ORIGINAL ARTICLE

Relationships between the antihypertensive effects of bisoprolol and levels of plasma atrial natriuretic peptide in hypertensive patients

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ABSTRACT

Previous studies have demonstrated that beta-blockade increases the levels of plasma atrial natriuretic peptide (ANP), but relationships between this effect and the antihypertensive action of beta-blockade remain unknown. In this study we investigated the amplitude and determinants of bisoprolol-induced ANP increase and the relationships between this increase and the antihypertensive effect of bisoprolol. Nineteen patients with mild to moderate hypertension were included in the study. In the first phase of the study (cross-over, placebo controlled, randomized phase), the effects of 10 mg bisoprolol on plasma ANP at rest and during exercise were compared to placebo. The antihypertensive action of bisoprolol was then evaluated after a 2-week period of treatment (10 mg/day) using ambulatory blood pressure monitoring. Bisoprolol significantly increased plasma ANP level at rest (from 30.6 ± 20.5 to 42.8 ± 35.6; $P < 0.05$) and also during exercise (from 54.7 ± 44.3 to 119.1 ± 159.9; pg/mL ± SD; $P < 0.05$). Plasma ANP at rest was not significantly correlated with left ventricular mass. After the 15 days of treatment, the bisoprolol-induced daytime diastolic blood pressure reduction was significantly correlated to the initial bisoprolol-induced plasma ANP increase ($r = 0.49$, $P = 0.035$). These results suggest that the antihypertensive effect of beta-blocking agents could be partly mediated by an increase of ANP release.

INTRODUCTION

Several studies in patients with essential hypertension [1,2] have demonstrated a positive correlation between blood pressure levels and plasma concentrations of atrial natriuretic peptide (ANP). With its natriuretic and vasorelaxant properties, ANP participates in the long-term regulation of blood pressure [3,4], but the interaction between ANP release and the antihypertensive action of drugs remains to be elucidated. Therapeutic strategies that could enhance ANP release of the atria could provide the best long-term results. Stretching of the atria appears to be the most important stimulus of ANP secretion [5–8]. ANP plasma levels increase during exercise both in healthy and in hypertensive subjects, with a significant correlation between ANP and blood pressure levels [9,10]. Such an increase is enhanced in both populations under beta-blocker treatment [9,11].

Relationships, however, between the amplitude of beta-blocker-induced ANP increase and the antihypertensive effects of beta-blockers have not been investigated. We therefore investigated, in hypertensive patients, the relationships between the antihypertensive effects of bisoprolol (a β1-selective beta-adrenoreceptor antagonist) and the amplitude of such induced ANP increases recorded at the initiation of treatment.

MATERIALS AND METHODS

Patient population

Patients of both sexes who were ambulatory, aged 18–70 years, and had mild to moderate essential hypertension, defined by a diastolic blood pressure (DBP) of 95–115 mmHg and/or systolic blood pressure (SBP) of 160–210 mmHg, were included in the study. Diagnosis of essential hypertension was made on the basis of patients' histories and routine diagnostic tests. Patients with hypertension that had already been treated were included only if the treatment had been ineffective or poorly tolerated. Patients had to be free of any exogenous form of intoxication as well as of any handicap limiting the performance of the exercise test. Women included in the study were required either to be in the menopausal phase or to carry out effective contraception. The main exclusion criteria were: severe or malignant stage of hypertension, secondary hypertension, diagnosed or suspected coronary artery disease, myocardial infarction in the previous 3 months, chronic heart failure corresponding to class II, III or IV of the New York Heart Association (NYHA), impaired hepatic or renal function (serum creatinine >180 μmol/L), and the existence of any contra-indications to beta-blocker treatment. The protocol was approved by the ethics committee of the Pitié-Salpêtrière Hospital (Paris, France) and each subject gave written informed consent to participate in the study.

Study design

Following a run-in period of 2 weeks, the study comprised two successive phases: the first was a 3-day double-blind, randomized, placebo-controlled cross-over phase with the administration of one dose of 10 mg bisoprolol or placebo on the 1st and 3rd days with 1 day between each administration. The second phase consisted of a single-blind chronic treatment of all patients with 10 mg bisoprolol, administered once daily for a period of 14 days (*Figure 1*). The 10-mg dose was chosen because it is the usually recommended dose in hypertensive subjects, providing maximal efficacy in most patients, and thus providing optimal conditions to detect effects on plasma ANP and relationships with blood pressure changes. During the run-in period all regular antihypertensive medications were withdrawn and the patients received one capsule of placebo daily. Before the cross-over phase the following examinations were carried out: medical history, blood pressure measurement, physical examination, electrocardiogram, pulsed Doppler echocardiography, 24-h ambulatory blood pressure monitoring, laboratory examination and collection of urine for 24 h for the determination of urinary sodium excretion and cyclic guanosine monophosphate (cGMP).

During the cross-over phase on days 1 and 3, 2 h after placebo or bisoprolol an exercise test was performed on a bicycle in order to assess the effect of bisoprolol on plasma ANP and catecholamine levels during exercise.

At the end of the second phase, in which 10 mg bisoprolol was taken once daily for 2 weeks, 24-h ambulatory blood pressure monitoring, urine collection for 24 h and a complete final clinical examination were again performed.

Assessments

Blood Pressure

Blood pressure was measured at rest and during exercise using a mercury sphygmomanometer (appearance of Korotkoff sounds for SBP and its disappearance for DBP).

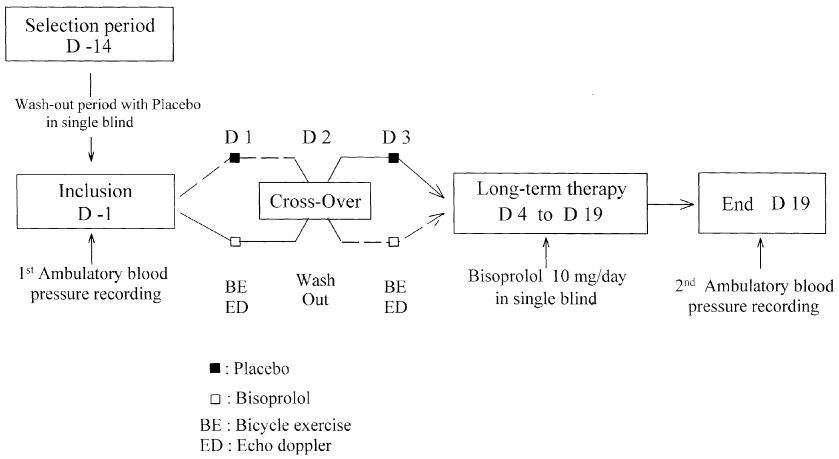

Figure 1 Study design. Following a run-in period of 2 weeks, the study comprised two successive phases: during days 1–3 of the first phase (cross-over, randomized), the effects of bisoprolol (10 mg) on plasma ANP at rest and during exercise were compared to placebo. During the second phase (days 4–19), bisoprolol (10 mg) was administered to all patients. Twenty-four-hour ambulatory recording was performed on days 1 and 19. The exercise test and echocardiography were carried out on days 1 and 3 of the first phase.

Twenty-four-hour ambulatory blood pressure monitoring
Twenty-four-hour ambulatory blood pressure monitoring was performed using a DIASYS (Novacor system; Novacor, Deerfield, IL, USA) type device that registered blood pressure every 20 min during the day (from 0700 to 2200 h) and every 30 min during the night (from 2200 to 0700 h). The mean 24-h, mean daytime and mean nighttime systolic and diastolic blood pressures and heart rates were calculated.

The exercise test
The exercise test was performed 2 h after drug intake (bisoprolol or placebo) and consisted of three workloads on an upright bicycle ergometer lasting 3 min each. The first two workloads were set at 1/3 and 2/3 of the last level corresponding to 66% of the theoretical maximal physical capacity [12]. The same workloads for each patient were used during the two exercise tests. Blood pressure was measured during the last 20 s of each workload and heart rate was determined by means of ECG recorder during the last 10 s. Following the exercise test, blood pressure and heart rate were measured at 2-min intervals until the 6th min of recovery, while patients still sitting on the ergometer.

Echocardiography measurements
Echocardiography measurements were performed with an annular phased array bidimensional Kontron Sigma 1 AC (Kontron Instruments, Montigny-le-Bretonneux, France) echocardiograph (3.5-MHz probe) during the run-in phase. Left ventricular dimensions were measured on the TM recording guided by 2D image on the parasternal short-axis view of the left ventricle. Left ventricular mass was calculated according to the formula of Devereux [13]. All echocardiography examinations were made by the same person.

Urine
Urine was collected for 24 h twice: on day 1 of the first phase and on the last day of the second phase, for determination of sodium excretion and cGMP levels. Urine samples were stored at − 20 °C until analysed.

Chemical determinations
Blood samples for plasma ANP and catecholamine determinations were taken 15 min before starting and at the end of the exercise test. They were immediately cooled and centrifugated. Plasma samples were stored at −80 °C for a mean duration of 6 months until assayed after completion of the study.

Plasma atrial natriuretic peptide and urinary cGMP concentrations were measured by radioimmunoassay (Amersham International, Freiburg, Germany) as previously reported [14]. It uses a highly specific activity [125I]2′-o-succinyl-cGMP tyrosine methyl ester tracer together with a highly specific and sensitive antiserum. Sensitivity was 0.5 fmol. Plasma norepinephrine and epinephrine concentrations were determined by a radioenzymatic method [15] with the CAT-A-KIT (TRK 995, Amersham International).

Sample size and Statistical analysis
We planned to include 20 subjects with a power of at least 80%.

This calculation was based on an ANP increase under bisoprolol of 20%, a baseline standard deviation of 20 pg/mL observed in previous studies in healthy volunteers [8] and a correlation coefficient ρ between individual values between the two phases of the cross-over of 0.8. Based on these hypotheses, a minimum number of eight patients per order group was required.

A paired *t*-test was used to analyse the significance of blood pressure changes between baseline values (end of run-in period) and following the 2-week period of treatment with bisoprolol.

Comparison of the effects of bisoprolol and placebo during the first phase, i.e. during the cross-over period, was performed according to the method of Armitage and Berry [16], evaluating treatment effect, order effect and the interaction between order of administration and treatment. However, as many data showed non-normal distribution and unequal variances, such analysis was performed with the non-parametric Wilcoxon test.

The relationship between the effect of bisoprolol on plasma ANP levels and the antihypertensive effect of bisoprolol was determined using correlation analysis (least error square method).

Statistical analysis was performed using SAS software (SAS Institute, Cary, USA).

RESULTS

Twenty patients were initially intended to enter the study. Complete data for ANP measurements could not be obtained in one patient, who was therefore excluded from analysis. The characteristics of the 19 patients included are shown in *Table I*. During the cross-over phase, nine received placebo first (group A), while 10 received bisoprolol first (group B). No significant

difference was observed between these two groups at baseline.

The cross-over phase

No treatment–order interaction was found for any parameters, allowing complete analysis of effects on the entire cross-over phase. Initial blood pressure and heart rate were similar between the two periods before drug intake.

Effects of bisoprolol on haemodynamic and echocardiographic parameters

Bisoprolol significantly decreased heart rate, both at rest and during maximal physical exercise, and also reduced the heart rate increase between rest and exercise (*Table II*). The blood pressure at rest (systolic, diastolic and mean) was not significantly affected by bisoprolol. During exercise, systolic blood pressure increased, but the increase was significantly less in the presence of bisoprolol ($P < 0.001$) (*Table II*).

Echocardiographic parameters did not change, with the exception of the left atrial minimal dimension, which increased under bisoprolol. The following dimensions were obtained: 50 ± 5 mm for end-diastolic diameter, 31 ± 4 mm for the end-systolic diameter with a fractional shortening of 38%. The minimal left atrial dimension was 27 ± 4 mm on placebo and 29 ± 4 on bisoprolol ($P < 0.05$). No significant correlation was found between plasma ANP level at baseline and left ventricular mass.

Neurohormonal results

Plasma ANP levels were significantly increased by exercise. Bisoprolol significantly increased plasma ANP concentration both in supine and in sitting positions at rest, and during exercise (*Table III, Figure 2*). For technical reasons, measurements of plasma ANP could not be obtained at the end of exercise in three patients.

Bisoprolol significantly increased plasma norepinephrine only during exercise. Plasma epinephrine, dopamine and aldosterone were not significantly changed at rest or during exercise by bisoprolol.

Plasma renin activity (PRA) increased from the supine position to the sitting position at rest and during exercise under placebo. Such an increase in PRA was significantly reduced by bisoprolol both in the sitting position at rest and during exercise.

Table I Characteristics of patients ($n = 19$) and baseline blood pressure values.

Sex ratio	10 females, 9 males
Age (years)	46.6 ± 14
Height (cm)	170 ± 8
Weight (kg)	75 ± 16
Mean duration of hypertension	5.4 ± 4.7
24-h heart rate (beats/min)	71 ± 11
SBP (mmHg)	165 ± 16
DBP (mmHg)	106 ± 9
24-h SBP (mmHg)	136 ± 17
Daytime SBP (mmHg)	139 ± 20
Nighttime SBP (mmHg)	126 ± 19
24-h DBP (mmHg)	93 ± 15
Daytime DBP (mmHg)	96 ± 16
Nighttime DBP (mmHg)	87 ± 14

Values are presented as mean ± SD; SBP, systolic blood pressure; DBP, diastolic blood pressure. Twenty-four-hour, daytime and nighttime blood pressure values were obtained by 24-h ambulatory blood pressure monitoring at the end of the 14-day run-in period.

The second phase

The effects of chronic bisoprolol treatment on blood pressure Holter monitoring parameters are summar-

Table II Cross-over phase: haemodynamic parameters.

	Placebo	Bisoprolol	Treatment effect	Interaction between order and treatment
HR at rest (beats/min)	68 ± 11	61 ± 9	$P = 0.001$	$P = 0.57$ NS
HR at maximum exercise	140 ± 22	117 ± 19	$P < 0.001$	$P = 0.97$ NS
Difference in HR between rest and exercise	61 ± 15	48 ± 18	$P = 0.003$	$P = 0.39$ NS
SBP at rest (mmHg)	156 ± 15	149 ± 19	$P = 0.23$ NS	$P = 0.71$ NS
SBP at maximum exercise	225 ± 22	199 ± 20	$P < 0.001$	$P = 0.97$ NS
Difference in SBP between rest and exercise	65 ± 21	46 ± 22	$P < 0.001$	$P = 0.59$ NS
DBP at rest	101 ± 8	101 ± 12	$P = 0.93$ NS	$P = 0.41$ NS
MBP at rest	119 ± 10	117 ± 14	$P = 0.29$ NS	$P = 0.59$ NS

Values are presented as mean ± SD; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure. NS, not significant.

Table III Cross-over phase. Results for plasma atrial natriuretic peptide level, plasma norepinephrine level and plasma renin activity at rest and during exercise ($N = 19$).

	Placebo	Bisoprolol	Treatment effect
ANP in supine position at rest (pg/mL)	30.6 ± 20.5	42.8 ± 35.6	$P = 0.049$
ANP in sitting position at rest	32.7 ± 20.2	45.9 ± 34.1	$P = 0.043$
ANP during exercise (maximum level)	54.7 ± 44.3	119.1 ± 159.9	$P = 0.008$
Difference in ANP between rest and exercise	22.6 ± 28.7	71.6 ± 12.8	$P = 0.01$
Norepinephrine at rest (pg/mL)	455 ± 178	417 ± 129	NS
Norepinephrine in sitting position at rest (pg/mL)	585 ± 177	619 ± 199	NS
Norepinephrine during exercise (pg/mL)	1244 ± 477	1662 ± 809	$P < 0.05$
Plasma renin activity at rest (pg/mL/min)	0.75 ± 0.4	0.63 ± 0.5	NS
Plasma renin activity in the sitting position at rest	0.98 ± 0.6	0.64 ± 0.4	$P < 0.02$
Plasma renin activity during exercise	0.96 ± 0.7	0.66 ± 0.5	$P < 0.02$

Values are presented as mean ± SD. ANP, atrial natriuretic peptide. NS, not significant.

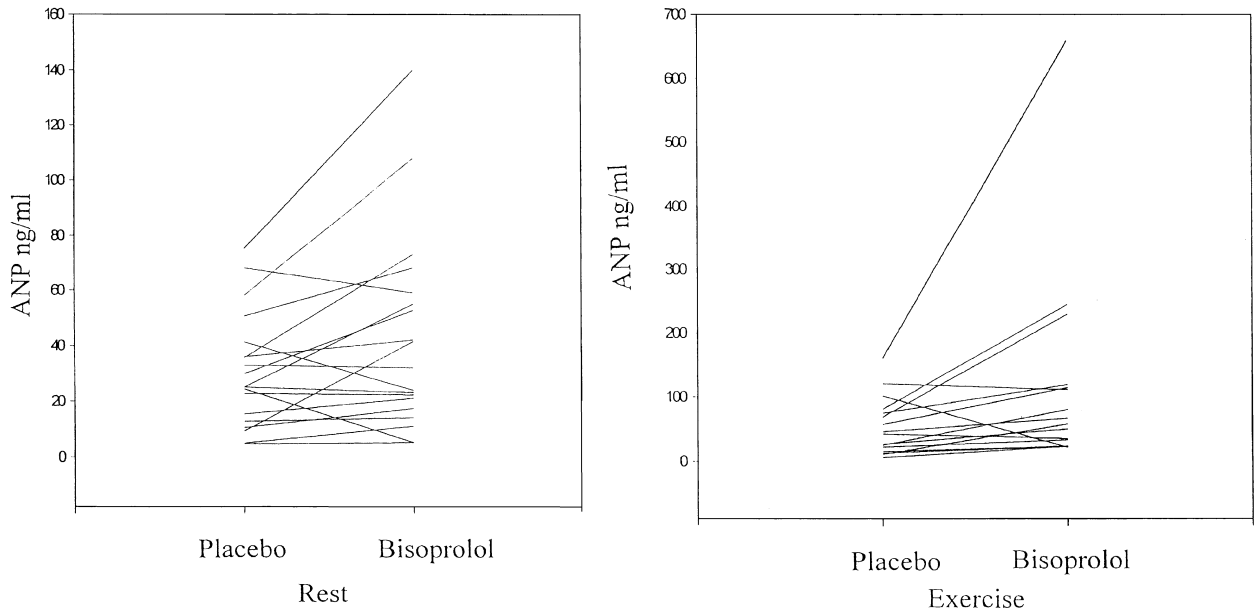

Figure 2 Plasma atrial natriuretic peptide concentration after administration of 10 mg bisoprolol and placebo in the supine position at rest and at the end of exercise.

ized in *Figure 3*. Bisoprolol significantly decreased mean 24-h, mean daytime and mean nighttime systolic and diastolic blood pressures, as well as heart rate.

A significant correlation was found between the reduction of the mean daytime diastolic blood pressure and the plasma ANP increase at rest after initial bisoprolol administration (*Figure 4*). No correlation was found between other haemodynamic effects and plasma ANP concentrations.

The total amount of 24-h urinary cGMP was significantly increased from baseline to the end of the 2-week beta-blocker treatment period (from 670 ± 335 to 1007 ± 392 ng/24 h, $P = 0.01$). Urinary cGMP concentration was also increased (from 0.514 ± 0.27 to 0.684 ± 0.26 ng/mL, $P = 0.05$) without a significant change of urinary volume (from 1333 ± 355 to 1482 ± 552 mL, $P = 0.05$).

At baseline, plasma ANP levels were not correlated to the mean 24-h level of cGMP.

Urinary cGMP changes were not correlated with 24-h blood pressure changes.

However, complete and reliable data on cGMP could only be obtained in 13 subjects.

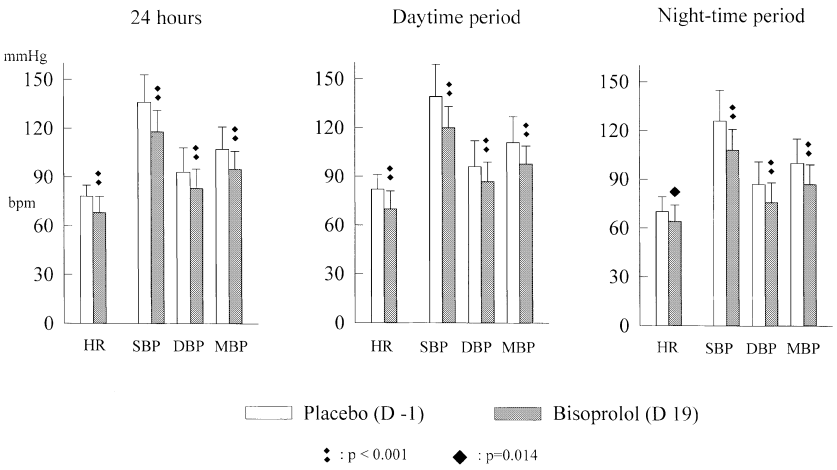

Figure 3 Diagram illustrating the effect of long-term bisoprolol treatment (10 mg/day) on the parameters of 24-h blood pressure ambulatory recordings. Mean ± SD values are shown. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure.

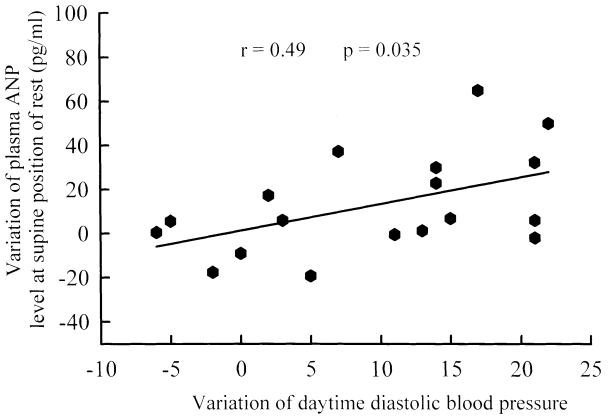

Figure 4 Relationship between variations in plasma atrial natriuretic peptide level in the supine position at rest (difference between placebo and bisoprolol) and those of daytime diastolic blood pressure after 14 days of bisoprolol treatment (10 mg/day).

DISCUSSION

Our study demonstrates that, in hypertensive subjects, beta-blocker treatment with bisoprolol increases plasma ANP at rest and potentiates the exercise-induced plasma ANP increase. Such an initial increase was correlated with a diastolic blood pressure decrease obtained after a 14-day period of treatment with 10 mg bisoprolol. In addition, the antihypertensive effect of bisoprolol was associated with a decrease in cGMP urinary excretion.

Beta-adrenoceptor blocking drugs have been found to increase plasma ANP levels during exercise in normotensive patients [17–19]. When the effects of beta-blockade on ANP in essential hypertension were compared to those in healthy subjects [17–19], beta-blockade was found to result in an ANP elevation of similar magnitude in the two populations. However, in the case of hypertension this elevation also occurred at rest, which was not the case in normotensive subjects. Our data on hypertensive patients after treatment with bisoprolol are in agreement with the findings of other authors, who obtained similar results at rest with other compounds such as atenolol [11] and propranolol [20].

Several factors should be considered in explaining the plasma ANP potentiating action of beta-blockers. Central blood volume, which increases during exercise, elevates atrial pressure and stimulates ANP secretion by atrial distension [21]. Beta-blockade reduces ventricular contractility and heart rate, which induces atrial enlargement as previously shown by echocardiographic measurement of atrial dimensions [9]. In the present study in hypertensive patients, bisoprolol was also found to increase significantly the minimal left atrial diameter, whereas it did not change the ventricular diameters or left ventricle fractional shortening. Atrial distension increases when left ventricular diastolic function is altered, explaining the observed relationship between plasma ANP and left ventricular mass in hypertensive patients [22]. ANP release potentiating the effect of beta-blockers at rest was found to be more marked in hypertensive patients with ventricular hypertrophy [22]. We did not, however, observe such a correlation

in our study, but this difference could be attributed to the fact that patients included in our study had no ventricular hypertrophy, whereas data from the literature showing the above positive relationship were obtained in patients with baseline ventricular hypertrophy.

ANP is cleared from the circulation by the kidney [23]. During prolonged exercise, renal blood flow is reduced and this could lead to decreased ANP clearance. As beta-adrenoceptor blockade decreases cardiac output, this may further diminish renal blood flow and ANP clearance. However, given the relatively short exercise period, it seems unlikely that a minor reduction in renal ANP clearance could account for the substantial increase in plasma ANP concentrations produced by treatment with beta-blockers.

Bisoprolol significantly increased plasma norepinephrine during exercise but reduced the plasma renin increase occurring from rest to the sitting position and exercise. Plasma epinephrine, dopamine and aldosterone levels were however, not affected by bisoprolol.

The well-established antihypertensive actions of beta-receptor antagonists appear to be the consequences of different mechanisms, including cardiac output reduction and renin release reduction. ANP interaction has also been recently suggested to play a role in this effect. [11,22]. Indeed, the inhibition of plasma renin increase under beta-blockade may be the direct consequence of beta-adrenoceptor blockade at the juxta glomerular level, but can also be mediated by ANP as ANP reduces renin release [24]. Both the increase of ANP secretion and the blockade of renin release may contribute to the antihypertensive effect of beta-blockade.

In agreement with this hypothesis, our study revealed a significant correlation between the mean daytime diastolic blood pressure reduction after 15 days of treatment with bisoprolol and the initial bisoprolol-induced plasma ANP increase at rest. Such an association could not be demonstrated for other antihypertensive agents such as angiotensin-converting enzyme inhibitors or diuretics [22]. However, new compounds, such as omapatrilate, have been developed with the aim of increasing circulating levels of ANP by preventing its degradation via inhibition of the neutral peptidase enzyme, and are also characterized by an angiotensin-converting enzyme inhibitor property. These compounds are a prospective class of new antihypertensive drugs that could also be useful in the treatment of other cardiovascular diseases (e.g. heart failure and primary pulmonary hypertension) [25].

Urinary cGMP excretion was significantly increased after 2-week treatment with bisoprolol, which could be related to the beta-blocker-induced stimulation of ANP excretion by atria. This result is consistent with our hypothesis, but such a urinary cGMP increase was not correlated with 24-h ambulatory blood pressure changes. Additional data obtained in a specific and more powerful study will be needed to elucidate such relationships.

CONCLUSION

This study confirms the interplay between beta-adrenergic compounds and the cardiac natriuretic peptide system. The data obtained suggest that natriuretic peptides may contribute to the therapeutic effect of beta-receptor antagonism, and that the combination of beta-blockade and drugs that increase plasma ANP levels may therefore produce an important synergic antihypertensive action.

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