70°C. The heart was removed and placed in ice Medium 199 (GIBCO Laboratories, Grand Island, N.Y.), and the left atrium was isolated. Atria were suspended at 0.7 g tension between an electrode and a force displacement trans...
ducer (model FT03, Grass Instruments, Quincy, Mass.) in a superfusion system described by Schiebinger and Linden and allowed to stabilize at 37°C for 65 minutes during perfusion with Medium 199 at a rate of 2 ml/min. Components of the apparatus included a multifunction solid state square wave stimulator (model S88, Grass Instruments), a stimulus isolation unit (model SIU5, Grass Instruments), a polygraph (model 7D, Grass Instruments), a heating bath/circulator (model D1-L, Haake Inc., Karlsruhe, FRG), a peristaltic pump (model 1203, Harvard Apparatus, South Natick, Mass.), and a fraction collector (Retriever II, ISCO Inc., Lincoln, Neb.). Atria were paced at a rate of 1 Hz for 30 minutes and 3 Hz for 35 minutes during the stabilization phase and at 3 Hz for the remainder of the experiment. Perifusate was collected at 2-minute intervals throughout the experimental protocol. A 10-minute basal collection period was followed by 20 minutes of stretch induced by increasing the external force applied to the atria to 1.7 g. After this procedure the force was returned to 0.7 g. After a 20-minute recovery period the atria were perfused with phenylephrine, 10⁻⁷ M, and propranolol, 10⁻⁶ M, for 30 minutes.

Perifusate samples and plasma were measured for immunoreactive ANF by radioimmunoassay. In that there were no significant differences between 16- and 24-month-old rats in right atrial pressure, plasma ANF levels, basal ANF secretory rate, and secretory rate during stretch and phenylephrine phases of the perfusion experiment, data from 16- and 24-month-old rats were grouped together and regarded as "aged" rats. Perifusate was analyzed for ANF in five basal samples, five samples taken between 6 and 16 minutes of external stretch, five samples from the last 10 minutes of the 20-minute reequilibration phase, and five samples from the last 10 minutes of the 30-minute phenylephrine phase. The samples from each phase were assayed and their values expressed as picograms ANF per minute. Results are expressed as mean±SEM. Comparisons within groups were performed by paired t test, and those between groups used an unpaired t test. Analysis of variance was used when data were divided into three groups. When more than one comparison was made, Bonferroni protection was used. Comparisons between groups for data from the perfusion experiment were done by analysis of covariance. Significant covariates were determined and included in the model. Because of technical problems with the radioimmunoassay, data are unavailable for plasma ANF levels in seven animals and for the phenylephrine phase of the in vitro study in two animals. Right atrial pressure was measured in 15 of 16 young rats, in four of five 16-month-old rats, and in all 24-month-old rats.

Results

Right atrial pressure was not different among the three groups (Figure 1). Right atrial pressure was 5.9±0.2 mm Hg in young rats, 6.3±0.3 mm Hg in 16-month-old rats, and 5.6±0.2 mm Hg in 24-month-old rats (p=NS). Arterial pressure was 125±2 mm Hg in young animals, 122±2 mm Hg in 16-month-old rats, and 101±1 mm Hg in 24-month-old rats (p<0.05 compared with 3- and 16-month-old rats). Plasma levels of ANF were greater in aged rats compared with young rats. Three-, 16-, and 24-month-old rats had plasma levels of 21±6, 51±8, and 53±16 pmol/l, respectively (p<0.05; Figure 2).

Atria from aged rats secreted significantly higher basal amounts of ANF during the perfusion experiment than atria from young rats. Three-, 16-, and 24-month-old rats had a basal secretory rate of 402±22, 830±47, and 920±51 pg/min, respectively (p<0.05, young versus 16-month-old and young versus 24-month-old rats). During the equilibration phase, atria from young rats relaxed to a greater degree from the originally applied external tension of 0.7 g than atria from aged rats (0.45±0.02 versus 0.56±0.04 g when basal samples were being collected; p<0.05). However, there was no correlation (r=0.32, p=NS by F test) between the amount of tension applied and the ANF secretory rate.

Among the variables analyzed (basal secretory rate, rat weight, atrial weight, right atrial pressure, and systolic arterial pressure), only basal secretory rate was found to be a significant determinant of ANF secretion throughout the protocol. Therefore, data from the stretch and phenylephrine phases are expressed as means after adjustment for basal secretory rate.

External stretch and phenylephrine each resulted in a significant increase in ANF secretion in both groups

Figure 1. Scatterplot shows distribution of right atrial pressure. There was no significant difference in right atrial pressure between young and aged rats.

Figure 2. Scatterplot shows distribution of plasma levels of atrial natriuretic factor (ANF). Higher plasma levels of ANF were found in 16- and 24-month-old rats than in young rats. *p<0.05 compared with 3-month-old rats.
Bar graph shows atrial natriuretic factor (ANF) secretory rates with stretch (solid bars) and phenylephrine (open bars) expressed as means after adjustment for basal secretory rate. *p<0.05 compared with 3-month-old rats.

(p<0.05 for each stimulus). During stretch, atria from 3-month-old rats had a secretory rate of 560±29 pg/min compared with 987±53 pg/min for 16-month-old and 1,042±63 pg/min for 24-month-old rats. Atria from aged rats showed a smaller increase in ANF secretion with stretch than atria from young rats after means were adjusted for basal secretory rate. Atria from 3-, 16-, and 24-month-old rats had a rate of 775±27, 697±44, and 643±48 pg/min, respectively, during stretch (p=0.13, Figures 3 and 4). When data from 16- and 24-month-old rats were combined and compared with data from 3-month-old rats, the ANF secretory response to stretch with aging was 673 ±37 versus 773 ±27 pg/min for young rats (p=0.07). Since mean basal tension was lower in atria from young rats, the net increase in tension was greater in atria from young rats (0.75±0.05 g increase versus 0.64±0.02 g for aged rats), but this difference was not significant. There was no correlation between change in ANF secretory rate and change in applied tension (r=0.21, p=NS by F test). During the time samples were collected in the stretch phase, the amount of tension on atria from all three groups was nearly equal (1.19±0.06, 1.16±0.05, and 1.23±0.05 g for 3-, 16-, and 24-month-old rats, respectively, as atria from all groups continuously relaxed from the initial tension of 1.7 g).

In contrast to the response to stretch, atria from aged rats demonstrated a greater secretory response to phenylephrine than those of young rats. Atria from 3-month-old rats had a secretory rate of 630±37 pg/min compared with 1,878±111 and 1,938±93 pg/min for 16- and 24-month-old rats, respectively. When adjusted for basal secretory rate, atria from 3-, 16-, and 24-month-old rats had a rate of 789±116, 1,693±176, and 1,678±193 pg/min, respectively (p<0.05, Figures 3 and 4).

Discussion

Many studies have documented increased plasma levels of ANF in healthy elderly humans compared with young individuals.1-5 Possible explanations for this finding include chronic volume overload and impaired renin-angiotensin-aldosterone system activity.16 There may also be a redistribution of blood volume resulting in increased atrial filling, as demonstrated by increased atrial volume in the elderly.17 In addition, due to loss of β-adrenergic sensitivity with increasing age,18 peripheral resistance and cardiac afterload may be increased. A relative tachycardia in the elderly could also contribute to elevated plasma levels as would increased ANF secretory responses to stretch or other stimuli. In addition a decrease in metabolic clearance rate in older individuals is also possible.

In the present study, aged rats had greater plasma ANF levels and an increased ANF secretory response to phenylephrine in vitro when compared with those in young rats. These results suggest that rats maintain an adequate, if not increased, ability to secrete ANF with normal aging. Since plasma ANF levels are elevated with aging in humans, aging rats appear to be an appropriate experimental model for physiological studies involving changes in ANF secretion with age.
The increase in plasma ANF with age may be due to increased responsiveness to catecholamines by atria of aging rats. This appears to be true in vitro as evidenced by increased responsiveness to phenylephrine. The effect of phenylephrine in the present study was unrelated to chronotropic stimulation since atrial rate was maintained constant by external pacing throughout the study. Although chronic sodium retention could also explain elevated ANF levels with aging, there was no difference in right atrial pressure to suggest this possibility. In addition, arterial pressure was equal or lower in aged rats, thereby excluding increased afterload as an explanation.

A role for adrenergic stimuli in maintaining increased plasma ANF levels with aging is also suggested by the fact that plasma norepinephrine levels are elevated in fact in older individuals. This may increase ANF secretion in the elderly through a heart rate-dependent mechanism or in a rate-independent manner, the latter being analogous to secretion stimulated by phenylephrine in vitro involving direct activation of cardiac adrenergic receptors. Since plasma catecholamines were not measured in our experiments, it is not possible to determine their role in increasing plasma ANF levels with aging.

Sanfield and his group reported that the ANF secretory response to epinephrine infusion in elderly humans is impaired compared with their young counterparts. Although it is not known exactly how epinephrine increases ANF secretion, we speculate that at the low doses of epinephrine used in that study, secretory responses may have been mediated through hemodynamic rather than direct effects. If this were true, that result is consistent with the present study in which stretch was a less effective stimulator of ANF secretion in older individuals.

Stretch-induced ANF secretion was decreased in atria from aged rats compared with young rats. Although this difference only approached statistical significance, the decreased response to stretch stimulation was in striking contrast to the increased secretory response to phenylephrine. Thus, there appears to be a dissociation of secretory responses with normal aging. This finding may be explained by previously reported observations that the stretch-secretion coupling mechanism is different and independent from other secretory coupling mechanisms. Known second messenger systems such as phosphatidyl inositol bisphosphate, protein kinase C, and cyclic adenosine 3',5' monophosphate, which are mechanisms of adrenergic stimulus-response coupling, do not appear to play a role in stretch-mediated secretion.

If the increase in plasma ANF levels observed in the elderly plays a significant role in mitigating volume expansion or sodium retention that occurs in some aging individuals, then this increased secretion may be important in preventing or limiting elevations in blood pressure in these patients. Diminished secretory responses to stretch may limit the effectiveness of this compensatory response.

The increased ANF secretory response to adrenergic stimulation in vitro may indicate the mechanism behind elevated plasma levels of ANF with aging. Whereas most studies have suggested that stretch is a major stimulus for ANF secretion in vivo, the results in our study show that adrenergic stimulation may play an increasing role in the regulation of ANF secretion with normal aging.

Acknowledgments

We thank Dr. Bennett Cohen for his advice and for his assistance in acquiring aged rats. We also thank Dr. Anthony Schork for providing his expertise in analyzing our data. In addition, we would like to thank Richard Sider, Trina Chen, and Marie Kung for technical assistance.

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Alterations in the secretion of atrial natriuretic factor in atria from aged rats.
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Hypertension. 1992;20:85-88
doi: 10.1161/01.HYP.20.1.85

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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