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Differential effects of antihypertensive drugs on neurohormonal activation: insights from a population-based sample

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Abstract. Schunkert H, Hense H-W, Bröckel U, Luchner A, Muscholl M, Holmer SR, Danser AHJ, Mayer B, Riegger GAJ (University of Regensburg, Regensburg; University of Münster, Münster, Institut für Epidemiologie, Munich-Neuherberg, Germany; Erasmus University, Rotterdam, The Netherlands). Differential effects of antihypertensive drugs on neurohormonal activation: insights from a population-based sample. *J Intern Med* 1998; **244**: 109–19.

Objectives. The clinical course of hypertension or heart failure may be modified by the extent of concurrent neurohormonal activation. Factors that regulate neurohormones in patients with these conditions are complex. In the present study, we examined the relative contribution of antihypertensive therapy to the variability of neurohormonal levels in a well defined population based sample.

Design and setting. Cross-sectional study of a mixed urban and rural population.

Subjects. Middle-aged individuals (n = 646) were analysed in order to elucidate determinants of neurohormone levels by uni- and multivariate comparisons. The assessment included anthropometric, echocardiographic and, if appropriate, genotype information.

Results. The intake of antihypertensive drugs was related to significant alterations of neurohormone levels that, in part, exceeded the contribution of all other variables studied. Multivariate analyses revealed that renin levels were independently related to the intake of beta blockers (n = 80; –8.4 mU L^{-1}; P = 0.001), angiotensin-converting enzyme (ACE)-inhibitors (n = 39; +15.9 mU L^{-1}; P = 0.0001), diuretics (n = 62; +14.3 mU L^{-1}; P = 0.0001), and calcium channel blockers (n = 45; +5.9 mU L^{-1}; P = 0.05). Aldosterone levels were related to ACE-inhibition (–156.5 pmol L^{-1}; P = 0.04) and diuretic treatment (+422.4 pmol L^{-1}; P = 0.0001) in an opposite fashion whereas beta blockers and calcium channel blockers had no significant independent effects. The levels of the atrial natriuretic peptide were significantly related to the use of beta blockers (+3.9 pmol L^{-1}; P = 0.002) and calcium channel blockers (+3.1 pmol L^{-1}; P = 0.05). Finally, serum angiotensinogen levels and ACE activity were not found to be significantly affected by antihypertensive medication but were rather related to gender or genotype.

Conclusions. The data emphasize that antihypertensive treatment with different classes of drugs may modulate serum levels of neurohormones substantially resulting in distinct patterns of activation. These drug-related effects may require consideration when neurohormonal activation is of functional relevance or when neurohormones serve as prognostic predictors in patients with cardiovascular disorders.

Keywords: aldosterone, angiotensin-converting enzyme, angiotensinogen, antihypertensive drugs, atrial natriuretic peptide, renin.

Introduction

Today, arterial hypertension can be treated by a variety of drugs that are equally effective in lowering blood pressure [1]. It is unclear, however, whether these drugs are also equally effective in improving the morbidity and mortality that is related to the disorder. In fact, recently published epidemiological

observations as well as a meta-analysis of clinical trials raised concerns since these studies suggested that the prognosis of patients with hypertension may depend, in part, on the dose and the class of the antihypertensive agent selected [2, 3]. Although the results of controlled trials must be awaited in order to clarify this pertinent clinical problem, it may be appropriate to further analyse the differential pharmacodynamic effects that accompany the antihypertensive action of these drugs. Clinical studies already suggested that antihypertensive agents may differ with regard to their effects on various neurohormones. On the other hand, many of these studies were relatively small and findings were, in part, contradictory [4–12]. In addition, it is unclear how these trials on healthy volunteers or selected patients translate to the general population. These data may be of specific interest since serum levels of various neurohormones have recently been related to the clinical outcome in patients with cardiovascular disease [13–15], or proposed to serve as a diagnostic marker [16], or as a guide in differential therapy [13, 17]. Therefore, the major aim of the present investigation was to assess, in a population based sample, the extent of neurohormonal modulation by various antihypertensive agents.

Study population

The subjects of this study originate from a sex-age-stratified random sample of all German residents that constituted the 1984/85 baseline survey of the MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) Augsburg study [18]. A second follow-up examination was carried out in 1994 that included anthropometric, echocardiographic, biochemical, and molecular genetic measurements. The survey was offered to a total of 1010 men and women, regardless of their hypertension status, aged 52–67 years, of whom 646 (64%) attended.

All subjects responded to a questionnaire on medical history with specific emphasis on current medication. Anthropometric measurements included blood pressure readings and measurements of body height and weight as previously outlined in detail [19, 20]. Resting blood pressure was measured after subjects remaining in a sitting position for a minimum of 30 min. Using a mercury sphygmomanometer, blood pressure was read three times at the right arm by two investigators. The mean of three measurements was used for this study. Hypertension was defined as blood pressure >140 (systolic) and/or >90 mmHg (diastolic) or chronic intake of antihypertensive agents. Drugs were considered to be used for treatment of hypertension when (i) subjects were aware of the diagnosis of hypertension or when (ii) blood pressure >160 mmHg (systolic) or >95 mmHg (diastolic). Antihypertensive combination therapy was considered when drugs acting via different blood pressure lowering mechanisms, e.g. diuretics and angiotensin-converting enzyme (ACE) inhibitors, were taken either as separate pills or in a fixed combination.

Methods

Echocardiographic measurements

A two dimensionally guided M-mode echocardiogram was performed on each subject by one of two expert sonographers (Sonos 1500; Hewlett Packard). M-mode tracings were recorded using standard procedures as previously described [21]. Only tracings that demonstrated optimal visualization of left ventricular interferences were used, a requirement that resulted in exclusion of 17% of potential subjects from analysis involving echocardiographic data. Left ventricular mass (LVM) was calculated from M-mode echocardiograms according to the formula described by Devereux *et al.* [22].

Biochemical measurements

Blood was drawn from nonfasting subjects who were in a supine resting position for at least 30 min. All determinations of neurohormones were carried out in duplicate. Immunoreactive renin was measured in a 200 μL plasma sample by means of an immunoradiometric assay kit (Nichols Institute, Wychen, The Netherlands), following the methods proposed by Derkx *et al.* [23]. Aldosterone and atrial natriuretic peptide (ANP) were quantified in 100 μL serum by standard radioimmunoassays (Peninsula, Belmont, CA, USA). For determination of angiotensinogen in 10 μL serum, 50 ng recombinant human renin (a generous gift of Dr Fischli, Hoffmann-LaRoche, Basel, Switzerland) was employed to generate angiotensin I as previously described [24]. Angiotensin I was measured by standard radioimmunoassay (Peninsula). ACE activity was determined

Table 1 Anthropometric data of participants according to intake of antihypertensive drugs

	Normotensive (n = 316)	Untreated hypertensive (n = 174)	Treated hypertensive (n = 156)
Age (years)	57.1 (0.1)	57.5 (0.2)	59.6 (0.3)*
Male gender (%)	43.5	53.4***	48.4
BMI (kg m^{-2})	26.3 (0.2)	27.7 (0.2)***	28.8 (0.3)*
Heart rate (min)	69.3 (0.6)	73.8 (0.9)***	70.1 (1.0)**
Systolic BP (mmHg)	135 (0.6)	164 (1.1)***	155 (1.5)*
Diastolic BP (mmHg)	84 (0.3)	100 (0.6)***	94 (0.8)*
LVMI (g m^{-2})	90.5 (1.6)	106 (2.9)***	118 (3.0)*
Fract. shortening (%)	36.7 (0.4)	36.8 (0.5)	36.2 (0.8)
Diabetes mellitus (%)	1.2	3.4	10.3*
Mycardial infarction (%)	1.5	0.6	3.2
Hormone replacement (%)	33.3	33.3	18.8*
Renin (mU L^{-1})	16.4 (0.6)	15.3 (0.9)	23.0 (3.2)*
Aldosterone (pmol L^{-1})	332 (11)	382 (19)	524 (58)*
Angiotensinogen (μmol L^{-1})	0.65 (0.02)	0.66 (0.02)	0.63 (0.02)
ACE activity (U L^{-1})	24.8 (0.3)	25.5 (0.5)	26.3 (0.7)***
ANP (pmol L^{-1})	17.1 (0.5)	17.6 (0.7)	22.2 (1.1)*

Values are expressed as mean (SEM).
ACE, angiotensin converting enzyme; ANP, atrial natriuretic peptide; BMI, body mass index; BP, blood pressure; Fract. shortening, fractional shortening by M mode echocardiography; LVMI, left ventricular mass index by M mode echocardiography (Penn formula).
*P < 0.05 vs. untreated groups; **P < 0.05 vs. untreated hypertensive subjects; ***P < 0.05 vs. normotensive subjects.

Table 2 Anthropometric data of participants according to class of antihypertensive drugs

	Beta-blockers (n = 80)	ACE inhibitors (n = 39)	Calcium channel blockers (n = 45)	Diuretics (n = 62)
Age (years)	58.7 (0.4)	59.6 (0.6)	60.6 (0.5)*	60.0 (0.5)*
Male gender (%)	48.7	53.8	62.2	34.0†‡§
BMI (kg m^{-2})	28.0 (0.3)	29.6 (0.7)*§	28.9 (0.6)	30.3 (0.5)*§
Heart rate (min)	66.7 (1.3)§	72.7 (2.0)*	71.4 (1.7)*	74.5 (1.6)*
Systolic BP (mmHg)	154 (2)§	158 (3)	155 (2)§	160 (2)*
Diastolic BP (mmHg)	94 (1.0)§	97 (1.7)	94 (1.5)§	95 (1.3)§
LVMI (g m^{-2})	121 (4.0)§	129 (7.7)§	124 (5.0)§	117 (5.1)
Fract. shortening (%)	35.8 (1.0)	33.0 (1.8)§	36.5 (1.3)	36.4 (1.5)
Diabetes mellitus (%)	3.8	20.5*§	17.8*§	11.7*§
Mycardial infarction (%)	2.2	7.7	0†	4.8
Hormone replacement (%)	24.4	16.7	11.8	9.8
Renin (mU L^{-1})	11.7 (0.8)	41.6 (12.0)*§	27.0 (9.2)§	35.1 (8.1)*§
Aldosterone (pmol L^{-1})	521 (105)	468 (42)	543 (47)	742 (147)*†
Angiotensinogen (μmol L^{-1})	0.64 (0.02)	0.65 (0.04)	0.64 (0.02)	0.67 (0.04)
ACE activity (U L^{-1})	26.3 (0.9)	ND	26.0 (1.3)	27.2 (1.4)
ANP (pmol L^{-1})	23.3 (1.2)§	18.1 (1.6)*	22.6 (2.4)§	21.2 (1.6)§

Values are expressed as mean (SEM).
ACE, angiotensin converting enzyme; ANP, atrial natriuretic peptide; BMI, body mass index; BP, blood pressure; Fract. shortening, fractional shortening by M mode echocardiography; LVMI, left ventricular mass index by M mode echocardiography (Penn-formula).
As a result of antihypertensive combination therapy, combined numbers in Table 2 are larger than the entire group of antihypertensively treated patients in Table 1.
*P < 0.05 vs. subjects on beta blockers; †P < 0.05 vs. subjects on ACE inhibitors; ‡P < 0.05 vs. subjects on calcium channel blockers; §P < 0.05 vs. untreated hypertensive subjects.

by a fluorometric assay as previously described in detail [25]. In patients taking ACE inhibitors, high levels of ACE activity may be measured due to the fact that *in vitro* the ACE inhibitor may dissolve from the enzyme. Furthermore, ACE inhibitors may induce ACE via feedback induction [25]. Therefore, no data are presented on ACE activity in patients receiving ACE inhibitors. The methods for determinations of the ACE insertion/deletion genotype as well as the angiotensinogen M235T genotype have

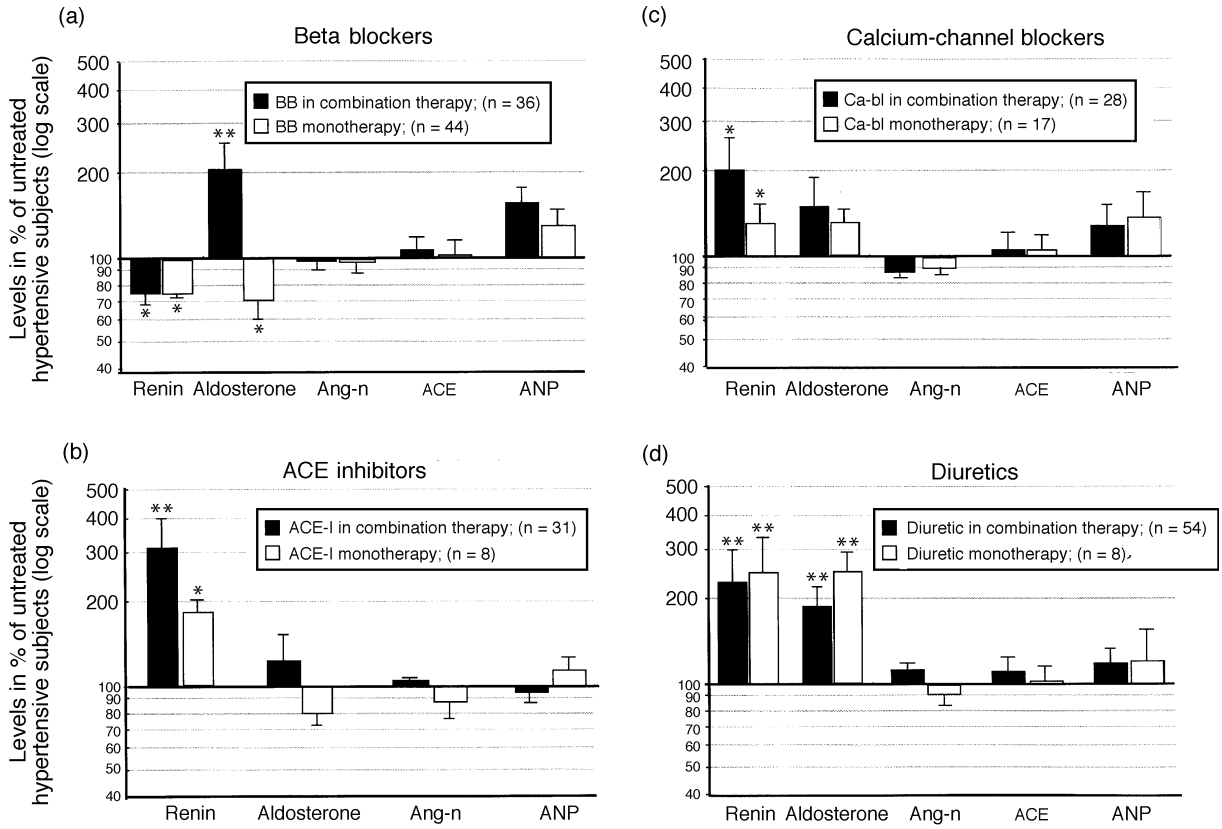


Fig. 1 Levels of circulating renin, aldosterone, angiotensinogen, angiotensin converting enzyme (ACE) activity, and atrial natriuretic peptide (ANP) in antihypertensively treated individuals expressed in percentage of untreated hypertensive subjects (logarithmic scale). Patients are presented in groups of those using the respective antihypertensive agent in form of combination therapy (closed bars) or monotherapy (open bars). The intake of beta blockers (BB) (a), ACE inhibitors (b), calcium channel blockers (c), and diuretics (d) is characterized with drug specific modulations of respective neurohormones. *$P < 0.05$; **$P < 0.005$ vs. untreated hypertensive subjects.

recently been published in detail [20, 26].

Statistical analysis

Drugs were combined according to their mode of action to form four classes, i.e. beta blockers, ACE inhibitors, calcium channel blockers, and diuretics. Only a small number of individuals received antihypertensive agents that did not fall into these principal categories, i.e. centrally acting drugs, alpha-blockers, or hydralazine (total of $n = 17$). These subjects were excluded from further analysis. ANOVA for multiple comparisons (continuous variables) or χ^2 tests (categorical variables) were performed to compare normotensive, untreated hypertensive, and treated hypertensive individuals or subjects taking one of the four major antihypertensive medications. In addition, Student's *t*-tests were carried out for comparisons between untreated hypertensive individuals and subjects receiving a particular antihypertensive class of agents or individuals receiving antihypertensive monotherapy. Data are presented as mean ± SEM.

Multivariate comparisons had two principal objectives. First, we compared neurohormone levels in patients receiving different classes of antihypertensive agents. This model included the four classes of drugs as main predictors as well as sex, age, heart rate, body mass index, systolic blood pressure, history of diabetes mellitus, and myocardial infarction by electrocardiography as obligatory covariates. The second aim was to compare in a broader fashion the relative impact of antihypertensive drugs with other potential determinants of neurohormone levels. Therefore, the initial model was expanded by a parameter of left ventricular systolic function (fractional shortening by echocardiography), and genotype information (insertion/deletion genotype of ACE and M235T genotype of angiotensinogen) as additional

covariates. With regard to the relative impact on neurohormonal modulation, there were no qualitative and only minimal quantitative differences between the first and second model such that only the second is reported. Data are presented as estimates from the regression model, i.e. beta-coefficients and their respective SEM. *P*-values <0.05 were considered significant.

Results

Anthropometric data

Anthropometric data of subjects are shown in Table 1 according to hypertension status. In total, 156 individuals received antihypertensive drug treatment, with similar numbers using antihypertensive combination therapy ($n = 79$) or monotherapy ($n = 77$). As a group, patients receiving drug treatment were characterized by substantial elevations of renin, aldosterone, and ANP serum levels (Table 1). In addition, ACE activity was slightly elevated whereas angiotensinogen levels were similar to individuals without antihypertensive medication. Furthermore, patients receiving antihypertensive drug treatment were slightly older, presented with higher body mass and left ventricular mass indices, were more likely to have diabetes mellitus, and, in women, less likely to receive oestrogen replacement therapy. In addition, patients receiving antihypertensive medication presented with lower blood pressure levels than untreated hypertensives. In Table 2, anthropometric data are presented according to the class of antihypertensive agents used in respective patients. Overall anthropometric, clinical, and echocardiographic data were similar in respective treatment groups although some statistically significant differences were observed (Table 2). In order to adjust for these differences and to further delineate the differential, drug-related pattern of neurohormonal modulation, these parameters, i.e. age, gender, body mass index, heart rate, systolic blood pressure, as well as diabetes mellitus and myocardial infarction disease status, were subsequently included in multivariate comparisons.

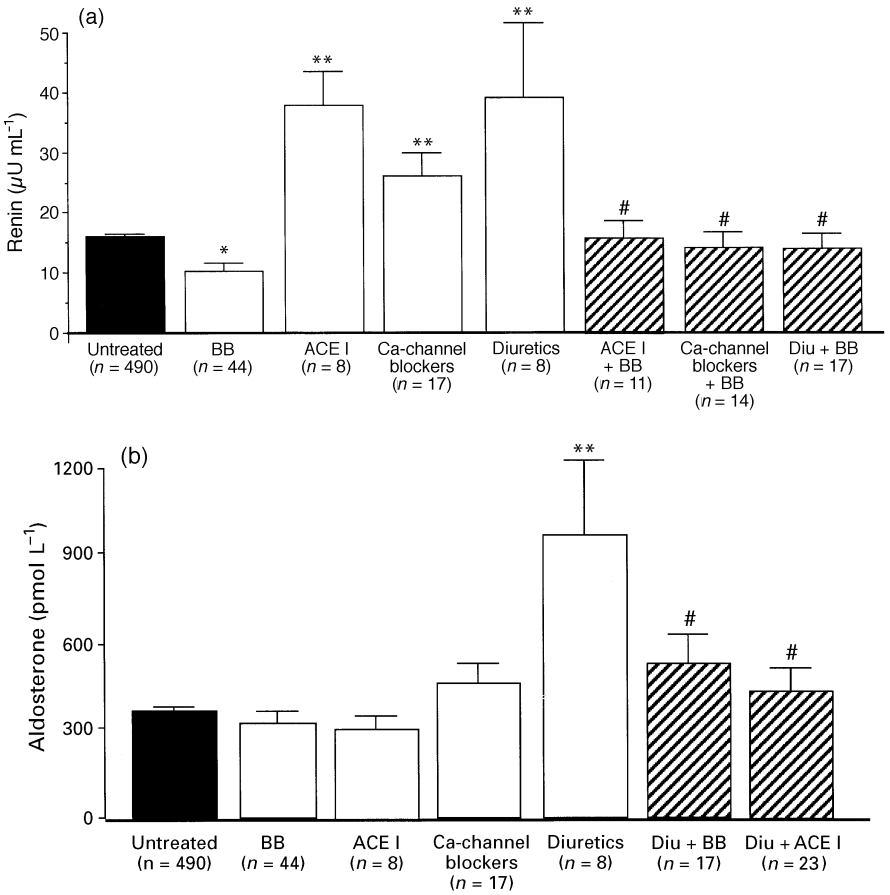


Fig. 2 Absolute levels of circulating renin (a) and aldosterone (b) in untreated subjects (closed bars), subjects on monotherapy (open bars), and subjects on frequently used antihypertensive combination therapies (hatched bars). BB, beta blockers; $^{**}P < 0.005$ vs. untreated subjects; $\#P < 0.05$ vs. subjects treated with respective monotherapy.

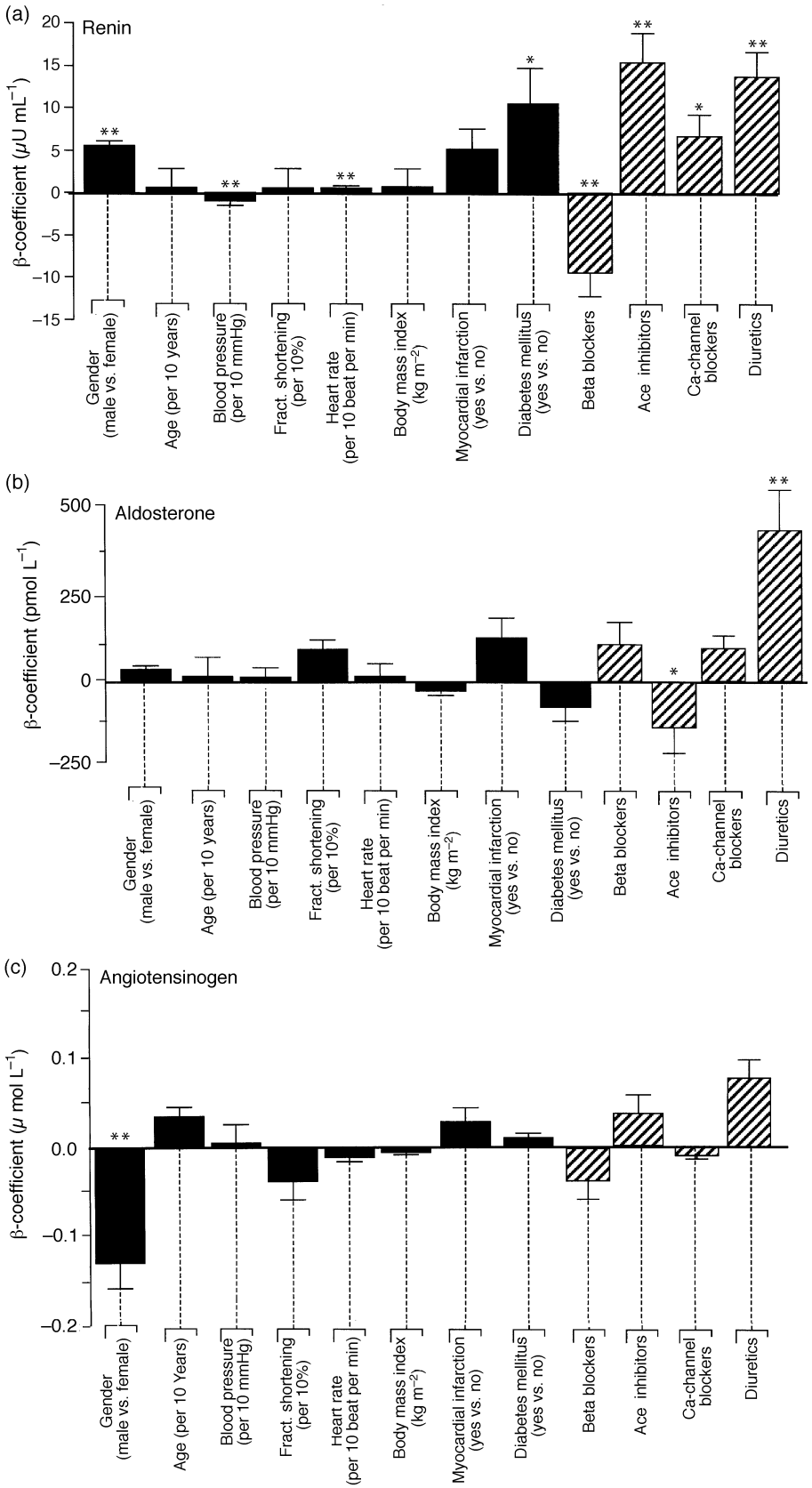
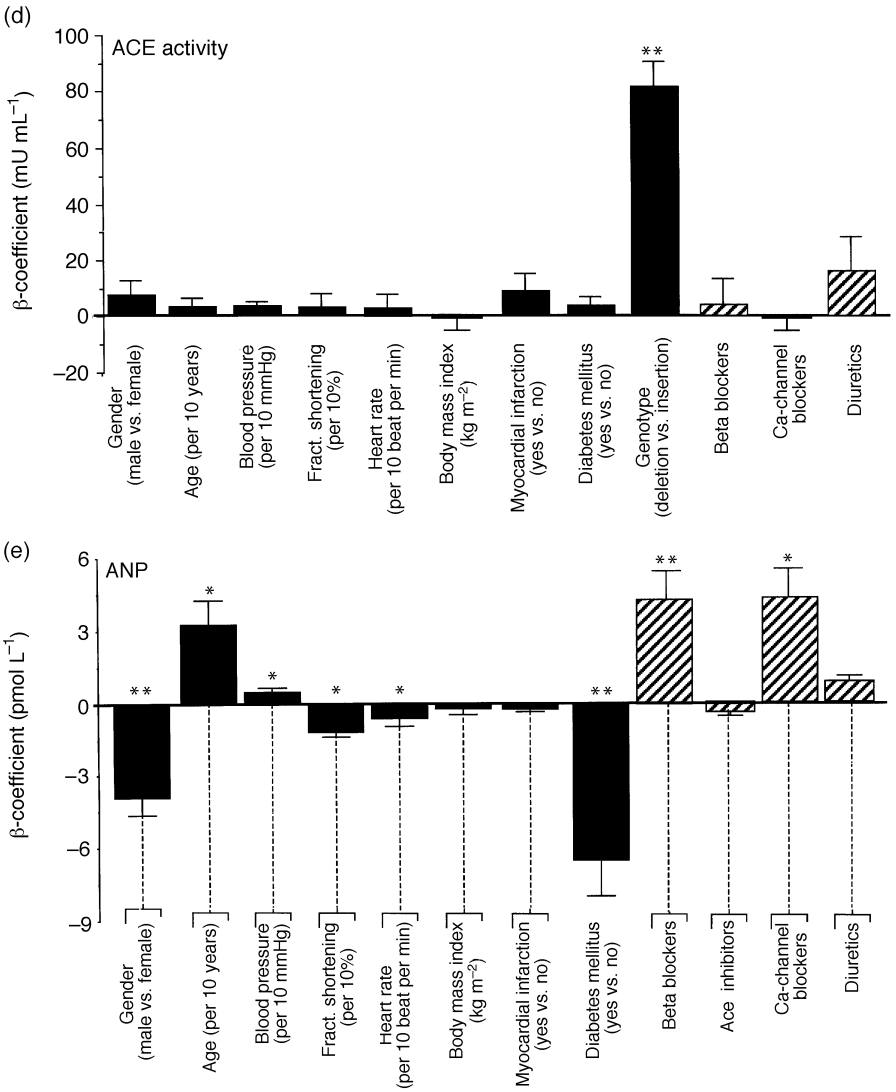


Fig. 3 Levels of circulating renin (a), aldosterone (b), angiotensinogen (c), angiotensin converting enzyme (ACE) activity (d), and atrial natriuretic peptide (ANP) (e) expressed in respective units that were related to a series of determinants by multivariate analysis. The intake of various antihypertensive drugs (hatched bars) is associated with specific modulations of respective neurohormones.



Beta-receptor blockers

Beta-blockers, used as either antihypertensive mono- ($n = 44$) or combination therapy ($n = 36$), were related to a pronounced decrease of renin levels (Fig. 1a). This trend was consistently detectable in comparisons with untreated hypertensive subjects (Figs 1a and 2a) or hypertensive individuals with other forms of hypertensive medication (Table 2; Fig. 2a). Multivariate analysis revealed that the decrease of renin levels persisted when a variety of covariates including other medications were included in the analysis (Fig. 3a). In contrast, aldosterone was not affected uniformly by beta-blockade (Figs 1a and Fig. 2b). Those patients using beta blockers in combination with other antihypertensive agents displayed elevated aldosterone levels whereas patients who received beta blocker monotherapy or in the combination with diuretics presented with slightly lower levels. No significant change of aldosterone was detectable by multivariate analysis. In addition, beta blockade had no perceivable effects on angiotensinogen or ACE levels (Figs 1a and 3c & d). However, beta blockers were related to a consistent and significant increase of ANP levels in uni- and multivariate analyses (Figs 1a and 3e).

ACE inhibitors

The intake of ACE inhibitors (monotherapy $n = 8$, combination therapy $n = 31$) was related to a substantial increase in renin levels that was detectable

in both uni- and multivariate analyses (Figs 1b, 2a, and 3b). Aldosterone levels were almost unchanged in patients receiving ACE-inhibitor monotherapy (Fig. 1b). However, a significant decrease of aldosterone was related to ACE inhibitors by multivariate analysis (Fig. 3b), an effect that is most likely to be related to a substantial decrease in aldosterone levels observed in patients treated with the combination of ACE inhibitors and diuretics (Fig. 2b). ACE inhibitors had no perceivable effect on serum angiotensinogen or ANP levels (Figs 1b and 3c & e). As outlined above, the effect of ACE inhibitors on ACE activity could not be measured reliably in the present sample.

Calcium channel blockers

Calcium channel blockers were used by 45 patients (monotherapy $n = 17$, combination therapy $n = 28$). This group was characterized by elevated serum levels of renin and ANP (Table 2; Fig. 1c). Interestingly, the effect of calcium channel blockers on renin was blunted in patients receiving the drug in combination therapy with beta blockers (Fig. 2a). Calcium channel blockers had no perceivable effect on serum aldosterone, angiotensinogen, or ACE levels (Figs 1c, 2b, and 3c & d). Multivariate analyses confirmed the elevations of renin and ANP in patients receiving calcium channel blockers (Fig. 3a & e).

Diuretics

The use of diuretics (monotherapy $n = 8$, combination therapy $n = 54$) was consistently related to an increase of both renin and aldosterone levels (Table 2; Figs 1d, 2a & b, and 3 a & b). In addition, some patients receiving diuretics had mildly elevated ACE activities (Fig. 1d). The effects of diuretics on renin were blunted in patients receiving a combination therapy with beta blockers (Fig. 2a). Likewise, the effects of diuretics on aldosterone were less pronounced in patients receiving a combination therapy with beta blockers or ACE inhibitors, respectively (Fig. 2b). In contrast, the combination of calcium channel blockers and diuretics was not associated with a significant reduction of aldosterone levels (combination therapy with calcium channel blockers, $n = 11$: 859 ± 258 pmol L^{-1}). Diuretics had no perceivable effect on serum angiotensinogen or ANP levels (Figs 1d and 3c & e).

Discussion

The present study shows that antihypertensive drug therapy, as used in the general population, is associated with substantial alterations of neurohormonal systems. Indeed, the use of antihypertensive agents was related to more profound changes of neurohormone levels than most anthropometric and functional parameters investigated. In the pooled group of antihypertensively treated patients, most neurohormones studied were found to be significantly stimulated. However, discrimination of principal substance classes highlighted distinctive patterns of neurohormonal modulation associated with respective medications.

The present data may extend the information of clinical studies that already document a strong modulation of neurohormonal activity by antihypertensive therapy [4–12]. In addition, the analysis of a population-based sample like the present may allow (i) comparison of the effects of antihypertensive drugs with other potential predictors, (ii) comparison of the various classes of antihypertensive drugs with each other, and (iii) extrapolation of experimental data on healthy volunteers or selected patients to the general population.

In the present study, renin was significantly elevated in individuals who received ACE inhibitors, diuretics, or calcium channel blockers whereas those patients who took beta blockers presented with lower levels. Furthermore, aldosterone was found to be elevated in many patients, in particular, when diuretics were part of the antihypertensive therapy. Finally, ANP levels were significantly elevated in patients who used beta blockers or calcium channel blockers whereas ACE inhibitors and diuretics were without perceivable effect on this cardiac peptide. The prominent and drug class-specific changes of renin, aldosterone, and ANP were in contrast with ACE activity and angiotensinogen levels that were not found to be significantly affected by medication but rather related to genotype or gender.

The most complex pattern of neurohormonal modulation was observed in patients receiving beta blockers. Renin, the rate limiting enzyme of the vasoconstricting and antinatriuretic renin angiotensin system, was suppressed whereas the diuretic, natriuretic and potentially vasodilating ANP was augmented. Overall, these beta-blocker-related neurohormonal effects are in good agreement with

observations reported from clinical studies. In particular, the suppression of renin by beta blockers is well documented [27, 28] whereas the modulation of ANP had been noted but received less attention clinically [11, 29]. The group of patients receiving beta blockers in combination with other antihypertensive agents was found to have elevated aldosterone levels. However, aldosterone induction was neither observed in patients receiving beta blocker monotherapy nor related to beta blockers by multivariate analysis. In fact, patients with beta blockers in combination with diuretics presented with lower aldosterone levels as compared to patients with diuretic monotherapy. The latter data also reflect the clinical experience with beta blockers that revealed either a mild reduction or no effect on aldosterone levels [11, 28, 30, 31]. The lack of a strong and consistent suppression of aldosterone by beta blockers is unexpected since renin, a powerful determinant of aldosterone levels ($r = 0.265$; $P = 0.0001$ in this sample), was lower in the beta-blocker group. However, it has been noted earlier that renin is no longer a predictor of aldosterone in patients receiving beta blockers [28]. Not investigated in the present study was the immediate inhibition of the beta-adrenergic system, a mechanism that may explain the lower heart rate in the beta-blocker group.

Patients who received ACE inhibitors and untreated hypertensive individuals presenting with similar aldosterone, angiotensinogen, and ANP levels (Fig. 1). In addition, the well documented feedback induction of renin is known to be of little functional relevance since the renin angiotensin system is at least partially blocked further downstream of the signalling cascade [32]. Thus, it might be concluded that ACE inhibitors have little effects on the neurohormones studied in the present cohort. However, the multivariate analysis suggested a suppression of aldosterone by ACE inhibitors. It may be of interest therefore that two out of three patients received ACE inhibitors in combination with diuretics; a class of drugs that was related to a substantial increase of aldosterone (see below). Thus, aldosterone may be only mildly affected in patients who receive chronic ACE-inhibitor monotherapy [33, 34], whilst these agents appear to partially prevent the aldosterone induction which is associated with the intake of other antihypertensive drugs [32].

Diuretics were related to substantial inductions of renin and aldosterone levels but were without effects on ANP and angiotensinogen. Furthermore, the group of all patients receiving diuretics, including those taking diuretics in combination with other antihypertensive agents, was found to have slightly higher ACE activity. Thus, as pointed out earlier [4, 32], treatment with diuretics is related to a pattern of neurohormonal activation that may be disadvantageous in patients with hypertension or heart failure. It needs to be considered, however, that the antinatriuretic and antidiuretic effects of the activated renin angiotensin aldosterone system are opposed by the intrinsic action of diuretics. Furthermore, in accordance with guidelines for treatment of more severe hypertension [1], diuretics were frequently coadministered with other antihypertensive agents (54 of 62 patients received combination therapy) that may potentially ameliorate neurohormonal activation. In fact, subjects receiving diuretics in combination with ACE inhibitors or beta blockers presented with substantially lower aldosterone levels [11, 32]. Likewise, normalized renin levels were found in patients taking diuretics and beta blockers concomitantly [11, 35].

Like diuretics, calcium channel blockers were related to an induction of renin levels, a finding that reflects the experience from most clinical studies [6, 9, 11, 12]. Most patients (32 out of 45) used dihydropyridines such that the data may specifically apply to these agents. Interestingly, we observed that general practitioners frequently combined calcium channel blockers with beta blockers and that this combination was associated with a potentially beneficial suppression of renin levels. Some smaller clinical studies on both dihydropyridines and newer long acting calcium channel blockers reported a suppression of aldosterone levels by these agents [9, 10]. The present study on a large population based sample is not in favour of this view and rather supports a series of earlier studies that noted no change or a mild induction of this neurohormone in subjects taking calcium channel blockers [6, 11, 36]. Finally, we observed that calcium channel blockers were related to an induction of ANP levels, an effect that was not noted in recent clinical studies [11, 12].

The analysis of the relation between anthropometric determinants and neurohormone levels was not the primary aim of the present study. Most effects observed reiterate previous reports [37–41] and confirm thereby the representative nature of the study sample. Other associations observed in the present study, like the ones between ANP levels and diabetes

mellitus or gender, have not been reported in this fashion and may require further investigation [42, 43].

Some limitations of the present observational study need to be considered. Most importantly, patient groups using different antihypertensive agents may be preselected for differential indications of respective drugs. However, anthropometric data were relatively similar in treatment groups and thus unlikely to explain the substantial differences of neurohormonal activation. Furthermore, we aimed to control for most of these determinants by multivariate analysis that confirmed most drug-related alterations of neurohormones. On the other hand, we were unable to include all potential determinants of neurohormone levels in the present analyses. In particular, information on sodium, potassium, or fluid intake as well as some comorbidities like renal artery stenosis were not available in the present cohort. However, given the extent and the class specificity of drug related neurohormonal modulation it seems unlikely that the present findings are due to unrecognized confounders. Finally, the construction of substance groups by pooling of different agents is a potential limitation. It should also be acknowledged that various drugs were used at different dosages. This diversity should tend to dilute specific findings rather than induce false positive results. Therefore, the drug related changes of neurohormones presented herein may be even an underestimate of effects seen under controlled conditions. Nevertheless, in order to obtain more detailed data, larger patient groups need to be investigated prospectively to confirm the present observations.

In summary, in the general population most commonly used classes of antihypertensive drugs are related to specific patterns of neurohormonal modulation. In addition, antihypertensive combination therapy was used frequently, resulting in reversal or pronunciation of some drug-associated alterations of neurohormones, a phenomenon with hitherto undefined functional implications.

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