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Plasma atrial natriuretic peptide concentration and platelet atrial natriuretic peptide binding site density in ageing and hypertension

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SUMMARY

1. Ageing and hypertension are associated with changes in the way in which the body handles sodium. This may involve changes in plasma atrial natriuretic peptide concentration, since atrial natriuretic peptide is a regulator of sodium handling by the kidney and the plasma atrial natriuretic peptide concentration is increased in both ageing and hypertension. An increase in the plasma atrial natriuretic peptide concentration could also be associated with a change in atrial natriuretic peptide receptor density, possibly involving down-regulation.

2. To investigate these possibilities plasma atrial natriuretic peptide concentration and platelet atrial natriuretic peptide binding site density were measured in 18 young, 11 middle-aged and 12 elderly healthy subjects and in 23 patients with mild to moderate essential hypertension.

3. In normotensive subjects, the plasma atrial natriuretic peptide concentration increased with age ($r=0.49$, $P<0.01$) and was significantly higher in elderly than young subjects (mean ± SEM, 31.9 ± 4.5 versus 18.3 ± 2.0 pmol/l, $P<0.05$). The plasma atrial natriuretic peptide concentration increased with the mean arterial pressure in normotensive subjects ($r=0.47$, $P<0.01$). Multiple regression analysis did not show independent relationships between the plasma atrial natriuretic peptide concentration and either age or mean arterial pressure in normotensive subjects alone. However, when normotensive subjects and hypertensive patients were considered together, multiple regression revealed both age and mean arterial pressure as independent predictors of the plasma atrial natriuretic peptide concentration ($P<0.05$, $P<0.01$, respectively). In normotensive subjects, the platelet atrial natriuretic peptide binding site density did not change with age ($r=0.19$, $P=0.27$).

4. The plasma atrial natriuretic peptide concentration was elevated in hypertensive patients (37.6 ± 2.5 versus 30.4 ± 3.1 pmol/l, $P<0.05$). There was no significant difference in the platelet atrial natriuretic peptide binding site density between hypertensive patients and normotensive subjects.

5. It is concluded that the plasma atrial natriuretic peptide concentration increases with age. The exact mechanism is uncertain, but it may play a role in the altered renal sodium handling seen with age. The elevation in the plasma atrial natriuretic peptide concentration with age is insufficient to induce a secondary reduction in atrial natriuretic peptide binding site density. Similarly, the elevation of the plasma atrial natriuretic peptide concentration in patients with mild to moderate hypertension does not lead to down-regulation of platelet atrial natriuretic peptide binding site density. It appears that increases in circulating atrial natriuretic peptide, greater than those observed in ageing and moderate hypertension, are required to induce down-regulation of platelet atrial natriuretic peptide binding site density.

Key words: ageing, atrial natriuretic peptide, hypertension, platelet binding sites.

Abbreviations: ANP, atrial natriuretic peptide; K_d, dissociation constant; MAP, mean arterial pressure; PRA, plasma renin activity.

INTRODUCTION

Ageing is associated with changes in the way in which the body handles sodium. The ability of the kidney to excrete

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a salt load declines with age [1]. Furthermore, the elderly have a reduced ability to conserve sodium in the face of salt restriction [2]. These differences in renal sodium handling may also be important in determining the rise in blood pressure which occurs with ageing. The mechanisms underlying these changes are not clear, but could be related to changes in plasma atrial natriuretic peptide (ANP) concentration with age. Preliminary reports suggest that the plasma ANP concentration is increased in both ageing [3] and hypertension [4]. Prolonged elevation of circulating hormone levels can lead to a secondary reduction in the density of receptors mediating the action of the hormone (down-regulation), and platelet ANP binding site density is reduced when plasma ANP levels are increased [5]. It is possible that the elevated plasma ANP concentration in ageing could be associated with down-regulation of ANP receptor density. The present investigation examined the effect of age and hypertension on plasma ANP concentration and platelet ANP binding site density.

METHODS

Patients and study design

The normotensive group comprised 41 subjects aged 20–74 years. All were healthy, living in the community and were medication free. Volunteers had a medical history taken and underwent physical examination and haematological and biochemical screening to confirm their status as normal subjects. The hypertensive groups comprised 23 patients aged 23–71 years recruited from the hypertension clinic of Beaumont Hospital, Dublin. They were either newly diagnosed or free of anti-hypertensive medication for at least 4 weeks before investigation. A diagnosis of hypertension was based on blood pressure levels above 160/90 mmHg or a diastolic (phase V) blood pressure in excess of 95 mmHg, measured by using a conventional mercury sphygmomanometer with the patient sitting for 5 min, on three separate occasions. Mean arterial pressure (MAP) was calculated as diastolic + 0.33 × pulse pressure. All subjects and patients gave their informed consent and the protocol was approved by the Beaumont Hospital Ethical Committee. All subjects and patients were studied at 09.00 hours after an overnight fast from 21.00 hours. A cannula was inserted into a forearm vein for the removal of blood samples. After 60 min of supine rest, blood pressure was recorded using a Hawksley random zero sphygmomanometer and a blood sample was withdrawn for measurement of plasma ANP concentration, plasma renin activity (PRA), platelet ANP binding site density and serum electrolyte concentrations. Sodium status was assessed by measurement of 24 h urinary sodium excretion.

Materials

^{125}I-ANP (2200 Ci/mmol) was obtained from Amersham (Amersham, Bucks, U.K.) and unlabelled ANP was purchased from Sigma (Poole, Dorset, U.K.). BSA, bacitracin, EDTA and Medium 199 were all purchased from Serva (Heidelberg, Germany).

Plasma ANP r.i.a.

ANP was extracted from plasma using Sep-Pak columns and was assayed as described previously [6]. The detection limit for plasma ANP was 2 pmol/l.

Preparation of platelets

Platelet ANP binding site density and affinity were measured by using a modification of the method described by Schiffrin *et al.* [5]. Peripheral venous blood (30 ml) was collected into plastic tubes and anticoagulated with 3.8% (w/v) tri-sodium citrate (tri-sodium citrate/blood, 1:5 ml). Platelet-rich plasma was prepared by centrifugation at 200 *g* for 15 min at 22°C. Platelet-rich plasma was removed, washed with 20 volumes of buffer A (Medium 199 containing 5 mmol/l EDTA, 0.2% BSA, 1 mg of bacitracin/ml, pH 7.4 at 22°C) and centrifuged at 1000 *g* for 10 min. Washing and centrifugation were repeated, and platelets were resuspended in 500 μl of buffer A. Platelets were counted manually, using a haemocytometer, and the volume was adjusted to yield a final concentration of 10^9 cells/ml.

ANP binding assay

Platelets were incubated with ^{125}I-ANP (5–60 pmol/l) in a final volume of 120 μl, in a shaking water bath, at 22°C for 120 min. Non-specific binding was assessed by the inclusion of ANP (10 μmol/l). Separation of bound from free radioactivity was performed by centrifugation after termination of the incubation by dilution with 2 ml of ice-cold buffer A. One millilitre of supernatant was recovered from each tube for confirmation of the free ANP concentration. The remainder of the supernatant was discarded and the pellet was resuspended in 2 ml of ice-cold assay buffer A and centrifuged as before. The supernatant was discarded and the radioactivity in platelet pellets was counted in a γ-counter at 77% efficiency. Saturation binding curves were analysed by non-linear regression using the computer program HYPMIC (Elsevier Scientific Publishers, Amsterdam, The Netherlands) to derive the ANP binding site density and the dissociation constant (K_d). Scatchard analysis of the data was also performed.

Other biochemical methods

PRA was measured by r.i.a. [7] using a standard kit (Sorin Biomedica, Saluggia, Vercelli, Italy; catalogue no.: SB-REN-1-M). Plasma and urinary electrolyte concentrations were measured by standard automated methods.

Statistical analysis

The data were analysed untransformed. Where there was evidence suggesting that a variable might not be

normally distributed, re-analyses were carried out by using an appropriate transformation or a non-parametric test as appropriate. In all cases the tests agreed on the significance or otherwise of a group of differences, so only the untransformed analyses are reported (except K_d values, which are presented as geometric means derived from logarithmic transformation of individual values). Linear regression was performed using the computer program MICROSTAT. Statistically identical regressions were obtained for both normotensive subjects and hypertensive patients (for age versus plasma ANP concentration), so it was considered valid to present pooled results for the two groups combined. Statistical comparison between the groups was performed by using analysis of variance or Student's *t*-test as appropriate. *P* values of less than 0.05 were regarded as significant.

RESULTS

Normotensive subjects

Subjects were arbitrarily divided into three age groups for the purpose of comparison: young, 20–32 years; middle-aged, 41–59 years; elderly, 60–74 years. Clinical, hormonal and platelet ANP binding site characteristics of the three age groups are shown in Table 1. Representative Scatchard plots for young and elderly subjects and a hypertensive patient are presented in Fig. 1. Correlation coefficients of Scatchard plots for young, middle-aged and elderly subjects were $r=0.99$, $r=0.98$ and $r=0.99$, respectively. The young and elderly groups did not differ significantly in their mean body weight, serum electrolyte concentrations or urinary excretion of electrolytes, whereas systolic blood pressure, diastolic blood pressure and MAP were significantly higher in elderly than in young subjects (Table 1). Plasma ANP levels were approximately twice as high in elderly as in young subjects, whereas PRA was lower in elderly than in young subjects (Table 1).

In normotensive subjects, plasma ANP concentration increased with age ($r=0.49$, $P<0.01$; Fig. 2*a*) and MAP ($r=0.47$, $P<0.01$). Multiple regression analysis did not show independent relationships between plasma ANP concentration and either age or MAP primarily due to the considerable variation in the plasma ANP concentration. When data from normotensive subjects and hypertensive patients were combined, however, multiple regression revealed both age and MAP as independent predictors of plasma ANP concentration ($P<0.05$, $P<0.01$, respectively). There was no significant relationship between age and the number of ANP binding sites (Fig. 2*b*), nor between PRA and plasma ANP concentration.

Hypertensive patients

Hypertensive patients were compared with a group of middle-aged and elderly normotensive subjects of similar mean age. All normotensive subjects and hypertensive patients were of Caucasian origin. Clinical, hormonal and platelet binding site data for both groups are presented in Table 2. Hypertensive patients and normotensive subjects did not differ significantly in mean age, weight, serum

Table 1. Baseline clinical, hormonal and binding site characteristics of the normotensive subjects

Values are means ± SEM, except those for K_d which are geometric means with 95% confidence limits in parentheses. $n=18$ for young subjects, $n=11$ for middle-aged subjects and $n=12$ for elderly subjects, unless indicated otherwise in parentheses. Statistical significance (analysis of variance): $*P<0.05$, $**P<0.01$ compared with young subjects. Abbreviation: ANG I, angiotensin I.

	Young	Middle-aged	Elderly
Age (years)	26.9 ± 0.9	50.2 ± 2.0	66.1 ± 1.2
Sex (M/F)	12/6	5/6	7/5
Weight (kg)	68.2 ± 2.7	73.4 ± 4.4	71.8 ± 4.2 (11)
Blood pressure (mmHg)			
Systolic	111.8 ± 2.3	118.7 ± 3.4	130.9 ± 5.5**
Diastolic	68.3 ± 2.4	77.3 ± 2.3	77.7 ± 2.7**
MAP	82.8 ± 2.0	91.1 ± 2.3	95.4 ± 3.2**
Plasma ANP concn. (pmol/l)	18.3 ± 2.0 (16)	28.6 ± 4.5 (8)	31.9 ± 4.5* (10)
Platelet ANP binding site density (fmol/10^9 cells)	9.6 ± 1.3 (15)	9.9 ± 1.1	12.8 ± 2.5
K_d (pmol/l)	3.54 (2.91–4.32)	3.63 (2.98–4.42)	3.56 (2.92–4.33)
Serum Na^+ concn. (mmol/l)	140.2 ± 0.4 (17)	140.0 ± 0.3	141.5 ± 0.6 (11)
Serum K^+ concn. (mmol/l)	4.4 ± 0.1 (17)	4.5 ± 0.1	4.5 ± 0.1 (11)
Urinary Na^+ excretion (mmol/24 h)	147.7 ± 12.3 (17)	156.2 ± 12.0	181.0 ± 19.3
Urinary K^+ excretion (mmol/24 h)	80.8 ± 7.4	85.8 ± 9.2	78.7 ± 8.1
PRA (pmol of ANG I h^{-1} ml^{-1})	0.92 ± 0.23 (11)	0.62 ± 0.15 (10)	0.39 ± 0.08 (11)

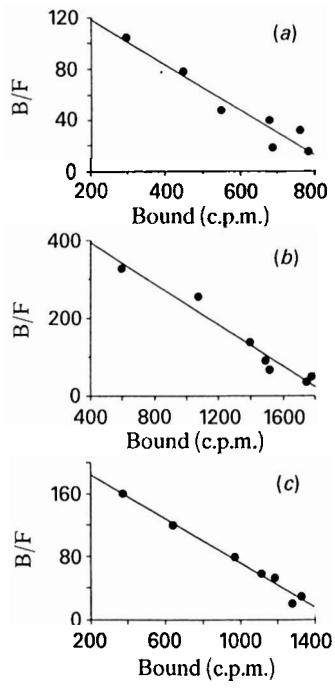

Fig. 1. Representative Scatchard plots of binding data from young (*a*) and elderly (*b*) subjects and from a hypertensive patient (*c*). B/F, Bound to free radioactivity ratio.

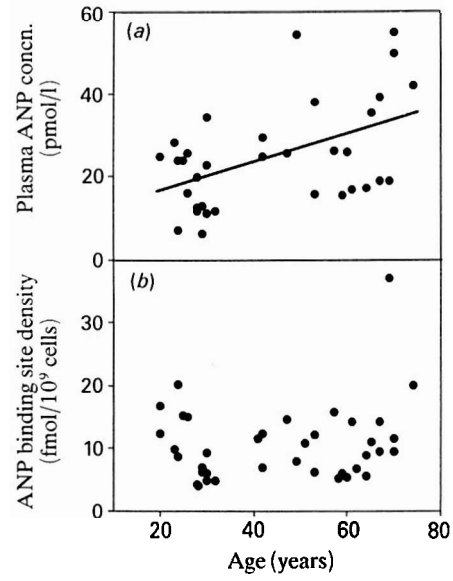

Fig. 2. (*a*) Relationship between age and plasma ANP concentration in normotensive subjects. $n=34$, $r=0.49$, $P<0.01$. (*b*) Relationship between age and platelet ANP receptor density in normotensive subjects. $n=38$, $r=0.19$, $P=0.27$.

electrolyte concentrations, urinary excretion of electrolytes or PRA. Plasma ANP concentration was significantly higher in hypertensive patients than in normotensive subjects, whereas platelet ANP binding site density was similar in both groups. Correlation coefficients of Scatchard plots of binding were $r=0.99$ for normotensive subjects and $r=0.98$ for hypertensive patients.

DISCUSSION

In this study the effects of age and hypertension on plasma ANP concentration and platelet ANP binding site density were examined. The findings confirm a rise in plasma ANP concentration with age [3, 8–10] and demonstrate that both age and blood pressure exert independent effects on plasma ANP concentration. This is the first investigation of the effect of age on platelet ANP binding site density to incorporate a substantial number of subjects aged greater than 60 years. The findings suggest that age does not influence ANP binding site density, despite the higher plasma ANP concentration in the elderly subjects. The group of patients with mild to moderate hypertension studied also exhibited elevated plasma ANP levels, but showed no reduction in ANP binding site density.

The exact mechanisms underlying the increase in plasma ANP concentration with age are not fully understood. Sodium restriction and sodium loading alter plasma ANP levels [11, 12], but there was no significant difference in sodium intake with age in the present study. Other possible contributing factors include elevated atrial pressure [13], higher noradrenaline levels [14, 15] and reduced metabolic clearance of ANP [16] with age. The physiological significance of the elevation in plasma ANP concentration with age is unclear. The increment is substantial, with the mean value in elderly subjects approximately double that of the young subjects in the present study and five to six times higher in other reports [3, 8]. These levels are similar to those attained during low-dose ANP infusion [17] and are sufficient to exert changes in the regulation of extracellular fluid volume. In addition, through opposing effects on the renin–angiotensin–aldosterone system [18–21], the raised plasma ANP concentration may contribute to the decline in plasma renin and aldosterone concentrations with age.

Elderly subjects have a diminished ability to conserve sodium in response to sodium restriction [2]. The elevation in plasma ANP concentration with age might contribute to this by increasing natriuresis. Paradoxically, prolonged elevation of the plasma ANP concentration could theoretically desensitize responses to ANP and contribute to the decline in renal sodium excretion with age [1]. Low-dose ANP infusion in young and middle-aged subjects, however, resulted in greater natriuresis, a larger fall in blood pressure and an increased plasma cyclic GMP concentration in the older group [22]. In contrast, saline infusion produced a larger increment in plasma ANP concentration in elderly subjects than in young subjects [3, 8] but a similar responsiveness of the plasma cyclic GMP concentration [8]. The evidence to date is conflicting and the role of ANP, if any, in altered sodium handling with age remains to be elucidated.

Plasma ANP concentration is elevated in most [4, 23–27] but not all [28] hypertensive patients, and from the present study there appears to be a large degree of

Table 2. Baseline clinical, hormonal and binding site data of the hypertensive patients and of normotensive subjects of similar mean age

Values are means ± SEM, except those for K_d which are means with 95% confidence limits in parentheses. $n = 23$ for normotensive subjects and hypertensive patients, unless indicated otherwise in parentheses. Statistical significance (Student's t-test): $^*P < 0.05$, $^{**}P < 0.0001$ compared with normotensive subjects. Abbreviation: ANG I, angiotensin I.

	Normotensive subjects	Hypertensive patients
Age (years)	58.5 ± 2.0	55.0 ± 3.0
Sex (M/F)	12/11	11/12
Weight (kg)	72.6 ± 3.0 (22)	70.8 ± 3.9
Blood pressure (mmHg)		
Systolic	125.1 ± 3.5	175.6 ± 4.2**
Diastolic	77.5 ± 1.7	100.7 ± 1.5**
MAP	93.3 ± 2.0	125.7 ± 2.0**
Plasma ANP concn. (pmol/l)	30.4 ± 3.1 (18)	37.6 ± 2.5* (19)
Platelet ANP binding site density (fmol/10^9 cells)	11.4 ± 1.4	10.1 ± 0.9
K_d (pmol/l)	3.74 (3.24–4.32)	3.04 (2.63–3.51)
Serum Na^+ concn. (mmol/l)	140.7 ± 0.4 (22)	140.9 ± 0.5
Serum K^+ concn. (mmol/l)	4.5 ± 0.1 (22)	4.4 ± 0.1
Urinary Na^+ excretion (mmol/24 h)	169.2 ± 13.2	144.1 ± 11.3 (20)
Urinary K^+ excretion (mmol/24 h)	82.1 ± 6.0	70.5 ± 6.1 (2)
PRA (pmol of ANG I h^{-1} ml^{-1})	0.48 ± 0.08 (21)	0.52 ± 0.08 (20)

overlap between normotensive subjects and hypertensive patients. In particular, plasma ANP concentration tends to be higher in hypertensive patients with echocardiographic evidence of left ventricular hypertrophy [29], although this is not essential for the elevation of plasma ANP concentration in hypertension [10].

The nature of the platelet ANP binding site with respect to the three known ANP receptor subtypes (ANP_A, ANP_B and ANP_C) has not been completely established. The platelet ANP binding site does exhibit guanylate cyclase activity, but is without effects on adenylate cyclase [30], and is probably therefore of the ANP_A receptor subtype. Furthermore, ANP facilitates platelet aggregation responses [30] and this effect is maximal at concentrations (10 pmol/l) reflecting maximal receptor occupancy in the present and other investigations. In addition, platelet ANP binding sites are susceptible to regulation by changes in circulating ANP levels [5], and this demonstrates the capacity of platelet ANP binding sites to interact with receptor-regulating processes. In the present study, however, despite the rise in plasma ANP concentration with age, no associated reduction in ANP binding site density was observed. This extends the work of previous investigators, who found no difference in platelet ANP binding site density between young and middle-aged subjects [22]. It appears, therefore, that the increase in plasma ANP concentration with age is insufficient to result in changes in platelet ANP binding site density. Furthermore, the increase in renal and cardiovascular responsiveness to ANP infusion observed in middle-aged [22] and elderly [31] subjects, which is reflected in animal studies [32, 33], does not support the concept of generalized ANP-induced desensitization in the elderly. Although in man these findings could be attributable to higher plasma ANP levels attained during infusion in older subjects [31], they suggest the presence of intact ANP responses in the elderly.

A non-linear inverse relationship has been found between plasma ANP concentration and platelet ANP binding sites in hypertensive patients with left ventricular hypertrophy, but not in those with mild hypertension [29]. In agreement with the latter findings, the slightly higher plasma ANP levels in patients with mild to moderate hypertension in the present study, as with the difference in the elderly, were insufficient to induce a secondary reduction in ANP binding site density. It appears, therefore, that hypertension must be severe to induce secondary changes in platelet ANP binding site density.

In conclusion, plasma ANP concentration increases with age. The exact mechanisms involved are uncertain, but increased circulating ANP may play a role in altered renal sodium handling in the elderly. The elevation of plasma ANP concentration with age is insufficient to result in down-regulation of ANP binding site density, at least in the platelet. The plasma ANP concentration is also elevated in mild to moderate hypertension with no associated change in platelet ANP binding site density. It

appears that increases in circulating ANP levels, greater than those observed in ageing and moderate hypertension, are required to induce an associated reduction in platelet ANP binding site density.

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