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Natriuretic Hormones in Hypertension

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Baseline and Stimulated ANF Plasma Levels: Is an Impaired Stimulus-Response Coupling Diagnostically Meaningful? *

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Summary. Plasma levels of ANF were determined and chromatographically analysed in normotensive controls, cirrhotic patients with and without ascites, hypertensive patients, patients with congestive heart failure and heart transplant recipients. A comparison of baseline plasma levels allowed for the conclusion that cirrhotic patients do not differ in this regard from control subjects (9.0 ± 1.3, $n = 41$ vs. $9.6 \pm 1{,}0$ fmol/ml, $n = 51$). Cirrhotic patients with ascites do not have lower plasma levels than cirrhotic patients without ascites (8.8 ± 1.4, $n = 8$ vs 8.6 ± 1.5 fmol/ml, $n = 10$). Stimulation of the ANF-system by head-out water immersion, however, revealed an impaired increase in ANF release in cirrhotic patients with ascites ($146 \pm 18\%$ vs $204 \pm 16\%$). Patients with cardiovascular disease display tonically-elevated ANF plasma levels. Heart failure patients displayed the highest plasma concentration (81.5 ± 32.7 fmol/ml, $n = 17$), whereas plasma levels in hypertensive patients ranged from normal to greatly elevated (61.7 ± 13.2 fmol/ml, $n = 36$). Heart transplant recipients also had significantly elevated plasma levels as compared to control subjects (31.2 ± 7.9 fmol/ml, $n = 14$) but levels were lower than in hypertensive patients in spite of a comparable arterial pressure. Short term ventricular pacing ($f = 150$/min for 5 min) revealed an impaired phasic activity of the ANF system in heart failure patients and heart transplant recipients. Two hypertensive patients without hypertensive heart disease displayed an increase to 367% of baseline levels immediately subsequent to ventricular pacing, heart transplant recipients showed an increase to $207 \pm 22\%$ above baseline whereas the response in heart failure patients was significantly less ($134 \pm 13\%$). The molecular weight pattern in transplant patients did not differ from heart failure patients prior to pacing; in heart failure, however, pacing may induce a release of higher molecular weight forms of ANF.

Key Words: Atrial Natriuretic Factor – Stimulus-Response-Coupling – Ventricular Pacing, Water Immersion – Heart Failure – Hypertension – Heart Transplantation – Cirrhosis

Peters and Gauer regarded the atria as being "the ideal site to sense the fullness of the blood stream" [23, 12]. Since the work of Henry and Gauer the atria have been known to bear volume/stretch-sensitive receptors [13, 17]. With the discovery of a natriuretic hormone in the atria (ANF), the mammalian heart acquired an endocrine function and ANF has emerged as a good candidate for the mediation of atrial distention-induced natriuresis. In distinction to other natriuretic hormones [8, 18, 20] it was not unreasonable to suppose that natriuretic hormone of atrial origin (ANF) should possess vasodilator properties [7, 24] enabling the heart to adapt to changes in extracellular volume (ECV) and blood pressure, thereby optimizing its performance by an appropriate ANF-effected decrease in pre- and after-load [1].

Remarkably quickly following the discovery of ANF, its molecular biology and physiology could be unravelled [5, 11, 21], and ANF plasma levels have been determined and characterized in various disease states [3, 14]. However, baseline plasma levels as such may not satisfactorily reflect the functional status and compensatory reactivity of the endocrine heart to alterations in volume/pressure homeostasis. A tonically-maintained high plasma level might induce a receptor down-regulation or be associated with other adaptive changes [5] and, paradoxically, eventually prove physi-

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ologically counter-productive. Further, the dynamics, responsiveness and magnitude of rapid and transient phasic alteration in ANF could be compromised in the case of a sustained very high rate of release. To investigate the phasic response of the ANF system to acute, rapid and transient stimuli we subjected, firstly, patients suffering from cardiovascular diseases and displaying chronically elevated levels of ANF to short-term ventricular pacing and, secondly, cirrhotic patients showing normal levels of ANF to head-out water immersion.

Methods

Extraction of plasma samples and RIA procedures were modified from [2] and have been detailed elsewhere [4]. Briefly, antibody Toni III is mid-molecule- and C-terminal directed. Cross reactivity has been reported previously [4]. The final titer was 1 : 120000 and the assay sensitivity was 0.5 fmol/assay tube. The 50%-binding intercept of the standard curve was 15 fmol. Synthetic standard was from Nova Biochem, Läufelfingen, Switzerland. 0.5 or 1 ml-plasma aliquots were extracted by adsorption to prerinsed Amberlite XAD-2 adorbent resin [2]. Recovery of synthetic ANF_{99-126} was approximately 67%. The intra-assay coefficient of variation ($n=6$) was $< 10\%$. Plasma extracts were subjected to high performance gel permeation chromatography (HPGPC) on a 7.5×600 mm TSK-125 Bio Sil column (Bio Rad, Munich, FRG), eluted with 0.09% TFA containing 0.005 *M* Na_2SO_4 plus 0.002 *M* NaH_2PO_4 and 30% acetonitrile as a solvent. Flow rate was 0.4 ml/min, and aliquots from column fractions were analyzed for immunoreactive (ir)-ANF. Ir-ANF following HPGPC was quantitated as 3 separate peaks coeluting with the void volume (69-kd-ANF), rat-Pro-ANF (15-kd- ANF), and human ANF_{99-126} (3-kDa-ANF). Results following HPGPC are given as % of total ir-ANF. Peripheral blood was drawn into pre-cooled syringes and immediately transferred to pre-cooled polystyrene tubes containing 500 kallikrein inhibitor units/ml aprotinin and 1 mg sodium EDTA. Plasma was separated and stored at –70° C until extraction.

Fifty normotensive control subjects showing no evidence of cardiovascular, renal, pulmonary or gastrointestinal disease, 36 patients with hypertension, 17 patients with congestive heart failure, 14 heart transplant recipients and 41 cirrhotic patients agreed to participate in the study. Heart transplant recipients were on immunosuppressive and antihypertensive therapy but remained hypertensive (MAP 115 ± 3 mm Hg).

Fourteen heart transplant recipients, 5 patients with congestive heart failure and 2 hypertensive patients (MAP 120 and 125 mm Hg) undergoing diagnostic heart catheterization were subjected to right ventricular pacing (3mA, f = 150/min for 5 min) after giving their informed consent. Venous blood was sampled prior to and immediately following electrostimulation.

22 healthy controls and 18 cirrhotic patients were subjected to head-out water immersion procedures. Cirrhotic patients were divided into subgroups with and without ascites. 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 (35.0 ± 0.2° C) for 1 hour 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 regular hospital diet. Data are presented as means $\pm$ S.E.M. Statistical analysis was performed by Student's unpaired or paired t-test.

Experimental protocols were approved by the institutional committee on the ethics of human investigation.

Results

Baseline ANF-plasma levels are given in table I. Whereas ir-ANF failed to differ between normotensive controls and cirrhotic patients, it may be seen that levels were significantly elevated in patients with cardiovascular disease. Hypertensive patients displayed a sixfold increase in plasma ANF as compared to normotensive controls. A subgroup of untreated patients with essential hypertension had comparably high levels. Patients with congestive heart failure displayed an 8-fold increase in plasma-ANF. Plasma concentrations in heart transplant recipients were 3-fold higher than in control subjects but significantly lower than in hypertensive patients ($p < 0.01$).

To evaluate the phasic response of the ANF system, cardiovascular patients were subjected to right ventricular pacing, a stimulus established to elicit a release of ANF [22]. Further, cirrhotic patients were subjected to head-out water immersion for augmentation of central venous pressure [6, 10, 15]. Thereby stimulated ANF plasma levels are given in table II. Electric pacing in heart transplant recipients increased ir-ANF to $207 \pm 22\%$ ($p < 0.01$) of baseline levels. In comparison, ir-

ANF in patients with congestive heart failure increased to 134± 13% of baseline levels. This increase was significantly less than in heart transplant recipients ($p < 0.01$) and considerably lower than in 2 hypertensive patients, in which ir-ANF levels increased to 367% of baseline values.

In control subjects, head-out water immersion induced an increase in ANF plasma levels to 207± 30% following 1 hour of immersion. The increase in ANF plasma levels was accompanied by a marked renal response. Urinary sodium excretion increased from 0.16± 0.02 to 0.31± 0.03 mmol/min and the volume of urine from 1.57± 0.25 to 5.28± 0.63 ml/min. The increase in ANF concentrations seen in cirrhotic patients without ascites (204± 16% of basal values) did not differ from that in normal subjects. However, cirrhotic patients with ascites displayed a lesser increase in ANF plasma levels to 146± 18%. This difference was paralleled by a lower increase in sodium excretion. Sodium excretion in cirrhotics without ascites increased by 0.11± 0.04 mmol/min as compared to cirrhotic patients with ascites (by 0.07± 0.02 mmol/min). Urine volume in patients without ascites increased by 2.34± 0.8 ml/min and in cirrhotics with ascites by 0.65± 0.32 ml/min.

An initial structural analysis of plasma ANF was performed by use of HPGPC in plasma extracts of 5 normotensive controls, 11 hypertensive and 13 patients with congestive heart failure. A cumulative analysis revealed that in controls 8± 5% of total ir-ANF eluted as 69-kd-ANF as compared to 16± 6% in hypertensive patients or 6± 2% in heart failure patients (not significant). However, in heart failure patients, 18± 3% of total ir-ANF co-eluted with the main peak from rat atrial extract (Pro-ANF or 15-kd-ANF); this portion was significantly greater ($p < 0.05$) than in control subjects or in hypertensive subjects (5± 2 or 9± 2%). More severely compromised patients tended to display higher amounts of 15-kd-ANF.

The chromotographic analysis of ir-ANF in a single, heart transplant recipient prior and subsequent to ventricular pacing is depicted in Fig. 1. It may be seen that the main fraction elutes as low molecular weight-ANF, in addition, two earlier peaks can clearly be separated. As yet, a total of 4 heart tansplant recipients have been thus analysed: 16.2± 7.4% or ir-ANF eluted as 15-kd-ANF; following pacing this fraction decreased slightly to 13.0± 4.2%. Conversely, a patient with congestive heart failure displayed an increase from 3 to 12% in 15-kd-ANF following electrical pacing.

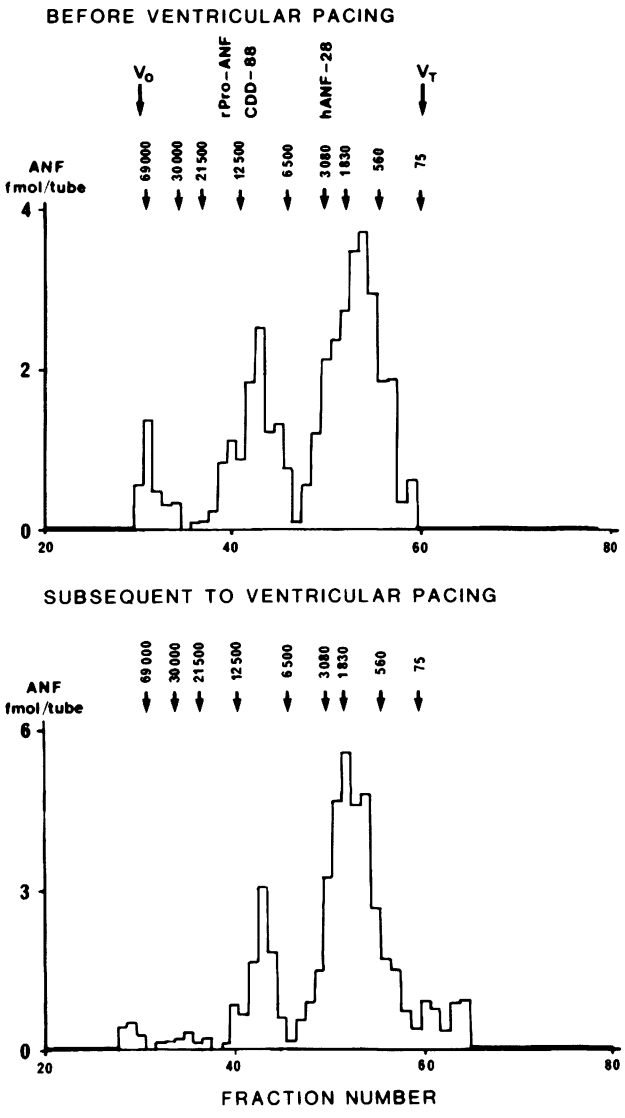

Fig. 1. Molecular weight pattern of ir-ANF in a single heart transplant recipient prior to (upper panel) and subsequent to (lower panel) ventricular pacing. The TSK-125 Bio Sil chromatography system separates compounds in accordance with their respective molecular size. V_o = void volume, bovine serum albumin; V_t = total volume, ^{3}H-glycin; *r* Pro-ANF = rat ANF_{1-126}; CDD-88 = bovine ANF_{1-88} (gift from Professor Forssmann, Heidelberg); *h* ANF-28 = human ANF_{99-126}. The molecular weight standards are indicated by arrows.

Discussion

Notwithstanding intensive study, mechanism of disturbed renal sodium handling in patients with cirrhosis are not, as yet, completely understood. It has been hypothesized that a failure to adequately elaborate a natriuretic hormone contributes to sodium retention in cirrhotics [9, 19]. However, our data demonstrate that baseline plasma levels of ANF in cirrhotic patients are not lower than in healthy counterparts (table 1).

It is possible, that despite the expanded extracellular volume in cirrhotics, splanchnic sequestration of fluids may prevent an increase in central volume and atrial pressure, and thus release may not be adequate to counteract the volume overload. It has, however, been reported that massive ascites may actually increase atrial pressure and thus should constitute a stimulus for ANF release [16]. However we were unable to demonstrate a difference in basal plasma levels between cirrhotic patients with and without ascites (table 2). Indeed, it may not be possible from an analysis of plasma levels alone to convincingly address the following questions: Firstly, is a stimulus which instigates a release of ANF in normal healthy subjects similarly capable of accelerating the secretion of ANF in cirrhotics? Secondly, is the ANF secretion elicited quantitatively adequate to exercise a functional effect? Thirdly, is the ir-ANF – material actually comprised of authentic ANF? Essentially, the central issue is as to whether the increased level of ANF in cirrhotic patients is sufficient to exert the desired effect in the target tissues. We and other have previously demonstrated that head-out water immersion in normotensive controls is an effective stimulus for ANF release [10, 15]. Water immersion elicits an increase in right atrial pressure via a distribution of blood to the intrathoracic venous bed [6] and may thereby evoke an increase in the release of ANF. As may be seen from table II, there was no difference in the response to water immersion between controls and cirrhotic patients without ascites. On the other hand, there was a blunted increase of ANF secretion in those cirrhotic patients exhibiting ascites. This diminished elevation in ANF corresponded to a blunted renal response (electrolyte secretion and urine volume) to immersion. Initial chromatographic analysis revealed no qualitative difference in the pattern of ir-ANF between cirrhotic patients and their normotensive counterparts [4]. The renal response, in this study, tended to be suppressed in the cirrhotic group, but not significantly so.

In contrast, patients suffering from various types of cavdiovascular disease (see table 1) manifested considerably elevated plasma levels of ir-ANF. The rise in the heart transplant group, was significantly lower than in the hypertensive patients although both groups displayed a comparable elevation in arterial pressure. The relatively lower levels in the transplant group may reflect the absent innervation of the grafted heart or be a consequence of immunosuppressive therapy.

In spite of overall, tonically elevated plasma levels in cardiovascular disease, the secretory atrial apparatus in these patients may, in fact, have surrendered its ability to react phasically to rapid, transient stimuli with a further facilitation of release. In addition, tonically maintained high plasma levels might result in a down-regulation of target receptors. Ventricular pacing mimics a rapid and transient increase in atrial pressure by atrial 'plugging' (Vorhofpfropfung) [22]. Pacing was performed in congestive heart failure patients, heart transplant recipients and 2 hypertensive patients without hypertensive heart disease exhibiting unaltered baseline levels of ANF. As shown in table 2, ANF levels rose significantly in transplanted patients, whereas the increase in heart failure pa-

Table 1. ANF-Immunoreactivity in Plasma

	Mean ± S.E.M. [fmol/ml]
Normotensive controls ($n = 50$)	9.6 ± 1.0
Hyertensive patients ($n = 36$)	61.7 ± 13.2*
Congestive heart failure patients ($n = 17$)	81.5 ± 32.7*
Heart transplant recipients ($n = 14$)	31.2 ± 7.9*
Cirrhotic patients ($n = 41$)	9.0 ± 1.3°

° n.s., * $p < 0.01$, t-test

Table 2. Stimulus-response-coupling of ANF

		Before	After
		[fmol/ml]	[fmol/ml]
a.)	Ventricular pacing		
	Heart transplant recipients ($n = 14$)	31.2 ± 7.9	54.8 ± 11.9**
	Congestive heart failure ($n = 5$)	42.1 ± 11.0	51.6 ± 8.0°
	Hypertensive patients with normal baseline levels ($n = 2$)	7.6	26.8
b.)	Water immersion		
	Control subjects ($n = 22$)	6.4 ± 0.6	13.9 ± 2.8*
	Cirrhotic patients without ascites ($n = 10$)	8.6 ± 1.5	16.8 ± 2.9*
	Cirrhotic patients with ascites ($n = 8$)	8.8 ± 1.4	12.8 ± 2.4

° not significant, ** $p < 0.01$, * $p < 0.05$, paired t-test

tients failed to achieve statistical significance. In both heart failure patients and transplant recipients the response was significantly less pronounced as in the 2 hypertensive patients. This may suggest that the ability of the ANF secretory mechanism to react to a phasic stimulus is compromised in heart failure and transplant recipients.

We have previously demonstrated that the molecular weight pattern of ir-ANF in patients with cardiovascular disease can differ from that in normotensive subjects suggesting a modification of post-translational processing of ANF [4]. In the present study, in which a larger population of patients were examined, the molecular weight pattern of ir-ANF also significantly differed from that shown by control subjects. 18% of total ir-ANF was comprised of 15-kd-ANF (Pro-ANF?) in heart failure patients. Four heart transplant recipients as yet analysed revealed a similar pattern: however subsequent to pacing this fraction decreased slightly whereas in one heart failure patient pacing even led to an increase in 15-kd-ANF.

We conclude that an analysis of baseline plasma levels of ANF may allow for the detection of an altered level of secretion of atrial secretory apparatus. However, in the presence of tonically elevated plasma levels in the pathological states of heart failure and transplant the phasic ability of the ANF system (perhaps physiologically more important) to swiftly react to transient stimuli may be impaired. In addition, heart failure patients may also show a qualitatively altered molecular pattern of ANF secretion. As performed here, a quantitative and qualitative (chromatographic) analysis of stimulated ANF plasma levels may eventually prove of value in the biochemical classification of heart disease.

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