Plasma Brain Natriuretic Peptide and Endopeptidase 24.11 Inhibition in Hypertension

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In contrast to the wealth of information available concerning the response of plasma atrial natriuretic peptide to changes in pressure and volume status and to inhibition of endopeptidase 24.11, very little is known of possible concomitant effects on brain natriuretic peptide. The effects of change in posture, pressor infusions of angiotensin II, or inhibition of endopeptidase 24.11 were documented in two groups of patients with essential hypertension receiving one of two orally active inhibitors (SCH 42495 or UK 79300) in double-blind, placebo-controlled, random-order crossover studies. Sustained (4 days) inhibition of endopeptidase 24.11 with either inhibitor significantly enhanced plasma atrial natriuretic peptide (P<.05, both groups) but suppressed plasma brain natriuretic peptide (P<.01, both groups) in association with significant falls in arterial pressure (P<.05, both groups). Assumption of the recumbent posture increased plasma atrial natriuretic peptide (20±5 vs 13±3 pmol/L, P<.05), whereas brain natriuretic peptide was unchanged (7±0.3 vs 7±0.4 pmol/L, NS). Pressor infusions of angiotensin II increased plasma levels of both atrial natriuretic peptide and brain natriuretic peptide (33±11 vs 17±4 pmol/L, P<.05, and 7.5±0.6 vs 5.5±0.4 pmol/L, P<.05, respectively). In contrast to atrial natriuretic peptide, brain natriuretic peptide probably is primarily regulated by left ventricular load rather than by atrial distending pressure. Thus, stimuli that incorporate substantial changes in arterial pressure may induce acute and chronic concordant changes in plasma brain natriuretic peptide, whereas other events that alter central blood volume and atrial stretch without major concomitant changes in arterial pressure will alter plasma atrial natriuretic peptide but not brain natriuretic peptide. (Hypertension 1993;22:231-236)

KEY WORDS • natriuretic peptides, brain • hypertension, essential • natriuretic peptides, atrial • blood pressure • posture • membrane metalloendopeptidase

Brain natriuretic peptide (BNP) is the second member of the family of natriuretic peptides to be discovered and characterized.1 Originally detected in porcine brain, it is present in greatest concentrations in cardiac tissue.2-4 Although small amounts of BNP may be cosecreted with atrial natriuretic peptide (ANP) from specific atrial myocyte granules,5 the predominant source of circulating BNP appears to be through constitutive secretion from ventricular myocytes.6 In healthy individuals, circulating plasma concentrations of BNP are lower than concurrent levels of ANP. However, in patients with cardiac impairment levels rise to equal or exceed plasma ANP, and, similar to ANP, increments in BNP are related to the severity of cardiac disease.6-7 It remains unclear whether hypertension is associated with any significant consistent disturbance in plasma BNP, but at least some reports indicate BNP is increased in proportion to left ventricular hypertrophy (LVH), left ventricular load, or both.8 BNP and ANP exhibit a similar array of biological actions, including natriuresis and suppression of the renin-angiotensin-aldosterone system (RAAS) in both healthy and hypertensive individuals.9-12 A substantial body of data indicates plasma ANP rises in response to acute stimuli such as assumption of the recumbent posture,13-15 infusion of pressor agents,16-17 or inhibition of the neutral endopeptidase EC 3.4.24.11, the enzyme recently discovered to initiate degradation of ANP.18-20 Together with the known biological actions of ANP these data point to an important role for ANP in pressure/volume homeostasis.21 In contrast, little is known of the response of plasma BNP to such events in either health or hypertension, and the role of this peptide remains obscure.

Accordingly, we have studied the acute and chronic effects of endopeptidase inhibition on plasma ANP and BNP. Since the source (atrium or ventricle) and mechanism of ANP and BNP secretion may differ, we also studied the differential effects of posture (largely affecting atrial pressure) and angiotensin II infusions (with substantial effects on arterial and hence ventricular pressure) in the presence and absence of endopeptidase inhibition.

Methods

The experimental protocols received approval from the Canterbury Area Health Board Ethics Committee. All patients gave written informed consent. Two groups of patients with uncomplicated mild to moderate essential hypertension took part in double-blind, placebo-
controlled, random-order crossover studies. All patients were monitored before studies for a minimum of 2 months while receiving no antihypertensive medication. Blood pressure, measured by a single observer (A.M.R.) with a standard mercury sphygmomanometer, was recorded repeatedly in all 20 participants, and it consistently exceeded 140/90 mm Hg (10 minutes seated) on at least four consecutive occasions before the participants entered into the studies. No patient had suffered myocardial infarction or stroke. None had evidence of renal impairment (normal plasma creatinine, normal urinary sediment, and negative dipstick tests for proteinuria, hematuria, or glycosuria). All underwent standard 12-lead electrocardiography and transthoracic echocardiography. Twelve of the patients met echocardiographic criteria for LVH. Body mass index exceeded 25 in 10 patients (mean 26.3±0.8; range 21.7 to 33.8). Patients or both exceeding 12 mm plus calculated left ventricular mass index exceeding 125 g/m². Five patients met standard electrocardiographic criteria for LVH. Body mass index (weight [kg]/height [m]²) exceeded 25 in 10 patients (mean 26.3±0.8; range 21.7 to 33.8). Patients were divided into two groups and studied according to the protocols illustrated in Fig 1.

Group 1 included eight male patients aged 38 to 65 years (mean 51.5 years) who weighed between 65 and 91 kg (mean 79 kg). Group 2 consisted of 12 male patients aged 31 to 61 years (mean 50 years) who weighed between 66 and 110 kg (mean 82 kg). Patients were placed on a constant sodium (150 mmol/d) and potassium (80 mmol/d), caffeine-free and alcohol-free diet between 66 and 110 kg (mean 82 kg). Patients were then seated or quietly ambulant. Lunch was provided at noon; patients were then again supine for 30 minutes before repeat blood pressure measurements and blood samples were taken at 3:30 PM. On day 4 of dosing, patients again presented at 8 AM. A venous sampling cannula was placed in a forearm vein. The brachial artery in the nondominant arm was cannulated for continuous recording of intra-arterial pressure by the Oxford method. Posture was carefully regulated throughout day 4 studies. Blood samples were obtained at 9:30 AM and 3:30 PM (as on day 1 of dosing) and at 9 PM.

Posture

In group 2, plasma samples for measurement of BNP were obtained during day 4 of dosing across changes in posture. Patients were seated upright for 30 minutes before blood samples were obtained at 10 AM, 1 PM, and 9 PM and were also upright (seated or standing) throughout the day from 9 AM. Subjects remained recumbent overnight (9 PM to 8 AM) and were then seated for an hour before a sample was taken at 9 AM on day 5 of dosing. Intra-arterial pressure recordings were performed through the inpatient study periods. Angiotensin II Infusions

On day 5 of dosing, subjects in group 2 received angiotensin II infusions. Subjects remained recumbent throughout from 10 AM to 12:30 PM. The angiotensin II (Hypertensin, CIBA-GEIGY, Basel, Switzerland) was dissolved in polygeline (Haemaccel, 500 ng/mL) and infused at 1, 2, and 4 ng/kg per minute for 30 minutes per dose (11 AM to 12:30 PM). Blood samples for measurement of BNP and ANP were obtained at 11 AM and 12:30 PM. Continuous recording of intra-arterial pressure continued throughout angiotensin infusions. Data from groups 1 and 2 were analyzed separately by analysis of variance using program P2V of the BMDP package with treatment (endopeptidase inhibitor or placebo) and time as repeated-measures factors. Where appropriate, paired t tests were also used. Data are presented as mean±SEM. P<.05 was taken to indicate statistical significance.
BNP (pmol/L) ANP (pmol/L) NEP (nmol/ml/min) 1000 1400 1800 2200 TIME (h) DAY 1 DAY 4

FlO 2.

Line graphs show plasma concentrations (mean±SEM) of brain (BNP) and atrial (ANP) natriuretic peptide and plasma neutral endopeptidase 24.11 activity (NEP) in eight patients (group 1) with essential hypertension before and after the first dose (day 1) of SCH 42495 (200 mg, △) or placebo (●) and on the fourth day (day 4) of treatment. Dose times are indicated by vertical arrows (↓). Falls in NEP (P<.005) and BNP (P<.005) and the rise in ANP (P<.05) were statistically significant.

Results

Compared with BNP values previously measured in an unmatched series of normotensive subjects (n=48; 6.3±0.3 pmol/L), levels in our hypertensive patients were slightly higher on average (7.5±0.4 pmol/L; P<.05). Mean resting plasma BNP concentrations in groups 1 and 2 fell within a narrow range (Figs 2 and 3). In this small sample no significant relations were observed between individual values of mean arterial pressure or left ventricular mass and concomitant levels of plasma BNP.

Acute and Chronic Endopeptidase Inhibition

In group 1, SCH 42495 profoundly suppressed plasma endopeptidase 24.11 activity (P<.005) throughout the dosing period (Fig 2). Plasma ANP rose briskly (P<.01) with the first dose of inhibitor and thereafter exhibited a sustained (though attenuated) increase above placebo values (P<.05). In striking contrast, plasma BNP tended to rise (NS) with the first dose of inhibitor, but on day 4 levels were significantly below placebo values (Fig 2, P<.005, treatment-time interaction). SCH 42495 induced a significant natriuresis on the first day of treatment with a mean excess excretion of sodium of 96±27 mmol above placebo values. Thereafter, sodium excretion fell slightly below placebo values. Blood pressure was unaffected by the first dose of SCH 42495 but fell significantly below placebo values (mean 24-hour intraarterial values, 152±5/89±1 mm Hg) on day 4 (mean falls in 24-hour values, −9.3±3/−3.6±1 mm Hg; P<.05 for both systolic and diastolic values).

In group 2, UK 79300 (candoxatril) also induced a significant short-lived natriuresis with an excess excretion of sodium of 81±19 mmol (P<.001) over the first 24 hours of dosing. UK 79300 also lowered blood pressure in comparison with matched placebo levels (140±4/86±3 mm Hg) as determined by continuous intra-arterial recordings on day 4 of dosing (mean falls in 24-hour values, −7.3±2/−2.1±1.5 mm Hg; P<.01 and P<.05 for systolic and diastolic pressures, respectively).

Posture

Plasma ANP rose significantly in the hour after assumption of the recumbent posture (9 to 10 PM; P<.05; Fig 3), whereas plasma BNP was unchanged (Fig 3). Plasma ANP continued to rise over the next 3 hours (P<.001 for overall change), and this additional increment in plasma ANP was significantly augmented (P<.05) by candoxatril (Fig 4). In striking contrast, plasma BNP failed to rise with either brief (9 to 10 PM) or prolonged (9 PM to 1 AM or 9 PM to 8 AM) recumbency. In further contrast to ANP and in accord with findings in group 1, mean BNP concentrations averaged over all seven sampling times (10 AM, 1 PM, 9 PM, 10 PM, 1 AM, 8 AM, and 9 AM) were significantly lower during the fourth day of candoxatril treatment (Fig 4, P<.001) when compared with time-matched placebo data.

Angiotensin II Infusions

Angiotensin II infusions increased blood pressure significantly from 134±4/75±3 to 155±5/90±3 mm Hg (P<.001 for both systolic and diastolic pressures) during
the placebo period, and the increment in pressure was enhanced (P<.05) by candoxatril with pressures rising (P<.001) from a lower preinfusion value of 129±4/74±2 mm Hg to final levels (156±5/92±3 mm Hg) similar to those observed with placebo. Plasma ANP rose significantly (P<.05, Fig 5), and plasma BNP also rose consistently (P<.05) although to a lesser extent. Candoxatril did not significantly alter the angiotensin-induced increment in plasma levels of either peptide (Fig 6). With candoxatril pretreatment, both preinfusion and peak intrainfusion plasma ANP levels tended to be greater than, and plasma BNP levels less than, time-matched placebo values (Fig 6).

Discussion

In summary, our data indicate that in patients with essential hypertension (studied under standardized conditions of dietary electrolyte intake and posture), plasma BNP concentrations are reduced by sustained inhibition of endopeptidase 24.11, are little affected by changes in posture, but do rise during pressor infusions of angiotensin II.

The small but consistent and highly statistically significant fall in plasma BNP in response to sustained inhibition of endopeptidase 24.11 was an unexpected finding. This observation was made in two separate groups of hypertensive patients (groups 1 and 2) receiving two distinct inhibitors (SCH 42495 and UK 79300). In vitro studies indicate endopeptidase 24.11 participates in degradation of BNP, although it may not play such a pivotal role as it does in clearance of ANP. In intact rats, Vanneste et al demonstrated delayed clearance of radiolabeled BNP by the endopeptidase inhibitor phosphoramidon. Seymour et
alP have demonstrated potentiation of the biological effects of BNP in cynomolgus monkeys pretreated with the endopeptidase 24.11 inhibitor SQ 28603. Furthermore, Lang et al12,13 have demonstrated an acute rise in plasma BNP in patients with heart failure receiving single doses of candesartan. However, no previous information is available concerning the effect of either acute or sustained endopeptidase inhibition on endogenous plasma BNP in animal models of hypertension or in human hypertension.

Our current data cannot provide definitive answers to reconcile our findings with these previous reports, and we must exercise caution in extrapolating from findings in patients with essential hypertension to those in either normotensive subjects or other patient groups with altered baseline BNP values (eg, heart failure). However, it is possible that with sustained endopeptidase inhibition, the initial enhancement of plasma BNP (secondary to slowed degradation) may be overwhelmed by a counteracting factor that lowers secretion of BNP sufficiently to result in a net lowering of plasma BNP concentrations. Lowering of blood pressure is a logical candidate for such a role. BNP is primarily a ventricular rather than atrial hormone, and indeed plasma BNP may be elevated in hypertension in proportion to LVH or left ventricular load.8 Thus, the significant fall in blood pressure with sustained endopeptidase inhibition (an effect absent in single-dose experiments in humans) observed in both study groups may have mediated reduced secretion of BNP through reductions in left ventricular wall tension. Further experiments documenting the responses of cardiac secretion and plasma concentrations of BNP to acute and sustained shifts in arterial pressure induced by a range of pressor and depressor agents may help clarify this issue. In addition, the effects of both short and longer term endopeptidase inhibition on plasma clearance of infused exogenous BNP should determine whether the effect of such inhibitors on the metabolism of BNP is constant over time.

The observed rise in plasma ANP with recumbency is consistent with our previous experience and with multiple published reports.13-15 That the changes in plasma ANP (Figs 3 and 4) are due to the effects of posture rather than diurnal or circadian patterns is supported by a consistent body of reports indicating that where the effects of posture are eliminated, diurnal or circadian changes in plasma ANP are trivial and may actually be absent below 65 years of age.34-37 In contrast, plasma BNP remained unaffected over an 11-hour period of recumbent posture (Fig 4, 9 PM to 8 AM). The distinction between responses by ANP and BNP to change in posture may, again, reflect their primary atrial and ventricular locations and their differing mechanisms of secretion. In health, right atrial pressures rise approximately 5 mm Hg with assumption of the supine posture. This constitutes a significant acute change in atrial distending pressure, which is well established as the primary regulator of ANP secretion.38 In contrast, arterial pressure, the prime determinant of left ventricular wall tension, exhibits very small proportional changes between lying and standing postures in healthy subjects.

Both peptides responded to pressor infusions of angiotensin II. The ANP response is consistent with previous reports16,17 and may reflect increases in central blood volume and atrial pressures, decreased blood flow to sites of ANP metabolism, or both, both effects being secondary to the peripheral vasoconstriction induced by angiotensin II. In the case of BNP, the acute significant increase in blood pressure and thus left ventricular load and wall tension may have enhanced BNP secretion, and (as for ANP) vasoconstriction may have impaired delivery of BNP to sites of metabolism. Consistent with this concept, blood pressure was similar at the top dose of angiotensin II in both the presence and absence of candesartan, and at this time point plasma concentrations of BNP were virtually identical for both study periods (see 12:30 PM, Fig 6).

From the preceding discussion, it seems reasonable to hypothesize that plasma BNP may rise with acute stimuli that significantly raise arterial pressure and thus ventricular load, whereas interventions inducing changes in atrial pressure but little change in arterial pressure may alter ANP but not BNP. Inspection of the few currently available reports appear to support this proposal. Both exercise39 and angiotensin II infusions (Figs 5 and 6) induce modest increments in plasma BNP in association with significant acute increases in arterial pressure. Conversely, lowering blood pressure in hypertensive patients with a converting enzyme inhibitor39 is associated with a reduction in plasma BNP. In contrast, changes in posture (Figs 3 and 4) and acute intravenous saline challenge40 alter atrial pressure and plasma ANP while both arterial pressure and plasma BNP are unaffected.

Angiotensin II infusions affected plasma ANP more than BNP. This observation may reflect the availability of larger intragranular stores of ANP available for an immediate secretory response, whereas BNP with its primarily constitutive ventricular secretion may only exhibit smaller and slower responses to such short-lived stimuli. With the passage of time, BNP responses may be substantial as in chronic heart failure where plasma BNP levels equal or exceed ANP. The slower response of BNP secretion may also explain the similar magnitude of change in plasma BNP that we observed with chronic endopeptidase inhibition (and modest falls in blood pressure) and with brief angiotensin II infusions (with substantial increases in pressure). In both situations plasma BNP changed by approximately 2 pmol/L (albeit in opposite directions). Conceivably more sustained increases in arterial pressure may have produced a further rise in plasma BNP. Future experiments in animal models and serial measurements in human pathophysiology should provide further insight into the temporal aspects of the plasma BNP response to changes in hemodynamic status.

The changes in plasma BNP observed in the current study and in previous reports12,13,39 are small, and their biological significance remains uncertain. Future observations of concurrent levels of plasma ANP and BNP in health and disease and during experimental manipulation of volume and pressure status should further define the relative roles of these two cardiac peptides in pressure/volume homeostasis and in the humoral response to cardiovascular disease.

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