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Atrial Natriuretic Factor and Renin-Aldosterone in Volume Regulation of Patients with Cirrhosis

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The role of the atrial natriuretic factor and of the main counteracting sodium-retaining principle, the renin-aldosterone system, in acute volume regulation of cirrhosis of the liver has been investigated. Central volume stimulation was achieved in 21 patients with cirrhosis, 11 without and 10 with ascites, and 25 healthy controls by 1-hr head-out water immersion. Immersion prompted a highly significant ($p < 0.001$) increase of atrial natriuretic factor plasma concentrations in cirrhotic patients without ascites from 8.5 ± 1.3 fmoles per ml to 16.5 ± 2.6 fmoles per ml, comparable to the stimulation in control subjects (6.0 ± 0.6 fmoles per ml to 13.6 ± 2.6 fmoles per ml). In cirrhotic patients with ascites, atrial natriuretic factor increase (from 7.7 ± 1.3 fmoles per ml to 11.4 ± 2.3 fmoles per ml) was blunted ($p < 0.05$). Plasma renin activity and plasma aldosterone concentration were elevated in cirrhotic patients, especially in the presence of ascites. Following immersion, plasma renin activity and plasma aldosterone concentration were reduced similarly in all groups. Water immersion induced a more pronounced natriuresis and diuresis in control subjects than in cirrhotic patients. Neither atrial natriuretic factor nor plasma renin activity nor plasma aldosterone concentration alone correlated to sodium excretion. However, atrial natriuretic factor to plasma aldosterone concentration ratios were closely correlated to basal and stimulated natriuresis in cirrhotic patients, particularly in those with ascites. These data suggest that atrial natriuretic factor and the renin-aldosterone system influence volume regulation in patients with cirrhosis.

Current concepts of the pathophysiology of renal sodium retention and ascites formation in patients with cirrhosis of the liver have progressively become more complex (1). Stimulation of the renin-aldosterone system is still considered important for the impaired volume regulation of cirrhosis (2), but there is increasing evidence for the involvement of other humoral factors (3). For many years, a deficiency of a putative natriuretic hormone in cirrhosis has been postulated (4, 5), but due to difficulties with precise characterization and quantification, the role of this hormone has not been satisfactorily elucidated (6). Since the discovery of the well-defined atrial natriuretic factor [ANF; for review, see Refs. (7–12)], the role of this hormonal peptide in cirrhosis has been of major interest. It has been demonstrated that plasma levels of ANF, determined by radioimmunoassay, were not lower in cirrhosis than in healthy subjects (13). Although several laboratories have confirmed that there is no deficiency of plasma ANF in cirrhosis [for overview, see Ref. (10)], reported plasma levels vary in a wide range and presently the importance of ANF in volume regulation in healthy man as well as in patients with cirrhosis is ill defined.

Several observations suggest a counteraction of ANF with the sodium-retaining renin-aldosterone system (14–17): ANF inhibits the synthesis of aldosterone and blocks the release of renin as well as of aldosterone. Upon ANF infusion, a decline of plasma renin activity (PRA) and of plasma aldosterone concentration (PAC) were observed (18). It has been speculated that the sodium retention and activation of the renin-aldosterone system in cirrhosis may result from derangements of ANF release or action (19). Therefore, in the present study the relationship of both hormonal systems in the volume regulation of cirrhosis has been investigated.

The characterization of the reactivity to stimulation of these hormonal systems may allow pathophysiological insights not provided by the determination of basal plasma levels alone. As a tool for acute volume stimulation, head-out water immersion (WI) increases central venous and atrial volume by shifting blood from the peripheral vessels to the intrathoracic venous bed (20), obviating the necessity of infusing plasma expanders that might alter plasma composition (21).

Using this approach, the role of ANF and the counteracting renin-aldosterone system in acute volume regulation of patients with cirrhosis have been examined.

SUBJECTS AND METHODS

Twenty-five healthy subjects, age 19 to 65 (mean: 35 ± 3) years and 21 patients with cirrhosis (12 alcoholic, six hepatitic,

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three cryptogenic) age 42 to 75 (mean: 59 ± 2) years were investigated after informed consent had been obtained. The protocol was approved by the Ethical Committee of the Faculty of Medicine, University of Munich. The subjects showed no evidence of cardiovascular, renal, pulmonary or endocrine diseases. The diagnosis of cirrhosis was established by biopsy, clinical and laboratory data. In 11 patients, no ascites could be detected by abdominal ultrasound. In this group, nine patients were ranked Grade A and two Grade B according to a modified Child-Pugh classification (22, 23). In 10 patients, presence of ascites was confirmed by clinical investigation and abdominal ultrasound; six of them were graded B, and four were graded C according to Child-Pugh. They all had a history of longstanding, stable ascites. Further clinical and laboratory data of patients are reported in Table 1. Cirrhotic patients had been withheld diuretic treatment for 1 week prior to the study. All subjects were prohibited alcohol, tobacco, tea and coffee the day before and during the experiment and were on a hospital diet containing approximately 150 mEq sodium per day.

In the morning, after complete emptying of the bladder, an intravenous catheter was placed into a forearm vein. Subjects were given 400 ml of water orally and assumed a seated position next to the immersion tank. After 1 hr, the subjects were immersed to their neck, maintaining the same seated position, into thermoneutral water (34.5 ± 0.2°C) for 1 hr. This was followed by a 1-hr period of sitting outside the tank. Throughout the investigation, 200 ml per hr of water were given orally to ensure adequate urine flow. All the urine voided by spontaneous emptying of the bladder as well as plasma samples were collected before (baseline), after 1-hr WI (immersion) and 1 hr subsequent to the end of WI (recovery).

Urine was analyzed for sodium, potassium (flame photometry) and creatinine (Jaffe reaction). PRA was measured as described before (3). PAC was determined by use of a commercial kit (Diagnostic Products, Los Angeles, CA). ANF was measured in XAD-extracted plasma samples by radioimmunoassay, as described in detail earlier (24). Briefly, the antibody is mid-molecule- and C-terminal directed. Cross-reactivity was 70% to rat ANF 99-126, 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 (bovine thyroglobulin). The final titer was 1:120,000 and the assay sensitivity was 0.5 fmole per assay tube. The 50% binding intercept of the standard curve was 10 fmol. One-ml plasma aliquots were extracted by adsorption to pre-rinsed Amberlite XAD-2 adsorbent resin (particle size: 0.3 to 1.0 mm; Serva, Heidelberg, Federal Republic of Germany). Recovery of synthetic human ANF 99-126 was approximately 67% in plasma of healthy subjects and 70% in plasma of cirrhotic patients. Validity of the radioimmunoassay for ANF determination in both normal and cirrhotic plasma was demonstrated by parallelism of serial dilution curves with the standard curve and by the absence of significant binding interference.

Clearance of creatinine as well as fractional sodium excretion (FeNa) were calculated by standard formulas. Mean arterial pressure was calculated as the sum of diastolic pressure and one-third of the difference between systolic and diastolic pressure.

Data are given as mean and S.E. Random distribution of data was tested by the Kolmogorov-Smirnoff test. Statistical evaluation of randomly distributed data was performed by unpaired t test between groups and by paired t test within groups. Nonrandomly distributed data were analyzed by Mann-Whitney test between groups and by Wilcoxon test within groups. A p value of 0.05 or less was considered statistically significant. The Pearson correlation coefficient was determined by the usual linear least-squares method.

TABLE 1. Clinical and laboratory data of the patients with cirrhosis

	Without ascites (n = 11)	With ascites (n = 10)
Age (years)	61 ± 2	56 ± 3
Sex (male/female)	8/3	8/2
Child-Pugh score (A/B/C)	9/2/0	0/6/4
Bilirubin (mg/100 ml)	1.3 ± 0.2	2.2 ± 0.4
Prothrombin time (%)	73 ± 3	64 ± 4
Serum albumin (gm/100 ml)	4.1 ± 0.2	3.7 ± 0.2
Serum creatinine (mg/100 ml)	1.0 ± 0.1	1.2 ± 0.1
Mean arterial pressure (mmHg)	84.5 ± 2.9	78.3 ± 2.6
Heart rate (beats/min)	74.2 ± 2.3	82.8 ± 2.8

RESULTS

Atrial Natriuretic Factor. Basal ANF plasma concentrations of cirrhotic patients without ascites, cirrhotic patients with ascites and controls were not significantly different (Fig. 1). Volume stimulation by WI prompted a highly significant increase of ANF from 8.5 ± 1.3 fmoles per ml to 16.5 ± 2.6 fmoles per ml (104 ± 16%) in patients without ascites, comparable to the stimulation from 6.0 ± 0.6 fmoles per ml to 13.6 ± 2.6 fmoles per ml (117 ± 29%) in controls. However, in patients with ascites, WI caused an increase from 7.7 ± 1.3 fmoles per ml to 11.4 ± 2.3 fmoles per ml (46 ± 18%) only. Thus, ANF stimulation by WI was significantly smaller in ascitic patients as compared to patients without ascites ($p < 0.05$). In the recovery period, ANF decreased toward baseline levels, although in cirrhotic patients with ascites no marked fall of stimulated values could be observed.

Renin-Aldosterone System. Basal PRA was elevated in cirrhotic patients with ascites (28.9 ± 9.4 ng angiotensin I (AI) per ml per hr) as compared to healthy subjects (5.3 ± 0.9 ng AI per ml per hr (Figure 2). PRA levels in cirrhotic patients without ascites were slightly higher than normal (8.6 ± 2.8 ng AI per ml per hr). WI reduced PRA rather uniformly to about one-half the baseline values in patients with ascites (by 50 ± 6% to

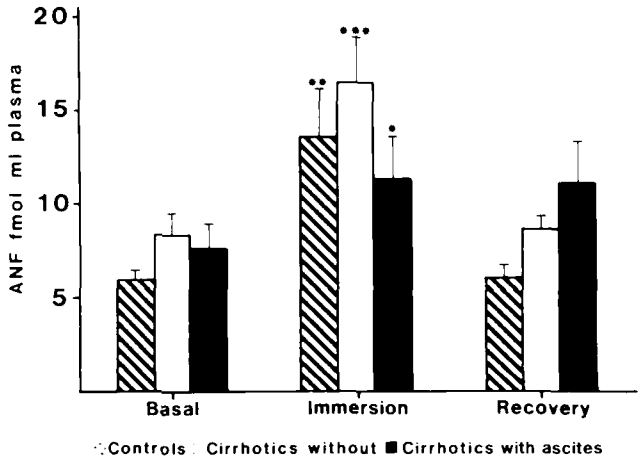

FIG. 1. ANF plasma concentrations in 25 healthy subjects, 11 cirrhotic patients without ascites and 10 cirrhotic patients with ascites before, during and following head-out water immersion. * = $p < 0.05$, ** = $p < 0.01$; *** = $p < 0.001$; significantly different from baseline values.

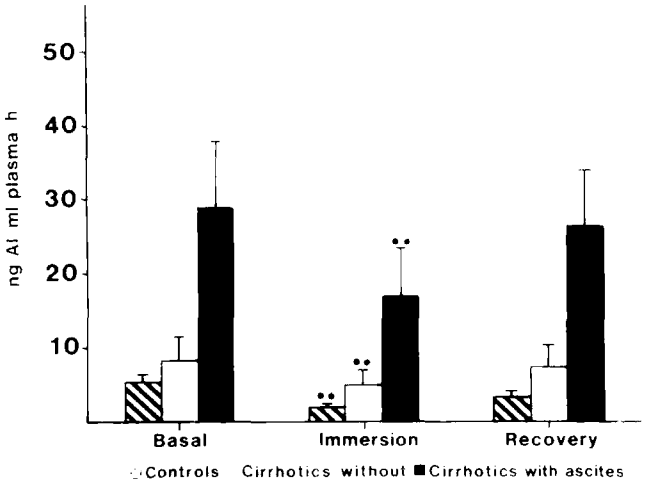

FIG. 2. Plasma renin activity in healthy subjects, cirrhotic patients without and cirrhotic patients with ascites before, during and following water immersion. ** = $p < 0.01$; significantly different from baseline values.

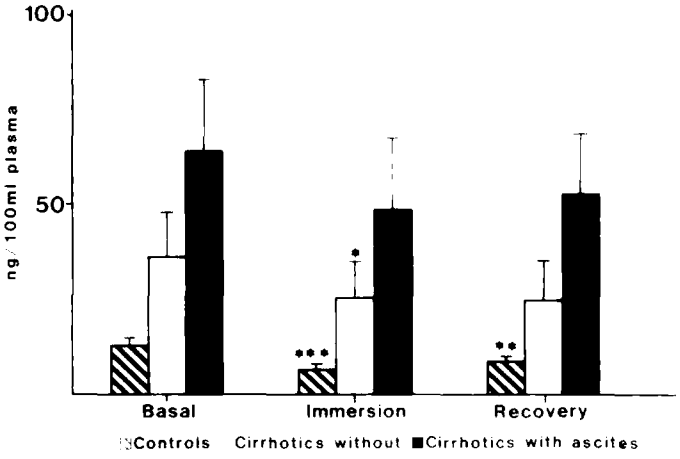

FIG. 3. Plasma aldosterone concentration in healthy subjects, cirrhotic patients without and cirrhotic patients with ascites before, during and following water immersion. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; significantly different from baseline values.

17.3 ± 6.9 ng AI per ml per hr), patients without ascites (by 43 ± 10% to 4.8 ± 1.8 ng AI per ml per hr) and controls (by 46 ± 8% to 2.4 ± 0.3 ng AI per ml per hr). In the recovery period, PRA returned to preimmersion levels in controls and cirrhotic patients.

PAC in cirrhotic patients with ascites was higher than in controls (63.9 ± 19.9 ng per 100 ml vs. 13.0 ± 1.7 ng per 100 ml), with levels of patients without ascites in between (38.1 ± 11.3 ng per 100 ml) (Fig. 3). WI significantly reduced PAC in controls (to 6.6 ± 0.8 ng per 100 ml) and in cirrhotic patients without ascites (to 26.6 ± 9.0 ng per 100 ml). The decrease in cirrhotic patients with ascites (to 49.1 ± 19.3 ng per 100 ml) did not quite reach significance ($p = 0.07$). When basal and immersion PRA values were plotted against the corresponding PAC values, significant correlations were seen in controls ($r = 0.56$, $p < 0.001$) as well as in cirrhotic patients ($r = 0.53$, $p < 0.001$).

Renal Response. The effects of immersion on renal excretion are displayed in Table 2. Mean increases of

TABLE 2. **Renal response to water immersion**

	Controls (n = 25)	Cirrhotics	
		Without ascites (n = 11)	With ascites (n = 10)
Urinary volume (ml/min)			
Basal	1.4 ± 0.2	1.3 ± 0.3	0.9 ± 0.2
Immersion	5.0 ± 0.6[c]	3.3 ± 1.0[a]	1.6 ± 0.4[f]
Recovery	2.8 ± 0.4[c]	1.6 ± 0.5	1.1 ± 0.6[d]
Urinary sodium excretion (μmoles/min)			
Basal	156 ± 19	74 ± 15[c]	28 ± 11[fg]
Immersion	302 ± 34[c]	149 ± 50[d]	71 ± 28[f]
Recovery	182 ± 28	102 ± 35	22 ± 7[fg]
Fractional sodium excretion (%)			
Basal	0.90 ± 0.12	0.56 ± 0.13	0.20 ± 0.05[fg]
Immersion	1.23 ± 0.16[b]	0.94 ± 0.29	0.35 ± 0.10[f]
Recovery	1.23 ± 0.17[a]	0.89 ± 0.24	0.20 ± 0.05[fg]
Creatinine clearance (ml/min)			
Basal	153 ± 26	119 ± 17	103 ± 26
Immersion	200 ± 32	109 ± 23[d]	96 ± 31
Recovery	117 ± 15	72 ± 14	63 ± 13[d]

$a = p < 0.05$; $b = p < 0.01$; $c = p < 0.001$; significantly different from baseline values.

$d = p < 0.05$; $e = p < 0.01$; $f = p < 0.001$; significantly different from corresponding values in controls.

$g = p < 0.05$; significantly different from values in cirrhotic patients without ascites.

urinary volume were highly significant in healthy subjects (3.64 ± 0.60 ml per min), still marked in cirrhotic patients without ascites (2.02 ± 0.81 ml per min) but not significant ($p > 0.1$) in cirrhotic patients with ascites (0.68 ± 0.35 ml per min). Similar differences were observed in urinary sodium excretion: WI induced a rise by 146 ± 38 μmoles per min in healthy subjects, by 75 ± 43 μmoles per min in patients without and by 43 ± 19 μmoles per min in patients with ascites. Increases of natriuresis as well as of fractional sodium excretion missed the level of significance in cirrhotic patients without (UNaV: $p = 0.13$, FeNa: $p = 0.10$) as well as in cirrhotic patients with ascites (UNaV: $p = 0.06$, FeNa: $p = 0.12$). Creatinine clearance was not significantly altered in any group throughout the experiment ($p > 0.1$). Neither basal nor stimulated natriuresis or diuresis nor the degree of renal response correlated to the corresponding values of ANF, PRA or PAC.

Relationship of Natriuresis with ANF and the Renin-Aldosterone System. The ratio of ANF over plasma aldosterone concentration (ANF/PAC) (Fig. 4) proved to be a useful marker of the apparent interaction of both systems. The ANF/PAC ratio increased from 0.65 ± 0.21 to 2.44 ± 0.73 fmoles per 10^{-2} ng in cirrhotic patients without ascites; this was not significantly different from the stimulation in controls (0.63 ± 0.11 to 3.29 ± 1.18 fmoles per 10^{-2} ng). In cirrhotic patients with ascites, however, the elevation from 0.38 ± 0.14 to 0.75 ± 0.24 fmoles per 10^{-2} ng was significantly ($p < 0.05$) smaller than in cirrhotic patients without ascites or in controls. In cirrhosis, basal natriuresis (Fig. 5, $r = 0.60$,

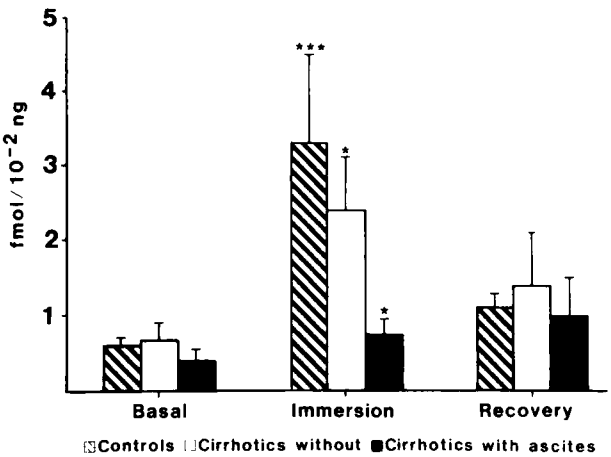

FIG. 4. Ratio of ANF over plasma aldosterone concentration in healthy subjects, cirrhotic patients without and cirrhotic patients with ascites before, during and following water immersion. * = $p < 0.05$; *** = $p < 0.001$; significantly different from baseline values.

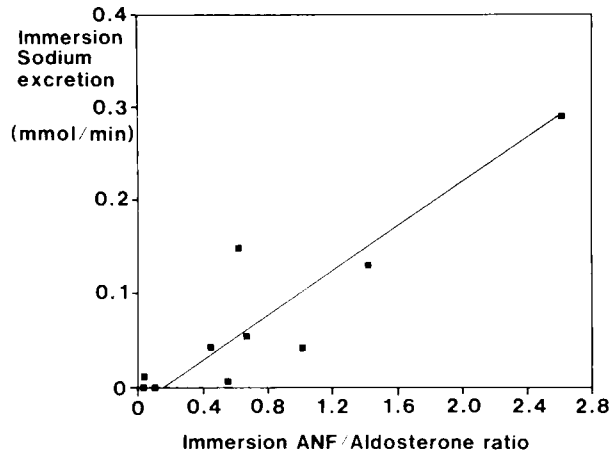

FIG. 6. Correlation of immersion-stimulated natriuresis to immersion-stimulated ratio of ANF over plasma aldosterone concentration in patients with cirrhosis and ascites; $r = 0.90$, $p < 0.001$.

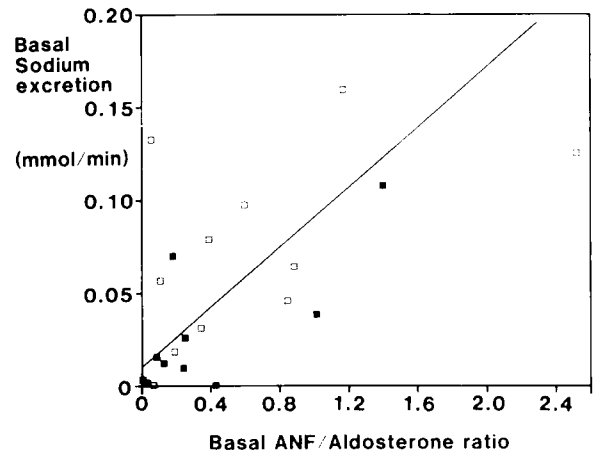

FIG. 5. Correlation of baseline sodium excretion to baseline ratio of ANF over plasma aldosterone concentration in patients with cirrhosis; $r = 0.60$, $p < 0.01$. *Open squares* = patients without ascites; *closed squares* = patients with ascites.

$p < 0.01$) as well as basal fractional sodium excretion ($r = 0.63$, $p < 0.01$) correlated to the baseline ANF/PAC ratio. The ratio of stimulated ANF/PAC was related to stimulated natriuresis ($r = 0.60$, $p < 0.01$). In the subgroup of cirrhotic patients with ascites, the relation between basal natriuresis ($r = 0.73$, $p < 0.02$), stimulated natriuresis (Fig. 6) ($r = 0.90$, $p < 0.001$) as well as between the increase ($r = 0.74$, $p < 0.02$) of natriuresis and the corresponding ANF/PAC ratios was more clearly expressed than in the subgroup without ascites.

DISCUSSION

In the present study, no significant difference of ANF plasma concentrations was observed between healthy subjects and patients with cirrhosis without and with ascites. Since substantial tissue extraction of ANF has been reported (25), similarity of venous plasma levels does not necessarily imply corresponding arterial plasma concentrations. However, it has recently been demonstrated (26) that arteriovenous extraction of ANF in various tissues is not altered in patients with cirrhosis and that arterial ANF plasma concentrations in cirrhosis equal those in healthy subjects.

The observation that acute volume stimulation by water immersion increases ANF plasma levels in healthy human subjects is in accordance with previous findings from our laboratory (27) and has been confirmed by several groups (28–31). Thus, immersion may be considered a useful tool to study the reactivity of the ANF system in volume regulation, as has been shown for the renin-aldosterone system (21). Patients with cirrhosis of the liver without ascites exhibited increases of ANF plasma levels upon stimulation by water immersion similar to controls. In cirrhotic patients with ascites, however, augmentation of plasma ANF after 1 hr of immersion was significantly blunted. Duration of immersion might have been too short to induce maximum ANF stimulation. Several 3-hr immersion studies reported mean maximum ANF stimulation within 1 hr immersion time (30–32), whereas Epstein et al., in a recently published investigation (29), observed individual peak ANF concentrations in eight of 13 healthy subjects only after 2 or 3 hr immersion time. Since the same author observed slower and less marked effects of immersion on volume-regulating hormones and on sodium excretion in subjects on a low-sodium diet (33), a 150-mEq sodium diet was chosen for the present study, approximately the average sodium intake of a normal diet. Following our studies on ANF stimulation by WI in patients with cirrhosis (34, 35), other investigators have submitted two (36) and eight cirrhotic subjects (37) to WI. In these studies, mean ANF plasma values reached a maximum after 1 hr immersion and remained elevated throughout the 3-hr immersion period. Thus, 1-hr immersion appears sufficient to assess the ANF response to volume stimulation, although the possibility cannot be excluded that some subjects did not attain maximal ANF augmentation.

Another explanation for the blunted ANF increase in cirrhotic patients with ascites might be a smaller degree of central volume stimulation in this subgroup of patients. However, plasma renin activity as an indicator of centrally effective blood volume decreased in cirrhotic

patients with ascites by the same percentage as in cirrhotic patients without ascites and in healthy subjects. The mean decrease of PRA by 50 ± 6% in patients with ascites in the present study compares well with the 45 ± 7% decrease observed in 16 cirrhotic subjects after 1 hr WI in an earlier study by Epstein et al. (38). Besides, it has been shown that intracardiac pressures are increased by immersion independent of the degree of ascites (39). Thus, although we cannot provide data on central hemodynamics, a diminished increase of atrial pressure does not seem a likely explanation for the blunted ANF stimulation in patients with ascites. Therefore, a defect of ANF release or a reduced cardiac ANF content may be suspected in cirrhotic patients with ascites.

Preliminary observations of other investigators found a normal or even exaggerated ANF response to immersion (37) in cirrhotic patients with ascites on a sodium-restricted diet as compared to healthy subjects without sodium restriction. This discrepancy to our results cannot be easily explained. Possibly, differences in dietary sodium intake and presence of edema in these patients with ascites might account for the different observations. Furthermore, in our patients with ascites, ANF plasma levels did not markedly decrease following the end of immersion, as was the case in the group without ascites and in the controls of our study as well as in the cirrhotic patients of the aforementioned study (37). Despite persistent elevations of ANF plasma concentrations, natriuresis returned to baseline levels in our patients with ascites. Although this observation might support the contention that renal sensitivity to ANF is blunted in cirrhotic patients with ascites (37, 40–44), stronger activation of sodium-retaining systems, not investigated in the present study, cannot be excluded.

So far, functional response of the renin-aldosterone system to volume stimulation in various stages of cirrhosis has not been well described. Whereas plasma renin activity was markedly elevated in ascitic patients, cirrhotic patients without ascites showed similar baseline values as did healthy subjects. Water immersion reduced PRA to a similar degree in all groups, indicating a rather uniform volume stimulus. Plasma aldosterone concentration in cirrhotic patients with ascites was significantly higher than in controls, with levels of compensated cirrhotic patients in between. Similar reductions of PAC in controls and cirrhotics patient were seen, with the changes in the subgroup with ascites not quite reaching significance. In view of considerable disagreement in the literature on the role of the renin-aldosterone system in volume regulation of cirrhosis (45–47), the rather unanimous activation of PRA and PAC in our subgroup with ascites and in particular the trend to elevated levels in the subgroup without ascites deserves attention. Possibly, the sitting position of the subjects in our study as compared to a supine position in many other studies might explain why we observed activation of the renin-aldosterone system in cirrhosis which is not generally agreed upon (45). However, hormone levels in the sitting position may more closely reflect the activity of the volume-regulating systems during about two-thirds of a regular day than concentrations taken in the supine position. Thus, our results underscore the importance of an activation of the renin-aldosterone system in volume regulation of patients with cirrhosis with and also without ascites.

Neither ANF nor renin-aldosterone plasma levels were found to correlate to basal or stimulated natriuresis. In view of the complex interactions in volume regulation, this is not surprising; it seems likely that several hormonal and neural factors act in concert and that several of them participate to induce sodium retention of cirrhosis. ANF is the only well-characterized natriuretic hormone that can be reliably quantitated as yet. ANF counteracts renin-aldosterone, the major sodium-retaining principle of cirrhosis, by inhibiting synthesis and release and by antagonizing the renal action. Therefore, the present study focussed on the possible role of ANF and renin-aldosterone in volume regulation of cirrhosis. Water immersion induced an increase of ANF and a decrease of plasma renin activity and plasma aldosterone concentration. In an attempt to characterize the balance of these opposing systems, the ratio ANF over aldosterone was calculated and correlated to the renal response. ANF/PAC was more markedly affected by immersion than ANF or PAC alone. In patients with cirrhosis, but not in controls, basal as well as immersion-stimulated natriuresis was significantly correlated to the corresponding ANF/PAC ratios. The closer correlations in the subgroup with ascites might be due to the stronger aldosterone activation in basal conditions and the blunted ANF stimulation by immersion. Even though these correlations do not necessarily imply a cause-effect relationship, they might suggest a role of ANF, counteracting aldosterone, in sodium handling. Thus, our results support the contention that these hormones are important for the volume regulation in patients with cirrhosis of the liver.

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