Effects of Aging and Hypertension on Plasma Angiotensin II and Platelet Angiotensin II Receptor Density

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Plasma renin activity (PRA) declines with age in normal individuals, but the effect of age on plasma angiotensin II (ANG II) is less clear. A decline in plasma ANG II with age could result in altered platelet ANG II receptor density since plasma hormone levels influence their target organ receptors. To investigate this possibility, PRA, plasma ANG II, and platelet ANG II receptor density were examined in 17 young, 12 middle-aged, and 14 elderly healthy normotensive volunteers. To assess whether hypertension altered receptor density, these variables were also examined in 23 hypertensive patients.

In normotensives, there was a negative correlation between age and PRA ($r = -0.43$, $P < .05$), no significant change in basal plasma ANG II with age, and a weak positive correlation between age and ANG II receptor density ($r = 0.34$, $P < .05$). Multiple regression analysis revealed that the relationship between age and ANG II receptor density was independent of the associated rise in mean arterial pressure with age ($P < .05$). Platelet ANG II receptor density was not significantly related to PRA or plasma ANG II. ANG II receptor affinity did not change with age. Neither PRA nor ANG II receptor density or affinity differed between hypertensives and normotensives of similar mean age, but plasma ANG II was significantly lower in hypertensives compared with normotensives.

We concluded that aging is associated with a decline in supine PRA. The small decrease in plasma ANG II was not significant. Platelet ANG II receptor density increased with age primarily due to a small group of elderly subjects with elevated receptor density. There was no change in ANG II receptor density or affinity in hypertensives despite apparently lower plasma ANG II in these patients. Am J Hypertens 1992;5:687–693.

KEY WORDS: Angiotensin II, platelet receptors, aging, hypertension.

Plasma renin activity declines with age in normal individuals.[1-3] This is associated with a fall in plasma aldosterone with age.[4-6] Most investigators have found no change in plasma angio-tensin II (ANG II) with age,[7-11] although there is a single report describing lower plasma ANG II levels in older normotensive subjects. This result was, however, obtained after stimulation of the renin-angiotensin system by standing for 2 h.[12]

Reduced plasma ANG II levels, if present in the elderly, could lead to a secondary increase in ANG II receptor density and responsiveness. Vascular smooth muscle is relatively inaccessible for study in humans, but platelets appear to provide an accessible model of changes in ANG II receptor density occurring in response to changes in plasma ANG II. Platelet receptor density increases and decreases in response to sodium loading and restriction, which decrease and

increase plasma ANG II levels[13,14] in a similar fashion to changes in ANG II receptor density in vascular smooth muscle.[15,16]

Hypertension is associated with enhanced pressor responsiveness to ANG II.[17] However, the mechanism underlying this effect is not known. It is possible that changes in ANG II receptor density or responsiveness associated with changes in circulating ANG II could be involved.

The present study was conducted therefore to ascertain the effect of age and hypertension on plasma ANG II and platelet ANG II receptor density.

METHODS

Patients and Study Design The normotensive group comprised 43 volunteers aged 20 to 74 years. All were healthy, living in the community, and were not taking any medications. Volunteers provided a medical history, and underwent a physical examination and routine hematological and biochemical screening to confirm their status as normal volunteers.

The hypertensive group comprised 23 patients aged 23 to 71 years recruited from the hypertension clinic of Beaumont Hospital, Dublin. They were either newly diagnosed or free of antihypertensive medication for at least the 4 weeks prior to the study. A diagnosis of hypertension was based on blood pressure levels above 160/90 mm Hg or a diastolic (phase V) blood pressure in excess of 95 mm Hg, using a conventional mercury sphygmomanometer with the patient sitting still for 5 min, on three separate occasions. The mean arterial pressure was calculated as diastolic + 0.33 × pulse pressure. All volunteers and patients gave informed consent and the protocol was approved by the Beaumont Hospital Ethical Committee.

On the morning of the study, volunteers and patients attended the department at 09:00 having fasted since 21:00. Following the insertion of an indwelling cannula for venous blood sampling and 60 min supine rest, the blood pressure was recorded using a Hawksley random zero sphygmomanometer. A blood sample was then withdrawn for the measurement of plasma renin activity (PRA), plasma ANG II, platelet ANG II receptor density, and affinity and serum electrolytes. Sodium status was assessed by 24-h urinary sodium.

Laboratory Methods *Plasma Renin Activity Assay* We collected 10 mL blood into prechilled tubes containing EDTA as anticoagulant. The sample was immediately chilled in ice and centrifuged at 4°C. Plasma was placed into prechilled cryotubes, frozen in liquid nitrogen and stored at −80°C until assayed. The PRA radioimmunoassay kits were obtained from Sorin Biomedica, Saluggia, Italy (Catalog No: SB-REN-1-M). For the assay of renin activity, samples were rapidly thawed into enzyme inhibitor solution containing 2,3-dimercaptopro-

pranolol and 8-hydroxyquinoline sulphate as enzyme inhibitors. Samples were then preincubated for 1.5 h at 37°C and at pH 5.5 to 6.0 to generate angiotensin I, or at 0°C to determine blank values. Assay of generated angiotensin I by radioimmunoassay was conducted in an ice-bath for 24 h. Separation of antibody bound angiotensin I from free angiotensin I was conducted using dextran-coated charcoal according to the instructions of the kit.

Plasma ANG II Assay We collected 10 mL blood into prechilled plastic tubes containing 0.5 mL of inhibitor solution (2% ethanol, 25 mmol/L phenanthroline, 125 mmol/L Na$_2$-EDTA, 2 g/L neomycin). Blood was immediately chilled in ice and centrifuged at 4°C. Plasma was frozen in liquid nitrogen and stored for several months at −80°C until assayed. Plasma ANG II was assayed as previously described using solid-phase extraction on phenylsilylsilica, high performance liquid chromotography, and a very sensitive radioimmunoassay.[18] The original description of the method mentions within- and between-assay precisions (coefficient of variation, CV) of 6% and 13%, respectively, for plasma containing 5.7 fmol/mL; in addition, the spiking of low renin plasma with ANG II at 2.5 and 5 fmol/mL gave recoveries with a CV of 12.5%. In the meantime, procedures have been improved and recoveries of 6 fmol/mL are now 89 ± 9% (n = 6) and a sample containing 0.68 fmol/mL recently gave a CV of 7.5% when measured five times in the same assay. The detection limit is at 0.1 fmol/mL.

Platelet ANG II Receptor Density and Affinity *Materials* ^{125}I-ANG II was purchased from New England Nuclear (Boston, MA) at a stated specific activity of 2,200 Ci/mmol. Bovine serum albumin (BSA), bacitracin, EDTA, and Medium 199 were all from Serva (Heidelberg, Germany). [Sar^1-Leu^8]-ANG II was from Sigma (St. Louis, MO).

Platelet ANG II receptor density and affinity were measured using a modification of the method described by Mann and colleagues.[19] We collected 30 mL blood into plastic tubes anticoagulated with 3.8% trisodium citrate (TSC) (TSC: blood, 1:5 mL). Platelet rich plasma (PRP) was prepared by centrifugation at 100 g for 15 min at 22°C. PRP was removed, diluted 20-fold with buffer (Medium 199, 5 mmol/L EDTA 0.2% BSA, 1 mg/mL bacitracin, pH 7.4 at 22°C) and centrifuged at 1000 g for 10 min. Washing and centrifugation were repeated once and platelets were resuspended in 0.5 mL of this buffer. Platelets were counted manually, using a hemocytometer, and the volume adjusted to yield a final concentration of 4 × 10^9 cells/mL. We incubated 8 × 10^7 platelets with ^{125}I-angiotensin (25 to 400 pmol/L) in a final volume of 80 μL for 120 min at 37°C mol/L in a shaking water bath. Duplicate samples were assayed for each concentration of labelled ANG II. Nonspecific binding was assessed by inclusion of [Sar^1-Leu^8]-ANG II

(10^{-5} mol/L) at each concentration of labelled ANG II in every experiment. Nonspecific binding ranged from 3 to 15% of the total binding at the region of the angiotensin II K_d, depending upon the level of specific binding (specific binding site density) for each individual. Incubations were terminated by dilution with 2 mL ice cold buffer followed by centrifugation at 2000 g for 5 min at 4°C. From each tube 1 mL supernatant was removed for confirmation of free ANG II concentration. The remainder of the supernatant was discarded and the pellet resuspended in 2 mL ice-cold buffer and centrifuged as before. The supernatant was discarded and the radioactivity in the pellets was counted using a γ-counter at 77% count efficiency. Saturation binding curves of specifically bound radioactivity were analyzed by nonlinear regression using the computer program HYPMIC (Elsevier Scientific Publishers, B.V., Amsterdam, The Netherlands) to determine ANG II receptor density (B_{max}) and affinity (K_d). The Hill slope for each saturation binding curve approximated to 1 (n = 66 [normotensive and hypertensive], median = 1.023, 95% confidence interval = 1.021, 1.035). This suggests the presence of one ANG II binding site on the platelet, as has been previously described by others.[19]

Other Biochemical Methods Plasma and urinary electrolytes were measured by standard automated methods.

Statistical Analysis Where indicated the data were analyzed untransformed. Where there was evidence that a variable might not be normally distributed, reanalyses were conducted using an appropriate transformation; the summary statistics presented for such variables have been detransformed into their original units. Linear regression was performed using the computer program Microstat (Ecosoft, UK). Multiple regression was conducted with ANG II receptor density as the dependent variable and age, weight, blood pressure, PRA, plasma ANG II, and urinary sodium and potassium as independent variables. Statistical comparisons between groups were performed using analysis of variance (ANOVA) or Student's *t* test as appropriate. Results are expressed as means with their 95% confidence limits. Significance was set at the conventional 5% level.

RESULTS

Normotensives Subjects were arbitrarily divided into three age groups for the purpose of comparison; young (20 to 32 years), middle-aged (41 to 59) and elderly (60 to 74). Baseline clinical and biochemical characteristics of the three age groups are shown in Table 1. The groups did not differ significantly in their mean body weight or serum or urinary electrolytes. Systolic, diastolic, and mean arterial pressures increased significantly with age. Plasma renin activity declined significantly with age (Table 1) and there was a negative correlation between age and PRA (r = .− 0.43, P < .05, Figure 1A). Plasma

ANG II was slightly but not significantly lower in elderly than young people (Table 1, Figure 1B). A single plasma ANG II measurement of 12.7 fmol/mL in a 58 year old subject was most likely due to a handling error and was therefore excluded from the analyses (the data were analysed with and without this point and the significance of the correlation between age and plasma ANG II was not altered). There was a positive correlation between PRA and plasma ANG II (r = 0.37, P < .01) and a positive correlation between age and ANG II receptor density (r = 0.34, P < .05, Figure 1C). Variation in ANG II receptor density increased with age. The positive correlation was primarily due to five patients over age 59 years with a substantially increased receptor density. Multiple regression revealed that age was significantly related to ANG II receptor density and when this was adjusted for, no other variables were related to ANG II receptor density. ANG II receptor affinity did not change with age. The analyses involving PRA, plasma ANG II, and platelet ANG II receptor density were repeated using only data from those subjects for whom all three measurements were available. The relationships remained unaltered, but because overall sample size was reduced, significance levels were also slightly reduced. Only those analyses using all available data are reported.

Hypertensives Hypertensive patients were compared with the middle-aged and elderly normotensive subjects who were of similar mean age. Clinical, hormonal, and receptor data of both groups are presented in Table 2. Plasma ANG II was significantly lower in hypertensives than in normotensives, but neither ANG II receptor density nor affinity differed significantly between the two groups. There was a positive correlation between PRA and plasma ANG II (r = 0.62, P < .01) in hypertensive patients. There was no significant correlation between age and PRA, age and plasma ANG II, or age and ANG II receptor density.

DISCUSSION

In this study the effects of age and hypertension on PRA, plasma ANG II, and platelet ANG II receptor density were examined. The results confirm that plasma renin activity falls with age. Supine plasma ANG II declined with age, but the relationship was not significant. Platelet ANG II receptor density increased with age independent of the associated rise in blood pressure, but this was primarily due to a number of patients over age 59 who had increased receptor density. Plasma ANG II levels were slightly lower in hypertensives than in normotensives, but platelet ANG II receptor density and affinity did not differ between groups.

This is the first investigation of the effect of age on basal plasma ANG II in subjects whose sodium status has been assessed. Previous investigations did not incor-

TABLE 1. BASELINE CLINICAL, HORMONAL, AND PLATELET ANG II
RECEPTOR DATA IN NORMOTENSIVES

	Young	Middle-Aged	Elderly
Age, years	26.7	50.9	65.3
	(24.8–28.6)	(46.6–55.2)	(62.8–67.8)
Weight, kg	67.9	73.5	71.3
	(61.9–73.9)	(64.6–82.4)	(63.6–79)
Blood pressure, mm Hg			
Systolic	112.2	118.7	129.6*
	(107–117)	(112–125)	(119–140)
Diastolic	68.2	77.0	78.1*
	(62.8–73.6)	(72.3–81.7)	(73.1–83.1)
Mean	82.9	90.9	95.2†
	(78.5–87.3)	(86.3–95.5)	(89.3–101)
PRA, ng/mL/h	0.98 [10]	0.64 [10]	0.30* [11]
	(0.60–1.61)	(0.41–1.02)	(0.14–0.63)
Plasma ANG II, fmol/mL	3.97 [16]	3.63 [8]	3.68 [10]
	(2.97–5.31)	(2.90–4.53)	(2.80–4.81)
ANG II receptor density, fmol/10^9 cells	1.61	2.43	2.75
	(0.88–2.94)	(1.16–5.10)	(1.10–6.89)
Affinity, pmol/L	56.1	66.6	65.7
	(42.4–74.3)	(56.1–79.7)	(46.4–92.6)
Serum sodium, mmol/L	140.5 [16]	139.9	140.8 [13]
	(140–141)	(139–141)	(139–142)
Serum potassium, mmol/L	4.4 [16]	4.5	4.5 [13]
	(4.2–4.6)	(4.4–4.6)	(4.3–4.7)
Urine sodium, mmol/24 h	144.9 [16]	157.6	169.2
	(118–172) ∣	(121–194)	(130–209)
Urine potassium, mmoL/24 h	81.8	87.1	74.8
	(65.2–98.4)	(68.4–106)	(58.9–90.7)

Mean (95% confidence limits for mean).

* P < .01.

† P < .001, ANOVA.

n = 17, young; n = 12, middle-aged; n = 14, elderly; unless otherwise indicated in square brackets.

porate the measurement of 24-h urinary sodium and differences in dietary sodium cannot be excluded as a reason for their findings of unchanged plasma ANG II with age.[7-11] In the present study 24-h urinary sodium did not differ significantly between young and old subjects and therefore could not be responsible for the finding of similar plasma ANG II in these groups. Previous investigations did not use high-performance liquid chromatography (HPLC) for the specific measurement of angiotensin-(1–8)octapeptide. In the present study, plasma derived ANG II was purified by HPLC prior to radioimmunoassay.[18] Thus, the findings could provide more accurate information concerning the effect of age on plasma ANG II in humans.

The PRA fell with age, while plasma ANG II fell slightly but not significantly. This is in agreement with the findings of Skott and associates in their study of normal 85 year old people.[11] On the basis of lower PRA, lower generation of ANG I and therefore ANG II would be expected. We cannot exclude a small amount of ANG II being generated in vitro after blood sampling,[20] since no renin inhibitor was added to the sampling cocktail.[21]

However, careful and rapid handling on ice and immediate freezing of plasma would have minimized this artifact. Furthermore, in vitro generation should accentuate, rather than diminish, age-related differences in ANG II concentrations, since less ANG II is formed in vitro when less PRA and ANG I is present,[20] as is the case in the plasma of older subjects. Plasma angiotensin converting enzyme was not measured in the present study, but is reported to be either unchanged[22,23] or decreased[24,25] in the elderly. Alternatively, the rate of ANG II metabolism may decline with age, but this remains to be established. Furthermore, it may be necessary to stimulate the renin-angiotensin system, for example by ambulation, to accentuate and thereby detect any difference in plasma ANG II between young and old.[12]

The finding of a positive correlation between age and platelet ANG II receptor density supports the results of the single previous study in this area.[26] However, in the previous investigation neither plasma ANG II nor urinary sodium were measured and the study was not conducted in healthy volunteers. The elderly group in the previous study were all hospital inpatients with dis-
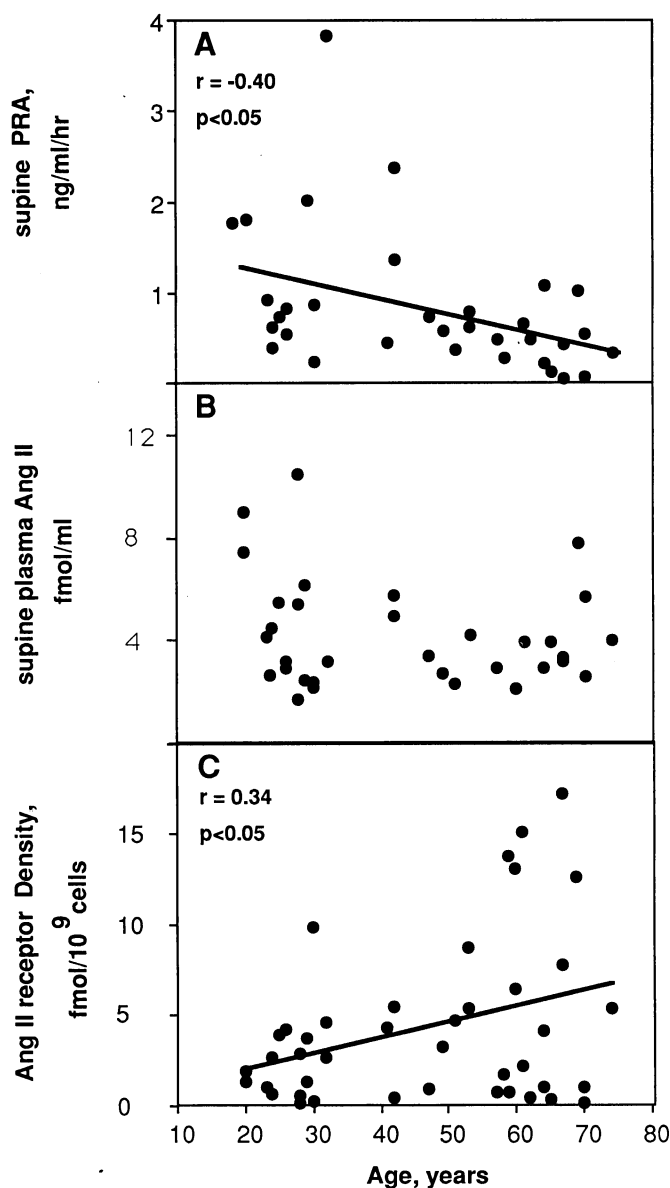
FIGURE 1. A. *Correlation between age and plasma renin activity (PRA) in normotensive volunteers (n = 31).* B. *Relationship between age and plasma ANG II in normotensive volunteers (n = 34).* C. *Relationship between age and platelet ANG II receptor density in normotensive volunteers (n = 43).*

subjects over age sixty, in a small group of these patients receptor density was elevated.

Another aspect of this study concerns the relationship between plasma ANG II and platelet ANG II receptor density. From studies involving the manipulation of plasma ANG II through changes in dietary sodium it appears that ANG II receptors, in common with many other receptor types and their respective hormones, undergo reciprocal regulation by circulating ANG II. Thus, dietary sodium restriction, which increases plasma ANG II, decreases vascular smooth muscle receptor density in the rat.[15,16] Human platelet ANG II receptors are regulated in a similar fashion, undergoing a reduction in receptor density ("downregulation") in response to sodium restriction. A corresponding increase in receptor density ("upregulation") occurs in response to sodium loading which is associated with reduced plasma ANG II levels.[13,14,28] The higher sodium intake in the elderly (169 v 145 mmol) in the present investigation could only have contributed minimally to lower PRA and increased receptor density. The difference in uri-

eases, including coronary artery disease, hyperthyroidism and hypothyroidism; in addition, five elderly patients were hypertensive and all older subjects were receiving medication. In the present investigation none of these potentially confounding variables was present. More recently, the same investigators have found the increase in ANG II receptor density to be independent of the accompanying age-related increase in ANG II uptake processes in the platelet.[27] In the present study, there was a correlation between age and platelet ANG II receptor density and increased variation with age. Although receptor density did not increase in many of the

TABLE 2. BASELINE CLINICAL, HORMONAL, AND PLATELET ANG II RECEPTOR DATA IN HYPERTENSIVES

	Normotensives	Hypertensives
Age, years	58.7	55.0
	(55.0–62.4)	(48.8–61.2)
Weight, kg	72.3	70.8
	(66.9–77.7)	(64.7–76.9)
Blood pressure, mm Hg		
Systolic	124.5	175.6 *
	(118–131)	(167–184)
Diastolic	77.6	100.7 *
	(74.4–80.8)	(97.6–104)
Mean	93.2	125.7 *
	(89.5–96.9)	(122–130)
PRA, ng/mL/h	0.43 [21]	0.43 [20]
	(0.28–0.67)	(0.24–0.76)
Plasma ANG II, fmol/mL	3.68 [18]	2.77 * [20]
	(3.11–4.29)	(2.19–3.49)
ANG II receptor density, fmol/10⁹ cells	2.60	3.71
	(1.48–4.53)	(2.22–6.23)
Affinity, pmol/L	66.1	66.5
	(54.5–79.7)	(60.8–72.8)
Serum sodium, mmol/L	140.4 [25]	140.9
	(140–141)	(140–142)
Serum potassium, mmol/L	4.5 [25]	4.4
	(4.4–4.6)	(4.3–4.5)
Urine sodium, mmol/24 h	163.8	144.1 [20]
	(139–189)	(121–168)
Urine potassium, mmol/24 h	80.5	70.5 [20]
	(68.9–92.1)	(57.8–83.2)

Mean (95% confidence limits for mean).

* *P < .001, hypertensives compared to normotensives.*

n = 26, normotensives; n = 23, hypertensives; unless otherwise indicated in square brackets.

nary sodium was not statistically significant and there was no correlation between urinary sodium and either PRA or ANG II receptor density. Furthermore, extremes of sodium restriction (10 mEq/day) and sodium loading (200 mEq/day) are required to demonstrate upregulation and downregulation in ANG II receptor density in platelets.[13,14] It appears, therefore, that regulation of platelet ANG II receptor density during aging is complex and that factors in addition to plasma ANG II are involved. The increase in receptor density is unlikely to be due simply to an increase in platelet size with age since platelet volume remains constant from early adulthood to old age.[29]

In agreement with others[14] our data suggests that platelet ANG II receptor density and affinity are similar in normotensives and hypertensives. In the present study, the hypertensives and normotensives had similar PRA, but plasma ANG II was slightly lower in hypertensives. ANG II receptor density was not, however, increased in hypertensives. There was no correlation between either PRA or plasma ANG II and ANG II receptor density in the hypertensive group. It may be that plasma ANG II in the hypertensives was not sufficiently low to result in upregulation of platelet ANG II receptor density or, as in normotensives, factors in addition to circulating ANG II may also influence receptor density in hypertensives. Some patients with hypertension demonstrate enhanced pressor responsiveness to infused ANG II,[17,30] but factors other than increased ANG II receptor density could contribute.

In conclusion, PRA falls with age. Plasma ANG II also fell with age, but the decrease was not significant. Platelet ANG II receptor density increased with age primarily due to a small group of elderly subjects with elevated receptor density. Hypertensives with lower plasma ANG II exhibit similar platelet ANG II receptor density as normotensives.

ACKNOWLEDGEMENTS

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