The vasodilator potency of human atrial natriuretic factor-(99-126) was investigated in the forearm vascular bed of 10 young and 10 elderly normotensive volunteers with venous occlusion strain gauge plethysmography. Atrial natriuretic factor was infused at six increasing dose steps into the brachial artery from 0.001 up to 0.3 μg/min/100 ml of forearm volume. This induced a mean±SEM increase in blood flow from 1.4±0.2 up to 6.0±1.0 ml/min/100 ml in the young and from 1.4±0.2 up to 3.9±0.6 ml/min/100 ml in the elderly. The dose-response curves of forearm blood flow and of forearm vascular resistance after increasing infusion rates of atrial natriuretic factor were shifted to the right in the elderly when compared with the young subjects. The mean percent decrease in forearm vascular resistance, induced by atrial natriuretic factor, during this dose-response curve averaged −31±3% in the elderly versus −56±3% in the young subjects (p=0.0002). The calculated forearm spillover of the second messenger of atrial natriuretic factor, cyclic guanosine monophosphate, significantly increased from baseline values of 1.2±1.1 and 0.7±0.5 pmol/min/100 ml in young and elderly subjects, respectively, up to 23.2±5.0 and 30.5±7.0 pmol/min/100 ml during the highest dose of atrial natriuretic factor (both p<0.01 versus baseline). There were no significant differences in the increments of the forearm spillover of this second messenger between both age groups. Further, both age groups demonstrated an equal vasodilator response to increasing infusion rates of the control vasodilator sodium nitroprusside, with a maximal decrease in forearm vascular resistance of 65±3% and 61±2% in the young and elderly group, respectively. This study demonstrates that advancing age in humans is accompanied by a specific reduction in vascular responsiveness to atrial natriuretic factor, without evidence for a diminished response of its second messenger. Therefore, we hypothesize that changes in postreceptor processes are involved in the age-related reduction in vascular sensitivity for atrial natriuretic factor. (Hypertension 1991;18:640–647)

Attenuated Forearm Vasodilator Response to Atrial Natriuretic Factor in the Elderly

Tim L.Th.A. Jansen, Paul Smits, Adriaan C.I.T.L. Tan, and Theo Thien

Atrial natriuretic factor (ANF) comprises a family of peptides of which the 28-amino acid ANF-(99–126), called human ANF-(99–126), is the major circulating form.1 ANF is considered to contribute importantly to the control of circulation homeostasis at physiological and pathophysiological conditions. One might wonder whether sensitivity to ANF changes during chronic exposure to elevated ANF levels in vivo. Contradictory reports have been published on the in vitro density of vascular binding sites after exposure to elevated ANF levels in rats. A group of individuals continuously exposed to elevated ANF levels are elderly subjects.6,7 Therefore it is of interest to know whether sensitivity to ANF has decreased in healthy elderly subjects. In vitro studies in rats clearly demonstrate that vascular smooth muscle relaxation induced by ANF is reduced with advancing age.8 Up to now, however, the few studies in this area were difficult to interpret for two reasons: 1) systemic administration of ANF yielded higher ANF levels in the elderly because of differences in the total body clearance of ANF between young and elderly, and 2) observed differences in the response to systemic ANF infusion may be explained by an altered cardiovascular reflex activity in aged subjects rather than by changes in vascular sensitivity to ANF.9–11 To avoid such difficulties in the interpretation of results, we compared the in vivo vascular sensitivity to ANF in young and elderly subjects in the local vascular bed of the forearm, and we hypothesized that the high baseline plasma ANF levels in the elderly are accompanied by a reduced forearm vascular sensitivity to ANF. Apart from the vasodilator
response to ANF, the forearm production of ANF's second messenger cyclic guanosine monophosphate (cGMP) was studied in both groups. To enable exclusion of a nonspecific loss of vascular sensitivity with advancing age, the vasodilator response to ANF was compared with the response to the control vasodilator sodium nitroprusside (SNP).

Methods

Subjects

The study protocol was previously approved by the ethics committee of our institute. Two groups of healthy young \((n = 10)\) and elderly \((n = 10)\) volunteers, recruited from the general population, were studied. Before participation, informed written consent was obtained from each subject. All subjects underwent clinical examination before admission to the study. Physical examination revealed no relevant abnormalities. The two groups were matched for blood pressure, which was noninvasively measured at screening examination. None of the subjects smoked, took any drugs, oral contraceptives included, or abused alcohol. Further, the subjects were instructed to eat their usual diet, not to drink any coffee or tea, and to refrain from hard labor during the 24 hours preceding the experiment.

Design of the Study

The study was performed during a 3-hour immobilization period in a climatized room (temperature, 22.6±0.2°C; \(\pm\) =20). The subjects were allowed to eat a light meal, but not within 2 hours before the test. The tests were performed after an equilibration period of at least 45 minutes with subjects in the supine position. The left brachial artery was cannulated with a 20-gauge Angiocath cannula (Deseret Medical Inc., Becton Dickinson and Co., Sandy, Utah) for intra-arterial blood sampling and drug infusion (automatic syringe infusion pump, type STC-521, Terumo Corp., Tokyo) as well as for blood pressure monitoring (Hewlett Packard monitor, type 78353B, Hewlett Packard GmbH, Boblingen, FRG). On the ipsilateral side an antecubital vein was cannulated for blood sampling. Since the dosages of all drugs were calculated per 100 ml of forearm volume (FAV), we measured the FAV in all participants by water displacement. The subjects remained supine throughout the 3-hour study period.

During the study, systolic and diastolic blood pressure and mean arterial pressure were recorded intra-arterially. Heart rate was monitored continuously by an electrocardiographic registration. Forearm blood flow (FBF) was measured by venous occlusion plethysmography using mercury-in-rubber strain gauges. The circulation of the hand was completely excluded during all FBF measurements by inflating a wrist cuff to 100 mm Hg above the systolic blood pressure. All these parameters were measured during the several infusions of the study. The infusions started simultaneously with the inflation of the wrist cuff, and FBF recordings were started from 1 minute after starting the infusion until the end of the infusion, during which at least three FBF recordings per minute were obtained. During the last minute of each infusion rate, a minimum of 30 consecutive values of systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate were registered and averaged to one representative mean value for that time point.

After the equilibration period, the experiment started with the infusion of placebo for 5 minutes (placebo 1). After a 10-minute pause, during which the wrist cuff was deflated, the ANF infusions were started with six increasing infusion rates from 0.001 to 0.3 µg/min/100 ml FAV. Each dose was administered during 5 minutes, and two consecutive infusion rates were applied within one 10-minute period of measurements. Afterward, the wrist cuff was deflated again for a period of 10 minutes to allow recovery of the hand circulation. Thus, three 10-minute periods were needed to complete the full dose–response curve for ANF (0.001–0.003; 0.01–0.03; 0.1–0.3 µg/min/100 ml FAV).

Forty-five minutes after cessation of the highest ANF administration, a second period with infusion of placebo was performed for 5 minutes (placebo 2). Ten minutes afterward, SNP was infused in one session using three increasing infusion rates (10, 30, and 100 ng SNP/min/100 ml FAV, 4 minutes per dose). The sequence of administration of the several drugs (placebo, ANF, SNP) was blinded for the subjects.

Blood for determination of ANF was sampled in prechilled EDTA-tubes from the antecubital vein and from the brachial artery at the last minute of the first placebo infusion and at the highest ANF dosage. cGMP was determined in plasma from prechilled EDTA-tubes, sampled from the antecubital vein and from the brachial artery at the last minute of placebo infusion and at the fourth and sixth ANF dosage.

Laboratory Determinations

The method for ANF determination was described previously. A specific radioimmunoassay based on prior extraction of the plasma on Seppak C-18 columns was used. Sensitivity of the assay was 0.8 pg/tube (corresponding to 4 pg/ml). Recovery averaged 96%; intra-assay and interassay coefficients of variation were 8.6% and 11.6%, respectively. Normal values in 66 healthy volunteers ranged from 8.3 to 86.8 pg/ml (mean±SD, 35.3±16.2 pg/ml).

Plasma cGMP concentrations were measured by radioimmunoassay as described previously.

Drugs

Human ANF-(99–126) was purchased from Bisendorf Peptide GmbH, Wedemark, FRG. Since we previously observed that adhesion of ANF to the Silastic infusion system was greatly reduced when ANF was dissolved in Haemaccel (Hoechst, Behring-Werke AG, Marburg, FRG) instead of sa-
line, we diluted ANF in Haemaccel immediately before use. Consequently, pure Haemaccel was used during the placebo 1 period. SNP was purchased from Hoffmann-La Roche, Mijdrecht, The Netherlands, and dissolved in glucose 5% solution just before use. Consequently, placebo 2 consisted of a pure glucose 5% solution.

Data Report

All results are expressed as mean±SEM, unless indicated otherwise. During the last 3 minutes of each dose, when steady-state effects were obtained, FBFs were averaged. These data were used to calculate absolute and percent changes from the preceding placebo values. The FVR was calculated as the quotient of mean arterial pressure and FBF and was expressed in arbitrary units (AU). Forearm extraction of ANF was calculated as the quotient of the arteriovenous difference in ANF plasma levels and the arterial ANF plasma concentration and was expressed as a percent. The local forearm spillover of cGMP was calculated as the product of the arteriovenous difference in plasma cGMP and the FBF and was expressed in picomoles per minute per 100 milliliters FAoV. The spillover was considered to be a valuable measure of the regional production of cGMP.

Statistical Analysis

To analyze the response to ANF infusion within each group of subjects, the mean percent ANF-induced effects were compared with placebo by paired Wilcoxon test. To evaluate the difference in response between young and elderly subjects, the mean percent ANF-induced change in FVR of the complete dose–response curve was calculated for both groups, and subsequently they were compared with each other by the unpaired Wilcoxon test. For correlations between parameters, the Pearson correlation coefficient was calculated. Significance was accepted at the 0.05 level of probability (two-sided).

Results

Table 1 presents the characteristics of the subjects of both age groups. Apart from the difference in age due to selection, the groups differed significantly (p<0.05) in mean arterial pressure and in the endogenous creatinine clearance, which was calculated from serum creatinine levels via the formula of Cockcroft and Gault. The latter finding can entirely be ascribed to the physiological decrease in renal function with age. The blood pressure levels presented in Table 1 concern measurements performed with a mercury sphygmomanometer in the supine position at the moment of screening.

Hemodynamic Effects

Table 2 presents steady-state hemodynamic parameters during each last minute of infusion of the placebo and the several ANF and SNP doses. Neither intra-arterial administration of ANF nor that of SNP induced a significant change in blood pressure or heart rate when compared with its preceding placebo value. Overall, blood pressure but not heart rate increased from the first to the second placebo infusion, but as shown in Table 2, this increase occurred during the equilibration period after ANF infusion rather than during the infusion experiments. The changes in blood pressure throughout the experiments were similar in both age groups.

Figure 1 shows the course of the mean FBF in relation to the time periods of ANF and SNP administration. FBF gradually increased from 1.4±0.2 during placebo to 6.0±1.10 ml/min/100 ml FAoV during the highest ANF infusion rate in the young subjects with corresponding values of 1.4±0.2 and 3.9±0.6 ml/min/100 ml FAoV in the elderly subjects. The administration of SNP induced comparable increments in FBF from 1.7±0.3 during placebo to 5.1±0.8 ml/min/100 ml FAoV during the highest SNP infusion in the young subjects and from 1.5±0.1 during placebo to 4.1±0.4 ml/min/100 ml FAoV during the highest SNP infusion in the aged subjects.

Figure 2 presents dose–response curves of the percent decrease in the calculated FVR, as induced by ANF administration on the left panel and by SNP administration on the right panel. At the lowest infusion rate, ANF failed to lower the FVR in the aged subjects, whereas the young showed an obvious response to this dosage. During the six ANF infusion rates the mean percent decrease in FVR measured 36±3% in the young versus 31±3% in the elderly group (p=0.0007). In contrast, the mean fall in FVR after the three SNP infusion rates was not different between the young and elderly groups (young versus elderly, 45±2% versus 40±3%, p>0.1).

Humoral Effects

The numbers for determination of ANF and cGMP levels were reduced, either because of insuf-
TABLE 2. Steady-State Hemodynamic Parameters During Each Last Minute of Infusion of the Placebo and the Several Atrial Natriuretic Factor and Sodium Nitroprusside Doses

| Infused drugs | Young | | Elderly | | | | | | | |
|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|               | SBP   | DBP   | MAP   | HR     | FBF   | FVR   |
|               | (mm Hg) | (mm Hg) | (mm Hg) | (beats/min) | (ml/min/100 ml) | (AU) |
| Young Placebo | 116±4 | 64±2 | 83±3 | 64±3 | 1.4±0.2 | 64±5 |
| ANF dose (µg/min/100 ml FAV) | | | | | | |
| 0.001 | 119±3 | 64±2 | 83±3 | 62±3 | 2.1±0.3 | 44±5 |
| 0.003 | 118±3 | 65±2 | 84±3 | 62±3 | 2.8±0.4 | 35±5 |
| 0.01 | 119±3 | 69±4 | 87±3 | 64±3 | 3.3±0.5 | 30±3 |
| 0.03 | 119±3 | 67±3 | 87±3 | 65±3 | 4.3±0.7 | 23±3 |
| 0.1 | 121±4 | 68±3 | 87±4 | 63±3 | 5.1±0.8 | 20±3 |
| 0.3 | 119±3 | 68±3 | 87±3 | 65±3 | 6.0±1.0 | 18±3 |
| Placebo 2 | 127±3 | 72±2 | 93±3 | 63±3 | 1.7±0.3 | 64±8 |
| Elderly Placebo | 143±5 | 65±2 | 95±3 | 64±3 | 1.4±0.2 | 75±9 |
| ANF dose (µg/min/100 ml FAV) | | | | | | |
| 0.001 | 143±5 | 66±3 | 96±3 | 62±3 | 1.5±0.2 | 75±9 |
| 0.003 | 141±4 | 65±2 | 95±3 | 62±3 | 1.7±0.3 | 65±8 |
| 0.01 | 145±6 | 66±2 | 97±4 | 64±3 | 2.1±0.3 | 50±5 |
| 0.03 | 146±6 | 68±3 | 98±3 | 62±3 | 2.3±0.3 | 49±6 |
| 0.1 | 147±5 | 67±2 | 100±4 | 63±3 | 3.0±0.5 | 37±4 |
| 0.3 | 145±6 | 67±2 | 97±3 | 63±3 | 3.9±0.6 | 30±5 |
| Placebo 2 | 149±6 | 70±2 | 102±4 | 64±3 | 1.5±0.1 | 71±7 |
| SN10 dose (ng/min/100 ml FAV) | 10 | 129±3 | 73±2 | 94±3 | 63±3 | 2.4±0.4 | 46±5 |
| 30 | 130±3 | 75±2 | 95±2 | 65±3 | 3.2±0.6 | 37±4 |
| 100 | 128±3 | 74±2 | 95±3 | 66±3 | 5.1±0.8 | 22±3 |

Values are mean±SEM of steady-state hemodynamic parameters calculated from measurements during the fourth to the fifth minute of intra-arterial administration of placebo 1 (Haemaccel) followed by atrial natriuretic factor and of placebo 2 (5% glucose solution) followed by sodium nitroprusside in a group of young (n=10) and elderly (n=10) volunteers. Blood pressure parameters were measured intra-arterially.

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; FBF, forearm blood flow; FVR, forearm vascular resistance; AU, arbitrary units; ANF, atrial natriuretic factor; SNP, sodium nitroprusside; FAV, forearm volume.

ficiency of plasma quantities (×3) or because of hemolysis of the plasma sample (×2). Baseline ANF levels, measured during the first placebo infusion, were elevated in the elderly when compared with the young subjects: arterial plasma concentrations 45.8±2.7 versus 21.1±2.7 pmol/l (p<0.001), and venous plasma concentrations 22.1±1.4 versus 11.9±1.5 pmol/l (p=0.02). After the highest ANF infusion rate clear increments in the ANF levels were observed, both in elderly and young subjects, leading to arterial plasma levels of 763±110 pmol/l and 644±260 pmol/l and to venous plasma levels at the side of intra-arterial infusion of 24,620±2,570 pmol/l and 14,040±2,940 pmol/l. Forty-five minutes after interruption of ANF administration, during the second placebo infusion, the ANF levels in the venous effluent were sharply decreased and no longer differ-

FIGURE 1. Graph shows time course of forearm blood flow (FBF) before, during, and after the infusion of six dose steps of atrial natriuretic factor (ANF) and of three dose steps of sodium nitroprusside (SNP) in young (n=10) and elderly (n=10) subjects. Mean±SEM. P1, placebo 1 (Haemaccel); P2, placebo 2 (glucose 5%). FAV, forearm volume.
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AFVR (Δ)
-20
-40
-60
p = 0.0002

Baseline

0 0.003 0.03 0.3
ANF infusion rate (μg/min/100 ml FAV)

10 30 100
SNP infusion rate (ng/min/100 ml FAV)

FIGURE 2. Line graphs show dose–response curves of the percent changes (Δ) in forearm vascular resistance (FVR) induced by cumulative intra-arterial infusion of atrial natriuretic factor (ANF) (left panel) and of sodium nitroprusside (SNP) (right panel) in young (n = 10) and elderly (n = 10) subjects. Mean ± SEM. Probability value indicates the level of significance obtained by comparing the means of both curves. NS, not significant; FAV, forearm volume.

ent from baseline: 46.7±3.4 versus 29.3±3.2 pmol/l in elderly and young subjects, respectively.

During the first placebo infusion, the forearm ANF extraction was calculated from arterial and venous plasma ANF concentrations. ANF extraction was 43±5% in the young and 48±8% in the elderly. When corrected for forearm volume, ANF extraction was 4.2±0.6%/100 ml FAV and 5.4±0.8%/100 ml FAV, respectively. Between young and elderly no significant difference in forearm ANF extraction was observed.

As was shown in Figure 2, the vasodilator response to ANF was reduced in the elderly. When the individual vasodilator responses to ANF from both young and aged volunteers were plotted against the baseline plasma ANF concentrations, there appeared to be a significant correlation between these variables. As expected, this correlation was most pronounced at the lowest ANF infusion rate (0.001 μg/min/100 ml FAV) because the difference in the vasodilator effect of ANF between both age groups was largest at that particular ANF infusion rate (Figure 2). Figure 3 shows the scatter diagram on the individual baseline ANF levels and the corresponding percent vasodilator response to the lowest ANF infusion rate in eight young and seven elderly subjects in which a basal forearm ANF level was obtained (n = 15, r = 0.79, p = 0.0005; linear regression, y = x - 49.7). When the mean forearm vasodilator response (calculated over all ANF dosages) was taken instead of the response to the lowest ANF infusion rate, there was still a significant correlation between the baseline ANF level and the response to ANF (n = 15, r = 0.72, p = 0.0025; linear regression, y = 0.7x - 67.4).

Table 3 demonstrates arterial and venous cGMP values in the young and elderly group. Venous cGMP values increased in the young from 14.1±1.3 nmol/l during placebo infusion up to 25.5±1.6 nmol/l at the highest ANF infusion rate. In the elderly, cGMP rose to significantly higher venous levels (from 15.0±0.9 nmol/l up to 31.8±1.7 nmol/l). Of course, to compare the local production of cGMP between both groups, the venous cGMP levels must be related to the local FBF, as has been done in the calculation of the cGMP spillover.

![Figure 3. Scatter diagram of individual baseline arterial plasma concentrations of atrial natriuretic factor (ANF) and the corresponding percent change (Δ) in forearm vascular resistance, induced by intra-arterial infusion of the lowest dose of ANF (0.001 μg/min/100 ml forearm volume) in eight young and seven elderly subjects.](http://hyper.ahajournals.org/)

![Table 3. Arterial and Venous Cyclic Guanosine Monophosphate Concentrations](http://hyper.ahajournals.org/)

<table>
<thead>
<tr>
<th>Infused drugs</th>
<th>Young (n=10)</th>
<th>p value</th>
<th>Elderly (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>12.6±0.8</td>
<td></td>
<td>14.5±1.0</td>
</tr>
<tr>
<td>Venous</td>
<td>14.1±1.3</td>
<td></td>
<td>15.0±0.9</td>
</tr>
<tr>
<td>ANF (0.03 μg/min/100 ml FAV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>13.8±0.8</td>
<td>&lt;0.01</td>
<td>15.8±1.0</td>
</tr>
<tr>
<td>Venous</td>
<td>17.5±1.2*</td>
<td></td>
<td>22.4±1.2*</td>
</tr>
<tr>
<td>ANF (0.3 μg/min/100 ml FAV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial</td>
<td>21.3±1.0</td>
<td>0.07</td>
<td>25.1±1.2</td>
</tr>
<tr>
<td>Venous</td>
<td>25.5±1.6*</td>
<td>&lt;0.01</td>
<td>31.8±1.7*</td>
</tr>
</tbody>
</table>

Values are mean±SEM of arterial and venous cyclic GMP concentrations (nmol/l) at the fifth minute of intra-arterial administration of placebo (Haemaccel) and atrial natriuretic factor in a group of young and elderly volunteers. ANF, atrial natriuretic factor; FAV, forearm volume. Probability values that were not significant are not demonstrated. *p<0.01 venous vs. arterial samples.
when compared with the young (Table 2). With sure level of the aged subjects appeared to be higher since, despite the well-matched blood pressures structural vascular changes. This is especially impor-
tant atherosclerotic changes, we checked whether forearm vascular bed is not very susceptible to age-
related atherosclerotic changes, we checked whether (p<0.01 versus placebo).
In the elderly, these values numbered 0.7±0.5 and 30.5±7.0 pmol/min/100 ml FAV (p<0.02 versus pla-
cebo). These ANF-induced increments in cGMP spillover from 1.2±1.1 at baseline to 23.2±5.0 pmol/min/100 ml FAV at the highest ANF infusion rate (p<0.01 versus placebo). In the elderly, these values numbered 0.7±0.5 and 30.5±7.0 pmol/min/100 ml FAV (p<0.02 versus placebo). These ANF-induced increments in cGMP spillover were not significantly different between the young and the elderly subjects.

Side Effects

During the local infusion of ANF into the brachial artery no hypotension, light-headedness, or bradycardia was observed. With regard to the diuretic effects of ANF none of the subjects had any urge to micturate during or directly after the immobilization period.

Discussion

In the present study, we observed elevated baseline levels of ANF in our group of healthy elderly subjects, and when compared with the young, these aged subjects obviously showed an attenuated forearm vasodilator response to ANF. Although the forearm vascular bed is not very susceptible to age-related atherosclerotic changes, we checked whether our selected elderly subjects showed any relevant structural vascular changes. This is especially important since, despite the well-matched blood pressures at screening (Table 1), the intra-arterial blood pressure level of the aged subjects appeared to be higher when compared with the young (Table 2). With respect to this item, it must be stressed that there were no differences in the vasodilator response to intra-arterial infusion of the control vasodilator SNP. Furthermore, it is important to realize that the highest dose of SNP induced trough values of FVR in the same range as after ANF, and therefore we think that within the applied range of ANF dosages, the currently observed age-related reduction in sensitivity for ANF cannot be attributed to structural forearm vascular changes in the elderly group. Consequently, aging seems to be accompanied by a specific reduction in vasodilator potency of ANF in humans. Until now, the human studies on ANF and aging were performed during systemic administration of ANF. As a result of the lower total body clearance of ANF in the elderly, similar doses of this peptide resulted in higher plasma ANF levels in those studies, complicating the interpretation of the results. Although in the present study baseline levels of plasma ANF were higher in the elderly when compared with the young, there were no differences in the baseline forearm extraction of ANF between the young and elderly subjects, suggesting that the handling of ANF across the forearm was similar in both groups. Consequently, the reduced vasodilator response to ANF in aged subjects seems to be of pharmacodynamic rather than of pharmacokinetic origin.

Despite relatively high systemic (arterial) plasma ANF levels, we did not observe a significant fall in blood pressure during the intra-arterial ANF infusion. This was probably due to the short infusion periods, never exceeding 5 minutes per dose. The absence of any significant drug-related fall in blood pressure or rise in heart rate demonstrates that the local vasodilator effects of ANF and SNP were not associated with systemic hemodynamic effects. Thus, the several procedures probably did not induce cardiovascular reflex activation. Therefore, it is unlikely that eventual differences in cardiovascular reflex activity between young and elderly subjects contributed to the observed differences in the vasodilator response to ANF. However, we should note that no time control studies were performed, and therefore it is not possible to exclude a systemic hemodynamic effect of ANF during the infusions.

Our human in vivo data are in agreement with the shifted dose–response curve of ANF-induced vasodila-
tion in aged rat thoracic aorta as demonstrated in vitro. Apart from the elderly, elevated endogenous ANF levels in humans have been found in several conditions, for instance in heart failure. Recently Hirooka et al showed that the forearm vasodilator response to intra-arterial ANF was attenuated in patients suffering from congestive heart failure. Combined with our current results, this suggests that the elevated plasma ANF levels rather than aging itself are responsible for the reduced vasodilator response to ANF. Observations on a reduced re-
sponse of mesentery artery smooth muscle cells to ANF after exposure to elevated ANF concentrations.
do also agree with this view. Although we realize that our results do not prove that baseline ANF concentrations have a primary role in reducing the responsiveness of the vascular system to ANF in the elderly, the significant correlation between the baseline plasma ANF concentrations and the vasodilator response to ANF as observed in our study may further argue for this hypothesis (Figure 3).

It has been demonstrated that even 24-hour exposure to elevated ANF levels induces a reduced response of mesentery artery smooth muscle cells to ANF. If this relatively short period also holds for the human situation, this may be relevant with respect to pharmacological agents that interfere with the endogenous ANF degradation such as the recently developed neutral metalloendopeptidase inhibitors. Furthermore, on account of the present results, it may be clinically relevant that the vasoactive effects of these neutral metalloendopeptidase inhibitors are expected to be less effective in aged subjects. Whether the physiological role of endogenous ANF becomes less important during aging remains to be established, especially since the plasma ANF levels in the venous effluent reached in our study are in the pharmacological rather than in the physiological range. However, the fact that the difference between young and aged subjects seems largest at the lowest infusion rate suggests that the reduced potency for ANF in the elderly may also be of relevance in the physiological range (Figure 2). From a theoretical point of view, a reduced responsiveness of the vascular wall to ANF in the elderly may contribute to the development of hypertension in these subjects. However, our data do not add much relevant information to this view because our elderly subjects were normotensive, and it would only be speculative to extrapolate the current results to aged hypertensive patients. Moreover, as a result of our experimental design with regional infusions of low dosages of ANF into the forearm vascular bed, the systemic blood pressure was not expected to show any change during the several infusions. Therefore in our study, systemic blood pressure must be considered as an irrelevant parameter with respect to this problem. An argument against an important role of the age-related fall in vascular responsiveness to ANF in age-related hypertension is the fact that the aged subjects have significantly elevated baseline plasma ANF concentrations. Together with a reduced vascular responsiveness to ANF, these elevated baseline plasma ANF concentrations may result in an ultimately unchanged ANF-mediated vascular tone in the elderly.

Theoretically, downregulation of the ANF receptor density is the most likely explanation for a reduced biologic response to ANF during states of elevated plasma ANF concentrations. Indeed, in vitro studies in rats have shown a reduced ANF-stimulated response of its second messenger cGMP during exposure to elevated ANF concentrations, and that may well reflect a lower ANF receptor density in those studies. However, we did not observe a reduced response of cGMP to ANF in the aged group (Figure 3). The ANF-induced increase in cGMP spillover was similar in the two groups during the two infusion rates. Within this context, it is interesting that a similar discrepancy between a reduced vasodilator response and cGMP production was found in patients with heart failure. Apparent, the elevated plasma ANF levels of aged subjects induce a defect in intracellular mechanisms that is located beyond the level of cGMP production and that reduces the transduction of the ANF receptor-mediated signal to the cellular response. Such an intracellular defect has also been hypothesized with respect to heart failure and may therefore be caused by the elevated plasma ANF levels rather than by the underlying (patho)physiological state. However, it should be noted that we did not study the intracellular mechanisms of ANF nor its transduction to biologic responses, and therefore the aforementioned interpretations still remain hypothetical.

In conclusion, short-term intra-arterial infusion of ANF induced acute increments in FBF. In aged subjects, the dose–response curve of the vasodilator effect of ANF clearly showed a significant shift to the right when compared with young volunteers, whereas the response to SNP was identical in both groups. No arguments were found for an ANF receptor downregulation with advancing age, since the production of ANF's second messenger cGMP was not diminished in the elderly when compared with the young subjects. Therefore we hypothesize that the age-related reduction in the vasodilator response to ANF is accompanied by alterations in postreceptor mechanisms.

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KEY WORDS • atrial natriuretic peptides • vascular reactivity • cyclic GMP • aging
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