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PRESENCE OF THE ATRIAL NATRIURETIC FACTOR (ANF) IN HUMAN ASCITIC FLUID

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Summary

Presence of atrial natriuretic factor (ANF)-like material was demonstrated by radioimmunoassay in ascitic fluid of 14 patients with cirrhosis of the liver. Immunoreactive ANF concentrations (M+SEM) were 2.4 + 0.5 fmol/ml in ascites, significantly lower ($p < 0.001$) than the corresponding plasma concentrations of 15.5 + 2.6 fmol/ml. High performance gel permeation chromatography and reverse phase high performance chromatography of the ascitic ANF immunoreactivity showed correspondence to the alpha human ANF(99-126). ANF levels in ascites were significantly ($p < 0.01$) correlated to levels in plasma ($r = 0.66$).

In patients with cirrhosis of the liver and ascites the pathophysiological role of ANF has not been fully elucidated (1,2). Following intravenous reinfusion of ascites elevated ANF plasma levels have been reported (3,4,5). This might be due to ANF release stimulated by the hemodynamic changes following ascites infusion or due to elevated ANF concentrations in ascitic fluid. Whereas the presence of atrial natriuretic factor (ANF) has been demonstrated in various fluids of the human body, such as plasma (6), urine (7) and cerebrospinal fluid (8), ascitic fluid has not been examined as yet for the occurence of ANF. In this study the presence of ANF in ascitic fluid was investigated .

Subjects and Methods

Fourteen patients (11 men, 3 women, aged 42 to 81 years) with biopsy proven cirrhosis of the liver and ascites were investigated.

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Determination of ANF by radioimmunoassay. Simultaneously obtained samples of ascitic fluid and peripheral venous blood were drawn into pre-cooled syringes and immediately transferred to pre-cooled polystyrene tubes containing 500 kallikrein inhibitor units aprotinin per ml and 1 mg sodium EDTA per ml. Samples were centrifuged at 4° C, the supernatant was immediately frozen and stored at - 80° C. Extraction of ascites aliquots as well as RIA procedures were performed in the same way as for plasma samples; procedures were modified from (6) and have been detailed elsewhere (9). Briefly, samples were extracted by adsorption to Amberlite XAD-2 adsorbent resin. The final titer of the C-terminal directed antibody Toni III was 1:120000, crossreactivity to rat-pro ANF was 48% and the assay sensitivity was 0.5 fmol alpha human ANF/tube. The 50% binding intercept of the standard curve was 10 fmol.

Chromatographic analysis of ANF immunoreactivity. Ascitic fluid extracts of 4 patients were subjected to high performance gel permeation chromatography (HPGPC) and reverse phase high performance chromatography (RP-HPC).
High performance gel permeation chromatography (HPGPC): Lyophilized ascitic fluid (10 ml) was dissolved in 25µl column eluens and applied to a Spherogel TM TSK, 2000 SW column (10µm, 7.5mm x 300 mm, Beckman Instruments, San Ramon, CA, USA), eluted with 0.09% trifluoroaceticacid (TFA) containing 0.005M Na_2SO_4, 0.002M NaH_2PO_4 and 30% acetonitrile (flowrate: 0.3 ml/min). Calibration was carried out with bovine serum albumin (BSA) (V_0), vitamin B12 (V_t), rat pro-ANF (2-126) and alpha human ANF (99-126). Immunoreactive ANF (IR-ANF) fractions (0.6ml), detected by RIA, were pooled and lyophilized.
Reverse phase high performance chromatography (RP-HPC): An aliquot of the pooled IR-ANF fractions of the HPGPC run was redissolved in 25 µl 0.1% TFA and loaded on a HPLC C 18 ODS Ultrasphere TM column (5µm, 2mmx150mm, Beckman Instruments, San Ramon, USA) according to (10). Elution was carried out with a linear gradient of acetonitrile (10-80%, 45 min) in 0.1%TFA (flowrate: 0.2ml/min). Calibration of the column was performed with synthetic atriopeptin I and III, alpha human ANF (99-126) and rat pro-ANF (2-126). Fractions (0.4ml) were assayed for ANF immunoreactivity.

Statistical evaluation. Differences between ascitic and plasmatic ANF concentrations were compared by paired t-test; the Pearson correlation coefficient was determined by the usual linear least squares test. Data are presented as mean and standard error (M±SEM).

Results

Radioimmunoassay

Serial dilutions of ascites were parallel to the standard curve, as shown in figure 1. Recoveries of 7.8 and 15.6 fmol synthetic human alpha ANF added to samples of ascites and plasma prior to extraction were 84% and 67% in ascites and 75% and 65% in plasma. Nonspecific binding, determined in the RIA without antiserum was less than 5% in both ascites and plasma. Ascitic ANF concentrations were 2.4 ± 0.5 fmol/ml, significantly ($p < 0.001$) lower than ANF concentrations in plasma (15.5 ± 2.6 fmol/ml). ANF levels

in ascites were significantly correlated to ANF plasma levels ($r = 0.66$, $p < 0.01$; figure 2).

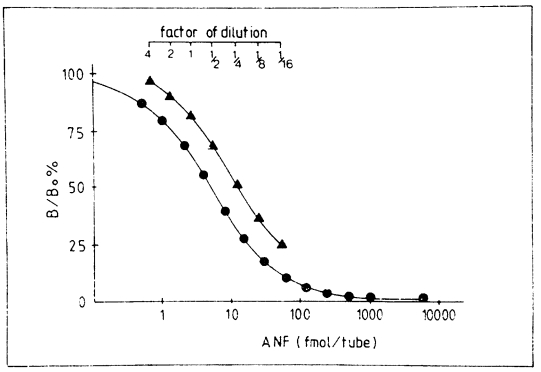

FIG. 1

A standard curve for radioimmunoassay of alpha human ANF (circles) in comparison to serial dilutions of ascitic fluid (triangles). Each point is the mean of two experiments. Dilution curves parallel the standard curves.

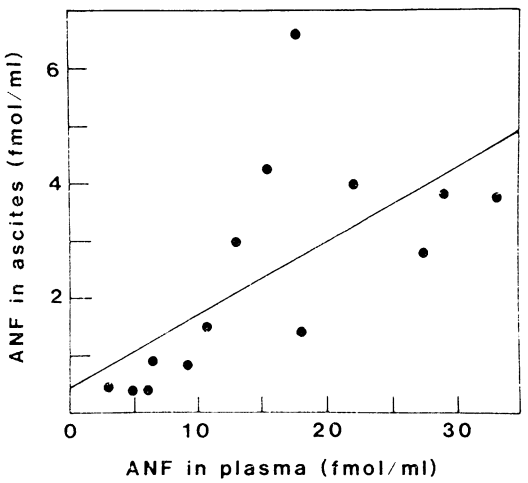

FIG. 2

Significant correlation of ANF concentrations in ascites and in plasma of 14 patients with cirrhosis of the liver ($r = 0.66$, $p < 0.01$).

HPLC characterization of immunoreactive ANF

When pre-extracted ascitic fluid was chromatographed on HPGPC and reverse phase HPLC, immunoreactive ANF was detected as a single peak coeluting with synthetic alpha human ANF. No significant amounts of higher molecular weight ANF-like material such as precursor pro-ANF was detected, as demonstrated in figure 3.

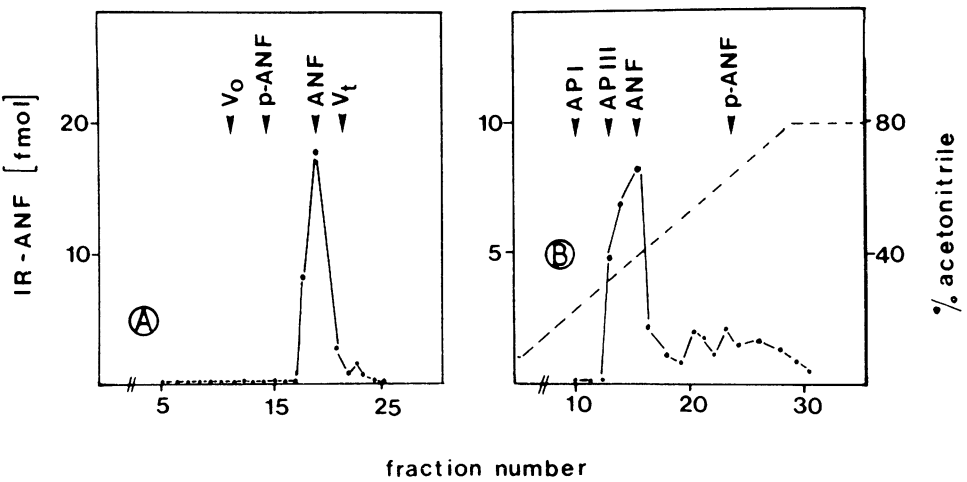

FIG. 3

HPLC analysis of immunoreactive ANF in ascitic fluid

A: HPGPC: The void (V_o) and total (V_t) volumes of the column were determined by BSA and vitamin B 12, respectively. The elution positions of synthetic rat-pro-ANF (2-126) (p-ANF) and alpha human ANF (ANF) are indicated by arrows. Fractions were tested for immunoreactive ANF by RIA.

B: Reverse phase HPLC: Arrows indicate the elution positions of atriopeptin I (AP I), atriopeptin III (AP III), alpha human ANF (ANF) and rat pro- ANF (2-126) (p-ANF). Immunoreactive ANF was evaluated by RIA.

Discussion

This study demonstrates the presence of ANF in ascitic fluid of patients with cirrhosis of the liver. Extraction procedures as well as characteristics of our highly sensitive and specific radioimmunoassay for determination of ANF in plasma have been previously described (6,9). Application of the extraction procedure to aliquots of ascitic fluid yielded high recovery rates, not different from those obtained in plasma. Validity of the RIA for ANF measurement in ascites was demonstrated by parallelity of serial dilution curves with the standard curve and by the absence of significant binding interference.

In an attempt to further characterize ANF immunoreactivity in ascites, the molecular weight pattern was investigated by high performance gel permeation chromatography and reverse phase HPLC. Whereas trace amounts of higher molecular weight ANF in plasma of patients with cirrhosis had been reported (9), ANF immunoreactivity in ascites coeluted with synthetic alpha human ANF, the main circulating form in human plasma. However, due to the low concentrations of ANF in ascites and to a 48% only crossreactivity of our antibody to pro-ANF (9), the presence of small amounts of higher molecular weight ANF in ascites cannot be excluded.

ANF concentrations in ascites were found to be significantly correlated to ANF plasma concentrations. This correlation as well as characterization of ascitic ANF as alpha ANF, the major circulating form of ANF, may allow the speculation that ascitic ANF is of plasma origin. ANF concentrations in ascites were found to be significantly lower than in plasma. Thus, elevations of ANF levels in plasma observed following infusion of ascitic fluid (3,4,5) seem not to be due to increased levels of ANF in ascitic fluid, but rather due to increased release of ANF following the hemodynamic changes of infusion.

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References

1. A.L. GERBES, R.M. ARENDT, D. RITTER, D. JÜNGST, J. ZÄHRINGER and G. PAUMGARTNER, N. Engl. J. Med. 313 1609-1610 (1985).
2. A.L. GERBES, R.M. ARENDT and G. PAUMGARTNER, J. Hepatology 5 123-132 (1987).
3. W. BURGHARDT, H. WERNZE and K.L. DIEHL, Klin. Wochenschr. 64 (Suppl VI) 103-107 (1986).
4. P. CAMPBELL, L.M. BLENDIS, K. SKORECKI, A. LOGAN, P.Y. WONG and P. GREIG, Hepatology 6 1120 (1986).
5. C.L. WITTE, A.P. MARTINEZ and M.H. WITTE, N. Engl. J. Med. 316 487 (1987).
6. R.M. ARENDT, E. STANGL, J. ZÄHRINGER, D.C. LIEBISCH and A. HERZ, FEBS Lett. 189 57-61 (1985).
7. F. MARUMO, H. SAKAMOTO, K. ANDO, T. ISHIGAMI and M. KAWAKAMI, Biochem. Biophys. Res. Comm. 137 231- 236 (1986).
8. F. MARUMO, T. MASUDA and K. ANDO, Biochem. Biophys. Res. Comm. 143 813-818 (1987).
9. R.M. ARENDT, A.L. GERBES, D. RITTER and E. STANGL, Klin. Wochenschr. 64 (suppl VI) 97-102 (1986).
10. A.M. VOLLMAR, R.M. ARENDT and R. SCHULZ, Eur. J. Pharmacol. 143 315-321 (1987).