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 perifusion 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, $10^{-4}$ 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,1-5 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.6 A number of clinical conditions such as essential hypertension,7 congestive cardiomyopathy,8 chronic renal failure,9,10 rapid volume expansion,11,12 and tachycardia syndromes13 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.14 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.

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 $-70^\circ$C. The heart was removed and placed in iced 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-
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, young versus 16-month-old and young versus 24-month-old rats). During the equilibration phase, atri from young rats relaxed to a greater degree from the originally applied external tension of 0.7 g than atri 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.

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).

Atri from aged rats secreted significantly higher basal amounts of ANF during the perifusion experiment than atri 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, atri from young rats relaxed to a greater degree from the originally applied external tension of 0.7 g than atri 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 3. 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 expansion due to occult congestive heart failure or impaired renal sodium excretion in the elderly. The latter possibility would be consistent with the observation that older individuals have decreased plasma renin and aldosterone levels. 16 There may also be a central 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.

FIGURE 4. Line plot shows atrial natriuretic factor (ANF) secretion by perfused rat atria over time. Atria from 16-month-old (small squares) and 24-month-old (circles) rats showed a greater increase in ANF secretion in response to phenylephrine than did atria from 3-month-old rats (large squares). Responses to stretch were not significantly different between the three groups. *p<0.05 compared with young (3-month-old) rats.
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 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.

Sanfeld 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 between secretory responses with normal aging. This finding may be explained by previously reported stretch 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.

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.

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13. Rankin AJ, Courneya CA, Wilson N, Ledsme JC: Tachycardia releases secretory responses with normal aging. This finding may be explained by previously reported observations that the secretory coupling mechanism is different and independent from other stretch-induced secretion mechanisms. Known second messenger systems such as phosphatidylinositol bisphosphate, protein kinase C, and cyclic adenosine 3',5' monophosphate, which are mechanisms of adrenergic stimulation—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.
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