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Different behaviour of the *N*-terminal and *C*-terminal fragment of proatrial natriuretic factor in plasma of healthy subjects as well as of patients with cirrhosis

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Gerbes AL, Vollmar AM, Thibault G, Arendt RM, Cantin M, Paumgartner G. Different behaviour of the *N*-terminal and *C*-terminal fragment of proatrial natriuretic factor in plasma of healthy subjects as well as of patients with cirrhosis. Scand J Clin Lab Invest 1990; 50: 195–198.

N-terminal (atrial natriuretic factor (ANF) 1–98) and *C*-terminal (ANF 99–126) fragments of proatrial natriuretic factor (NTA and CTA, respectively) were determined in plasma of healthy subjects adopting different postures and in patients with cirrhosis. Seven healthy subjects were investigated while seated and 30 min after assuming a horizontal position. NTA plasma concentrations increased in subjects in the horizontal position (from 734±250 (SE) fmol/ml to 902±227 fmol/ml; $p<0.05$). In contrast, CTA plasma concentrations remained unchanged (9.2±1.3 fmol/ml *vs* 8.9±1.6 fmol/ml). In 10 patients with cirrhosis of the liver, NTA concentrations were markedly ($p<0.001$) elevated compared to 11 healthy subjects (2334±291 fmol/ml *vs* 743±155 fmol/ml). However, there was no difference of CTA plasma levels between cirrhotic patients and healthy subjects (8.7±1.3 fmol/ml *vs* 8.2±0.9 fmol/ml). These data demonstrate changes of the plasma concentration of the N-terminal fragment of proatrial natriuretic factor by posture and in liver disease, in contrast to unchanged levels of the *C*-terminal fragment.

Key words: atrial natriuretic factor (ANF); cirrhosis; posture

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Proatrial natriuretic factor (atrial natriuretic factor (ANF) 1–126) is stored in atrial granules, released and cleaved upon appropriate stimulation; the *C*-terminal fragment, CTA (ANF 99–126) has been identified as biologically active circulating peptide (for review see [1, 2]).

Recently, presence of the *N*-terminal fragment NTA (ANF 1–98) has been demonstrated in plasma of rat and man [3, 4] and there is increasing evidence for cosecretion of NTA with CTA [3–5]. Plasma concentrations of NTA have been found several-fold higher compared to

respective CTA concentrations [3–6], probably owing to a longer half-life of NTA [3]. Therefore, an increase of pro-ANF release may be more sensitively detected by determining the *N*-terminal than the *C*-terminal fragment in plasma. To test this hypothesis, in the present study NTA and CTA plasma concentrations were determined upon a change of posture, which may be regarded as a subtle stimulus of pro-ANF release.

More pronounced elevations of NTA than of CTA have been observed in plasma of patients with chronic renal failure, suggesting a role of the kidney in eliminating NTA from the circulation [4]. The possible role of the liver, the other major organ for clearance of endogenous compounds, in the elimination of NTA has not yet been addressed. Therefore, in the present study NTA and CTA plasma concentrations were determined in patients with chronic liver disease.

MATERIALS AND METHODS

Subjects

Eleven healthy subjects, aged 25 to 65 (mean 37±4) years and 10 patients with cirrhosis of the liver (histologically confirmed, mostly in advanced stage with ascites), aged 47 to 75 (mean 58±3) years, were investigated (sitting position) after their informed consent had been obtained. The subjects showed no evidence of cardiovascular, renal or pulmonary disease. They were on a regular diet, containing approximately 150 meq sodium/day.

Change of posture

Seven healthy subjects, aged 20 to 65 (mean 35±6) years were investigated. Blood samples were taken after they had been sitting comfortably on a chair for 30 min. Thereafter, they assumed a horizontal position, lying on a bed in supine position. Thirty min after this change of posture another blood sample was taken. Heart rate and blood pressure were recorded in both positions.

Determination of ANF

CTA was determined by radio-immunoassay (RIA) with antibody 'Toni III' (no evidence of cross-reactivity with NTA), as described in detail previously [7]. The RIA procedure for NTA with antiserum 'GT 23' (no cross-reactivity with CTA detectable) has been reported in detail [3]. Immunoreactivity has been identified as CTA and NTA, respectively, by high-performance liquid chromatography (HPLC) techniques [5, 7]. Plasma renin activity was determined as described by Wernze *et al.* [8].

Statistical evaluation

Differences of CTA and NTA plasma concentrations, of plasma renin activity, heart rate and mean arterial pressure, induced by the change of posture were calculated by paired t-test. CTA and NTA plasma levels of healthy subjects and controls were compared by unpaired t-test. A value of $p<0.05$ was considered significant. Data are presented as mean and standard error.

RESULTS

Change of posture

NTA concentrations in seven healthy subjects were about a hundred-fold higher ($p<0.001$) than the corresponding CTA concentrations (Table I). The change of posture to the supine position did not affect CTA levels, whereas NTA concentrations significantly increased ($p<0.05$). Mean arterial pressure did not change, the heart rate decreased ($p<0.01$) and plasma renin activity tended to decrease ($p<0.08$).

ANF in patients with cirrhosis

In sitting position, CTA concentrations in 10 patients with cirrhosis were not different from those in 11 healthy subjects (8.7±1.3 fmol/ml *vs*

TABLE I. Effects of change of posture in seven healthy subjects

	Sitting	Supine	p
C-terminal ANF (fmol/ml)	8.9±1.6	9.2±1.3	—
N-terminal ANF (fmol/ml)	734±250	902±227	<0.05
Plasma renin activity (ng AI/ml/h)	2.6±0.5	1.6±0.3	<0.08
Heart rate (min-1)	69.7±3.6	61.1±2.1	<0.01
Mean arterial pressure (mmHg)	93.1±3.6	95.7±6.1	—

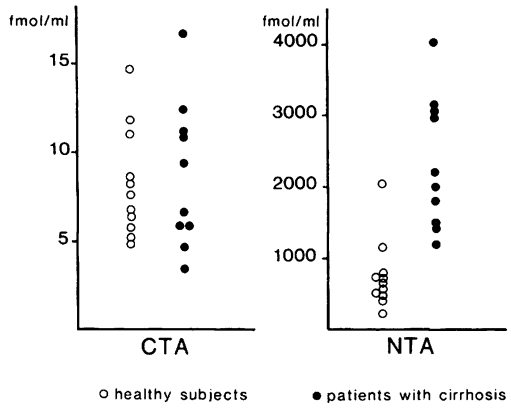

FIG. 1. Plasma concentrations of *C*-terminal (CTA) and *N*-terminal (NTA) fragments of proatrial natriuretic factor in 11 healthy subjects (○) and 10 patients with cirrhosis (●).

8.2±0.9 fmol/ml). However, mean plasma concentrations of NTA were markedly ($p<0.001$) higher in cirrhosis than in controls (2334±291 fmol/ml *vs* 743±155 fmol/ml) (Fig. 1). Renal function, as estimated by serum creatinine concentrations was no different in patients with cirrhosis when compared with healthy subjects (1.10±0.03 mg/100ml *vs* 1.03±0.05 mg/100ml).

DISCUSSION

The influence of posture on plasma concentrations of *C*-terminal ANF has been the subject of some controversy, with some authors observing differences [9, 10] while others did not [11, 12]. In the present study, the concentration of the *C*-terminal fragment was not influenced by the change from sitting to supine position. However, the change of posture initiated a significant increase of *N*-terminal fragment concentrations. The decrease of heart rate as well as that of plasma renin activity in the supine position suggests a subtle increase of central vascular filling, which may be regarded as a stimulus of pro-ANF release. Thus, the likely augmentation of ANF release by the change of posture was reflected by the change of NTA concentrations, and not reflected by CTA determinations.

Whereas many authors agree that CTA plasma levels are unchanged in cirrhosis [13], some report elevated levels (for review see [14]). In the present study, we found no difference in the plasma concentrations of CTA in patients with cirrhosis or healthy subjects. However, concentrations of the *N*-terminal fragment were elevated in cirrhosis by about threefold. This finding might be compatible with the contention of increased release of ANF in cirrhosis [15], but only in the presence of an enhanced clearance of *C*-terminal ANF.

Since clearance of CTA seems unaltered in cirrhosis [16], this hypothesis is rather unlikely. Another explanation for increased NTA at normal CTA concentrations could be an impaired elimination of the *N*-terminal fragment in cirrhosis. Renal elimination of NTA has been suggested, based on the observation of more marked elevations of NTA than of CTA in patients with chronic renal failure [4]. In the cirrhotic patients of this study, normal serum creatinine levels make significant renal impairment rather unlikely. However, in cirrhosis, renal blood flow can be reduced at unchanged serum creatinine concentrations; thus, the possibility cannot be excluded that the observed increase of NTA in cirrhosis could be partly caused by decreased renal elimination. Furthermore, influence of age cannot be excluded since patients and controls were not age-matched. However, the striking elevations of NTA observed in patients with cirrhosis suggest that the liver plays a role in the elimination of NTA from the circulation.

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REFERENCES

1 Cantin M, Genest J. The heart as an endocrine gland. Sci Am 1986; 254: 76–81.
2 Needleman P. Atriopeptin: biochemical pharmacology. Federation Proc 1986; 45: 2096–100.

3 Thibault G, Murthy KH, Gutkowska J *et al.* NH_2-terminal fragment of rat pro-atrial natriuretic factor in the circulation: identification, radioimmunoassay and half-life. Peptides 1988; 9: 47–53.
4 Sundsfjord JA, Thibault G, Larochelle P, Cantin M. Identification and plasma concentrations of the *N*-terminal fragment of proatrial natriuretic factor in man. J Clin Endocrinol Metabol 1988; 66: 605–10.
5 Gerbes AL, Vollmar AM. Water immersion increases the concentration of the immunoreactive *N*-terminal fragment of proatrial natriuretic factor in human plasma. Biochem Biophys Res Comm 1988; 156: 228–32.
6 Gauquelin G, Thibault G, Cantin M, Schiffrin EL, Garcia R. Glomerular atrial natriuretic factor receptors during rehydration: plasma NH_2- and COOH-terminal levels. Am J Physiol 1988; 255: F621–5.
7 Arendt RM, Gerbes AL, Ritter D, Stangl E. Differential processing of plasma atrial natriuretic factor in cardiovascular disease. In: Brenner BM, Laragh JH, eds. Biologically Active Atrial Peptides, Vol I. New York: Raven Press, 1987: 544–7.
8 Wernze H, Spech HJ, Müller G, Schweinsfurth H, Schöner H, Brügel H. The renin-angiotensin-aldosterone system (RAAS) in chronic liver disease. In: Addison GM *et al.*, eds. Aldosterone Antagonists in Clinical Medicine. Amsterdam: Excerpta Medica 1978: 394–404.
9 Larose P, Meloche S, du Souich P, De Léan A, Ong H. Radioimmunoassay of atrial natriuretic factor: human plasma levels. Biochem Biophys Res Comm 1985; 130: 553–8.
10 Ogihara T, Shima J, Hara H, Kumuhara Y, Kangawa K, Matsuo H. Changes in human plasma atrial natriuretic polypeptide concentration in normal subjects during passive leg raising and whole-body tilting. Clin Sci 1986; 71: 147–50.
11 Hodsman GP, Phillips PA, Ogawa K, Johnston CI. Atrial natriuretic factor in normal man: effects of tilt, posture, exercise and hemorrhage. J Hypertension 1986; 4 (Suppl. 6): 503–5.
12 Jüppner H, Brabant G, Kapteina U, Kirschner M, Klein H, Hesch RD. Direct radioimmunoassay for human atrial natriuretic peptide (hANP) and its clinical evaluation. Biochem Biophys Res Comm 1986; 139: 1215–23.
13 Gerbes AL, Wernze H, Arendt RM, Riedel A, Sauerbruch T, Paumgartner G. Atrial natriuretic factor and renin–aldosterone in volume regulation of patients with cirrhosis. Hepatology 1989; 9: 417–422.
14 Gerbes AL, Arendt RM, Paumgartner G. Editorial review. Atrial natriuretic factor — possible implications in liver disease. J Hepatol 1987; 5: 123–32.
15 Gines P, Jiménez W, Arroyo V *et al.* Atrial natriuretic factor in cirrhosis with ascites: plasma levels, cardiac release and splanchnic extraction. Hepatology 1988; 8: 636–42.
16 Henriksen JH, Schütten HJ, Bendtsen F, Warberg J. Circulating atrial natriuretic peptide (ANP) and central blood volume (CBV) in cirrhosis. Liver 1986; 6: 361–8.

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