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Biologically Active Atrial Peptides

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Differential Processing of Plasma Atrial Natriuretic Factor in Cardiovascular Disease

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The recent discovery of the long-elusive natriuretic hormone, atrial natriuretic factor (ANF), has emphasized the importance of the heart in blood pressure regulation and volume homeostasis (1). Its smallest endocrine unit is the atrial myoendocrine cell (2), the biological function of which is determined by (a) substrate uptake, (b) biosynthesis of the pre-prohormone, (c) cleaving of the signal peptide, (d) storage of the prohormone in specific granules, and, upon the appropriate stimulus, (e) secretion of the biologically active material by exocytosis. Under normal conditions, the posttranslational processing of the prohormone is likely to be coupled to the secretion stimulus and may occur at the secretion site. We have recently demonstrated the presence of the processing product ANF-28 in the circulation of normotensive subjects (3). Surprisingly, it has also been shown that patients with cardiovascular disease characterized by volume/pressure loading have plasma levels of ANF that are considerably higher than those of their normotensive counterparts (3–6). Cirrhotic patients showing a similarly increased extracellular volume, though mainly confined to an extravascular subcompartment, also tended to have increased plasma levels, though not significantly so in all studies (7–9). Head-out water immersion has recently been shown to be an important investigative tool in the examination of the secretory function of the heart (10,11). In addition to alterations in stimulus-response coupling, a defective posttranslational processing of pro-ANF (ANF-126) or a modified target-tissue responsiveness to circulating ANF might be important features in the pathophysiology of volume homeostasis. We have recently demonstrated that the molecular weight pattern of plasma ANF in hypertensive patients differs from that seen in normotensive controls (4). Elevated plasma ANF levels in patients with congestive heart failure consisted predominantly of such higher molecular weight forms not apparent in normal subjects (12). In this study, we document plasma levels of ANF in hypertensive patients, patients with congestive heart failure before and under converting-enzyme-inhibitor therapy, and in normotensive subjects and cirrhotic patients prior to and during head-out water immersion. An initial structural analysis of circulating ANF was achieved by use of high-performance gel permeation chromatography of plasma extracts from these patients.

METHODS

Extraction of plasma samples and radioimmunoassay procedures were modified from ref. 3. Briefly, antibody Toni III was used instead of the less sensitive antibody Toni II. This antibody is mid-molecule- and C-terminal-directed. Cross-reactivity with the N-terminally extended cardiodilatin-88 (gift from Dr. W. G. Forssmann, Heidelberg, Federal Republic of Germany) was 29.7%, 35.8% to rat ANF-28, 13% to atriopeptin III, and 0.03% to atriopeptin I or II. It did not cross-react with a wide variety of peptides and proteins, including its immunization conjugate, which is bovine thyreoglobulin. The final titer was 1:120,000 and the assay sensitivity was 0.5 fmol/assay tube. The 50%-binding intercept of the standard curve was 7 fmol. Synthetic standards and iodinated labels were from NovaBiochem, Läufelfingen, Switzerland. Plasma aliquots (0.5–3 ml) were extracted by adsorption to pre-rinsed Anberlite XAD-2 adsorbent resin (particle size 0.3–1.0 mm, Serva, Heidelberg, Federal Republic of Germany) (3). Recovery of synthetic ANF-28 was approximately 67%. Levels were not yet corrected for recovery (the results of the "International Collaborative Study of the Proposed International Standard for Atrial Natriuretic Factor On Behalf of The AHA/ISH/WHO" pending). The intra-assay coefficient of variation ($n = 6$) was <5%. Plasma extracts (5 ml) were subjected to high-performance gel permeation chromatography on a 7.5 × 600-mm TSK-125 Bio Sil column (Bio Rad, Munich, Federal Republic of Germany) and eluted with 0.09% trifluoroacetic acid containing 0.005 M Na_2SO_4 plus 0.002 M NaH_2PO_4 with 30% acetonitrile as a solvent. Flow rate was 0.4 ml/min, and aliquots from column fractions were analyzed for immunoreactive ANF (ir-ANF). Peripheral blood was drawn into precooled syringes and immediately transferred to precooled polystyrene tubes containing 500 KIU/ml aprotinin and 1 mg/ml sodium EDTA. Plasma was separated and stored at −70C until extraction. Forty-one normotensive control subjects showing no evidence of cardiovascular, renal, pulmonary, or gastrointestinal disease took part in the study. Twenty-seven patients with essential hypertension were examined; at the time of examination, their mean blood pressure was 176 ± 4.1 over 101 ± 3.5 mm Hg. In addition, 14 patients with congestive heart failure functional class NYHA II to IV were studied. Patients were hospitalized 1 week before catheterization of the heart, and all medication was discontinued except for diuretics and digitalis. Measurements were taken before, immediately following, and 6 months after institution of therapy with an angiotensin-converting-enzyme inhibitor (enalapril, usually 5 mg twice daily). Thirty-one patients with cirrhosis of the liver, confirmed by biochemical and histological examination, were investigated. Cirrhotic patients were divided into subgroups with and without ascites. Twelve healthy controls and 11 cirrhotic patients were subjected to head-out water immersion procedures. After voiding, subjects assumed a seated position next to the immersion tank for the first hour of the experiment. Subsequently, they were immersed up to their necks, maintaining the same seated position in thermoneutral water (34.0 ± 0.2°C) for 1 hr, followed by an additional hour sitting outside the tank. Throughout the experiment, 250 ml/hr of tap water was given orally. All patients were on a hospital diet allowing 150 mmol of NaCl daily. Experimental protocols were approved by the institutional committee on the ethics of human investigation.

RESULTS AND DISCUSSION

Hypertensive patients displayed a sevenfold increase in plasma ANF as compared to normotensive controls (62.2 ± 16.8 versus 8.8 ± 1.1 fmol/ml, mean ± SEM, $p < 0.001$, Student's t-test). A subgroup of untreated patients with essential hypertension had comparably high levels. Patients with congestive heart failure displayed an 18-fold increase in plasma ANF (158 ± 56 fmol/ml, $p < 0.001$). Plasma ANF levels in cirrhotic patients (10.3 ± 1.3 fmol/ml) were not lower than in normotensive controls. Plasma ANF levels in congestive heart failure patients were positively and significantly correlated to increased right atrial pressure and pulmonary capillary wedge pressure ($r = +0.72$, $p < 0.01$; and $r = +0.73$, $p < 0.01$, respectively) and reversely related to cardiac index ($r = -0.73$, $p < 0.01$). In the course of 6 months' therapy with an angiotensin-converting-enzyme inhibitor (enalapril), plasma ANF levels in congestive heart failure patients fell, in parallel with the hemodynamic improvement, to 63% of pretreatment levels (from 158 ± 56 to 100 ± 50 fmol/ml, $p <$ 0.02, Wilcoxon paired-sample test). Head-out water immersion induced an increase in plasma ANF levels by 83% following 1 hr of immersion (from 6.5 ± 0.8 to 12.0 ± 2.6 fmol/ml, $p < 0.01$). This response was comparable to that seen in cirrhotic patients without ascites, whereas cirrhotic patients with ascites displayed a blunted response (a 50% increase as compared to a 98% increase in patients without ascites) (9). An initial structural analysis of plasma ANF was performed by use of high-performance gel permeation chromatography in all plasma extracts. As previously reported (3), in normotensive individuals, ir-ANF consisted exclusively of authentic 3,080-dalton ANF-28, the biologically active processing product of pro-ANF (13). Such a molecular analysis in cirrhotic patients yielded a similar

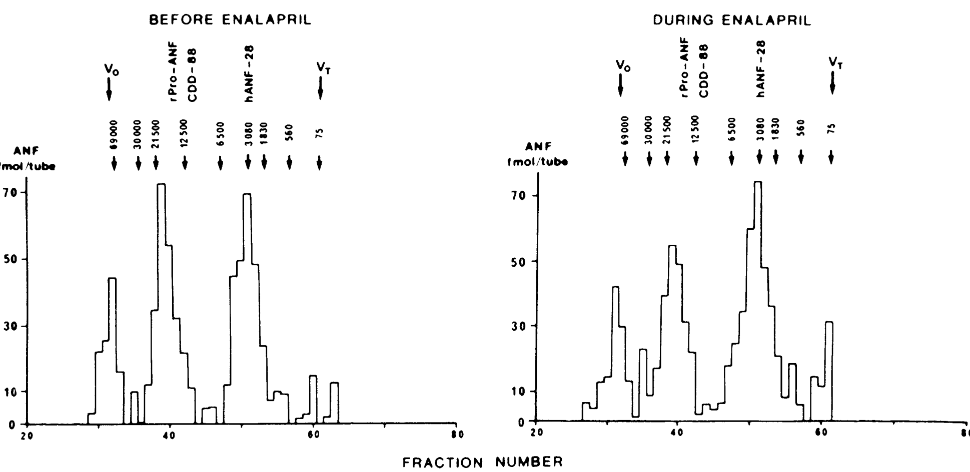

FIG. 1. Molecular weight pattern of ir-ANF in the plasma of a representative patient with congestive heart failure before and following institution of therapy with enalapril. The TSK-125 Bio Sil column was calibrated with bovine serum albumin (V_0), leu-enkephalin (V_T), a series of opioid peptides, and synthetic ANF-28. Fraction aliquots were assayed for ANF-immunoreactivity.

pattern. However, in these patients, trace amounts of a higher-molecular-weight material (~13,000 daltons) was also present. This higher-molecular-weight portion of plasma ir-ANF did not increase in parallel to the rise in authentic ANF-28 induced by head-out water immersion in cirrhotic patients. This finding is in favor of the contention that in these patients the stimulus-response coupling is intact and a maximal converting capacity of the putative pro-ANF-converting enzymes is not exceeded. Ir-ANF in hypertensive patients comprised a peak coeluting with synthetic ANF-28 as in normotensive subjects and cirrhotics; in addition, a 13,000-dalton-ANF immunoreactivity and an ANF immunoreactivity eluting in the void volume (molecular weight > 50,000 daltons) were also present. The void-volume ANF immunoreactivity most likely represents ANF-28 bound to carrier proteins (a differential recovery of which may, in part, explain the vast differences between various extraction procedures and between extracted and unextracted assays). The elevated plasma ANF levels in congestive heart failure were primarily composed of such higher-molecular-weight forms (Fig. 1). Interestingly, therapy with an angiotensin-converting-enzyme inhibitor effected a reduction in total plasma ANF immunoreactivity in parallel with a hemodynamic improvement. Molecular weight analysis revealed that it was primarily the higher-molecular-weight forms that were decreased rather than the authentic ANF-28. This may indicate that the increased demand in these patients that cannot be met, subsequently leading to the release of immature ANF forms. We conclude that a putative dysregulation of posttranslational processing of pro-ANF may be an important feature in the pathophysiology of cardiovascular disease states.

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