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Disposal of atrial natriuretic factor (ANF_{99–126}) in patients with cirrhosis: effect of beta-adrenergic blockade

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Bendtsen F, Gerbes A L, Henriksen J H. Disposal of atrial natriuretic factor (ANF_{99–126}) in patients with cirrhosis: effect of beta-adrenergic blockade. Scand J Clin Lab Invest 1993; 53: 547–554.

To test a possible effect of blood flow change on disposal of atrial natriuretic factor: ANF_{99–126} (ANF), we determined renal, azygos, hepatic and cubital venous, and arterial plasma concentrations of ANF in 18 patients with cirrhosis before and after ingestion of propranolol 80 mg. Arterial ANF was similar to that of controls (9.4 vs. 10.9 pmol l⁻¹, NS) and was positively correlated to cardiac output ($r = 0.49$, $p < 0.02$) and to right atrial pressure ($r = 0.44$, $p < 0.01$). All the vascular beds examined extracted ANF significantly. The renal ($n = 17$), hepato-enteric ($n = 16$), and splanchnic superior collateral (azygos) beds ($n = 13$) had significantly higher extraction ratios (0.34–0.39) than that observed in the cubital vein (0.24, $n = 15$, $p < 0.05$). Arterial ANF showed no significant change (9.6–11.0 pmol l⁻¹, NS) after reduction of cardiac output (–25%, $p < 0.001$) by propranolol. Only insignificant changes in ANF extraction and a small decrease in azygos and hepato-enteric clearance occurred during beta-adrenergic blockade. Our results show a substantial extraction of ANF in the kidney, in the splanchnic bed drained through superior portosystemic collaterals, and in the hepato-enteric bed. Only minor effects on ANF extraction were observed after reduction of the blood flow with propranolol.

Key words: atrial natriuretic factor (ANF); beta-adrenergic blockade; cirrhosis; extraction; propranolol

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Atrial natriuretic factor: ANF_{99–126} (ANF) is a circulating hormone with natriuretic and vasodilating effects [1, 2]. In patients with cirrhosis, different levels of plasma ANF has been reported: from below normal to highly elevated values [3–9]. However, the circulating level of ANF is

not only governed by release, but by the dynamic equilibrium between release and disposal, and recent reports document major extraction (15–60%) of this peptide in various tissues [5, 10–13].

Treatment with beta-adrenergic blockers is common in patients with cirrhosis [14]. Propran-

olol reduces cardiac output and hepatic blood flow in most of the patients [15]. As the overall clearance of a high extraction substance may be flow dependent, it could be anticipated that a reduction in blood flow would result in decreased disposal and elevated circulating levels. However, the effect of a change in blood flow on disposal and extraction of ANF has hitherto not been investigated in patients with cirrhosis.

Therefore, the present study was undertaken to determine circulating ANF in patients with cirrhosis before and during beta-adrenergic blockade. We measured the concentration of ANF in arterial, renal, azygos, hepatic, and cubital venous plasma before and during propranolol ingestion in order to examine whether a decrease in blood flow affects the disposal of ANF.

PATIENTS AND METHODS

The study comprised a total of 18 patients (2 women, 16 men) with cirrhosis, as verified by biopsy, aetiology for all was alcohol. The age range was 30–69 years, mean 58 years. All the patients were considered to be in a stable condition and had abstained from alcohol for at least one week. There were no signs of current consumption of alcohol or of withdrawal symptoms. Endoscopy revealed oesophageal varices in all patients. None had experienced gastrointestinal bleeding or hepatic encephalopathy above grade I. Six patients were in Child-Turcotte Class A, 7 in Class B, and 5 in Class C. Fluid retention and ascites were present in 10 patients. Patients without fluid retention were not restricted as to diet, whereas those with ascites were put on a sodium diet of 40 mmol day⁻¹. Ten patients received diuretics (spironolactone 100 mg day⁻¹ and additionally bumetanide 1–8 mg day⁻¹). The biochemical data are summarized in Table I. Six patients, described in [16] with disorders of minor degree (one intercostal neuralgia, one dermatomyositis, one chronic hepatitis, two irritable bowel syndrome, one post cholecystectomy pain) and unrestricted diet served as controls. Their age range was 50–68 years, mean 62 years.

Patients and controls consented to participate in the investigation after receiving a thorough explanation verbally and in writing. The study was approved by the Ethics Committee for

Medical Research in Copenhagen. No complications or side-effects were encountered during the investigative procedures.

Catheterization

Patients and controls were studied in the morning after an overnight fast. Catheterization was performed under local anaesthesia with the subject lying supine. Azygos venous blood was collected from the femoral route with either a double thermistor coronary sinus catheter (Webster Laboratories, Altadena, CA) or a Swan-Ganz catheter as described elsewhere [17]. Hepatic and right renal venous blood was collected with a Cournand 7F catheter as described elsewhere [18]. A small indwelling polyethylene catheter was introduced to the femoral artery and cubital vein by the Seldinger technique.

The azygos venous blood flow was measured by the continuous infusion thermodilution technique [16, 17], and the hepatic blood flow by the indocyanine green constant infusion technique [19]. Arterial and right atrial pressures were measured with a capacitance transducer (Simonsen & Weel, Copenhagen). The mid-axillary line was taken as zero pressure reference. Heart rate was recorded by ECG.

Protocol

Blood samples for determination of endogenous ANF in plasma were taken simultaneously from artery/azygos vein, artery/hepatic vein, artery/right renal vein, and artery/cubital vein. Blood samples were collected and the blood flow and pressures were measured to obtain baseline values without medication and procedures were repeated 90 min after oral administration of propranolol 80 mg [20].

Analysis of endogenous human alpha-atrial natriuretic factor

After discharging the catheter dead space, 5 ml of blood was collected on ice into test tubes containing aprotinine 2500 KIU and heparin 250 IU. The samples were immediately centrifuged at 4°C and the plasma was stored at –25°C until assayed. Plasma was extracted and ANF was determined by radio-immunoassay as described elsewhere [21, 22]. Intra-

Table I. Laboratory data of 18 patients with cirrhosis.

	Haemoglobin (mmol l ⁻¹)	S-bilirubin (μmol l ⁻¹)	S-aspartate amino- transferase (U l ⁻¹)	S-alkaline phosphatase (U l ⁻¹)	Factor 2, 7, 10 Index	S-Na ⁺ (mmol l ⁻¹)	S-K ⁺ (mmol l ⁻¹)	S-creatinine (μmol l ⁻¹)	S-albumin (μmol l ⁻¹)	Plasma volume (ml kg ⁻¹)
Cirrhosis										
Mean	8.3	33	67	384	0.70	137	3.9	80	492	56
(range)	(6.9–9.8)	(7–115)	(29–150)	(211–807)	(0.29–1.51)	(130–144)	(3.4–5.2)	(55–135)	(288–666)	(41–79)
Reference interval	F: 7.1–9.9 M: 8.1–10.9	2–17	10–40	50–275	0.7–1.3	136–147	3.5–5.0	49–121	540–800	35–65

Table II. Haemodynamic values in patients with cirrhosis before and after oral ingestion of propranolol 80 mg.

	Heart rate (min ⁻¹)	Right atrial pressure (mmHg)	Mean arterial blood pressure (mmHg)	Systemic vascular resistance (dyn s cm ⁻⁵)	Cardiac output (l min ⁻¹ m ⁻²)	Hepatic blood flow (l min ⁻¹)	Azygos venous blood flow (l min ⁻¹)
Cirrhosis (n)	(18)	(18)	(18)	(17)	(17)	(18)	(9)
Before propranolol	76 ± 2.6 ^b	2.6 ± 0.54	84 ± 3.3 ^a	1020 ± 68 ^c	3.70 ± 0.23 ^a	1.16 ± 0.07	0.98 ± 0.19
After propranolol	62 ± 2.0*	3.8 ± 0.69†	79 ± 2.6	1275 ± 80†	2.82 ± 0.15†	0.92 ± 0.08†	0.71 ± 0.07*
Controls (n = 16)	68 ± 2.3	3.5 ± 0.68	95 ± 3.3	1490 ± 128	2.99 ± 0.18	-	-

Mean ± SEM

Compared to value before propranolol: * $p < 0.025$; † $p < 0.005$

Compared to control value: a $p < 0.05$; b $p < 0.02$; c $p < 0.01$.

assay and inter-assay coefficients of variation were below 10% and 15%, respectively. The detection limit was 0.5 pmol l⁻¹.

Calculations and statistical evaluation

Extraction ratio (E). This was assessed as $E = (C_a - C_v)/C_a$, where C_a and C_v are the plasma concentrations of ANF in the artery and organ vein, respectively.

Clearance (Cl). Clearance in azygos and hepato-enteric beds was assessed as $Cl = F \cdot (1 - Hct) \cdot E$, where F is the azygos and hepatic blood flow, and Hct is the haematocrit fraction.

Paired and grouped differences were compared by Student's paired and unpaired two-sided t -tests, and the Bonferroni correction was applied in multiple group comparison. Corre-

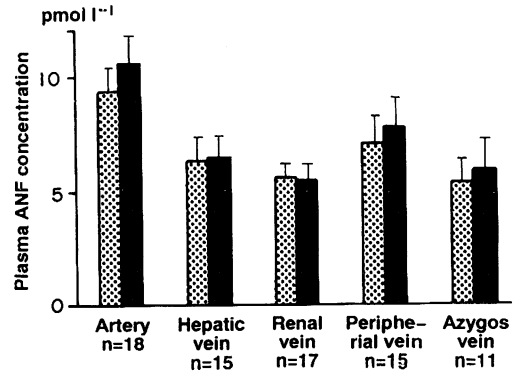


Fig. 1. Plasma ANF in different vascular beds in patients with cirrhosis before \square and after propranolol ingestion \blacksquare . Mean + SEM.

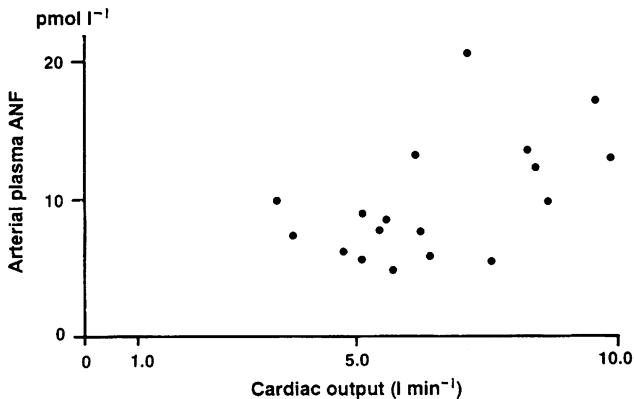


Fig. 2. Relation between cardiac output and arterial plasma ANF in 18 patients with cirrhosis ($r = 0.49$, $p < 0.02$).

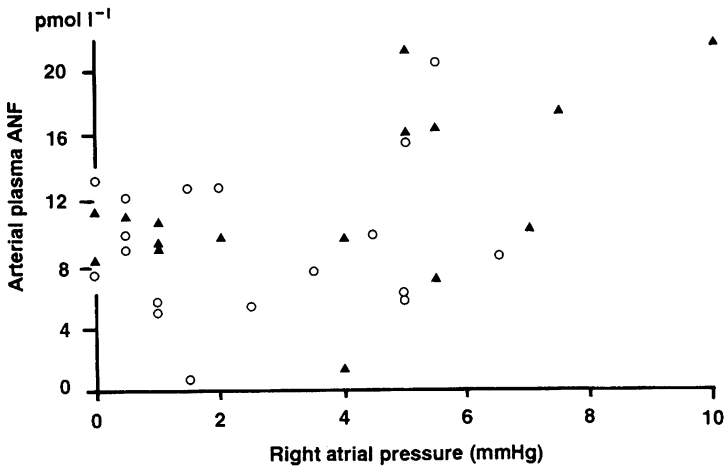


Fig. 3. Relation between right atrial pressure and arterial ANF in 18 patients with cirrhosis, before ○ and after ▲ propranolol ingestion ($r = 0.44$, $p < 0.01$).

lations were analysed by the Pearson correlation coefficient. $p < 0.05$ was considered significant. Values are given as mean \pm SEM.

RESULTS

Table II summarizes the haemodynamic results. During beta-adrenergic blockade, a substantial

reduction was observed in the heart rate, cardiac output, and hepatic and azygos blood flow, whereas a slight, but significant, increase was observed in the right atrial pressure.

The mean arterial concentration of ANF in the patients was similar to that of the controls (9.4 vs. 10.9 ± 3.1 pmol l⁻¹ NS). There was only an insignificant increase after propranolol ingestion (9.4 to 11.0 pmol l⁻¹ NS), and no significant change was observed in ANF from the venous beds (see Fig. 1). Arterial ANF in the cirrhotic patients was positively correlated to cardiac output ($r = 0.49$, $p < 0.02$, Fig. 2) and to the right atrial pressure ($r = 0.44$, $p < 0.01$, Fig. 3). No relation to the heart rate could be demonstrated.

All the vascular beds examined showed a significant extraction of ANF (Fig. 4). Renal

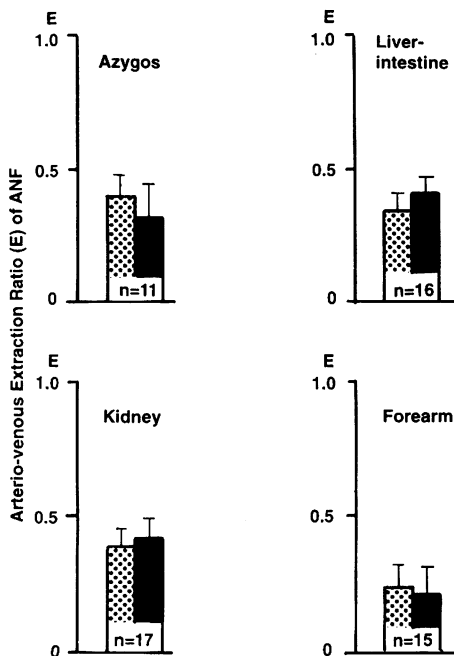


Fig. 4. Arterio-venous extraction ratios in different vascular areas in patients with cirrhosis before □ and after propranolol ingestion ■.

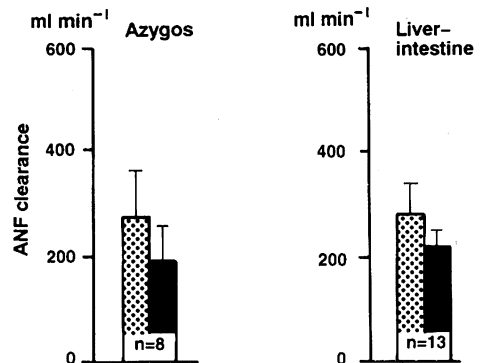


Fig. 5. Azygos and hepato-enteric clearances of ANF in patients with cirrhosis before □ and after propranolol ingestion ■.

(0.39 ± 0.06), hepato-enteric (0.34 ± 0.06), and azygos bed (0.38 ± 0.07) extraction ratios were significantly higher than that for the cubital vein (0.24 ± 0.08 , $p < 0.05$). No significant changes in extraction ratios were observed during beta-adrenergic blockade (Fig. 4). A non-significant change in azygos and hepato-enteric clearance of ANF was seen after propranolol ingestion (Fig. 5).

DISCUSSION

The arterio-venous extraction technique is a well-established method of obtaining information on regional disposal of a variety of bioactive substances [10, 12, 18, 23, 24]. The present study shows substantial extraction of ANF in the azygos, hepato-enteric, and renal venous beds. Removal of ANF was smaller in the forearm circulation.

The high extraction of ANF in renal, hepatic, and azygos venous plasma probably reflects the presence of peptidases and specific degradation receptors [13]. It has been demonstrated that ANF is rapidly hydrolysed by endopeptidase 24.11, a membrane-bound metallo-peptidase that hydrolyses bonds involving the amino-group of hydrophobic amino acid residues (i.e. cysphe) [25, 26]. Two types of receptors with specific affinity for ANF have been described: B and C receptors [27, 28, 29]. The B receptor appears to mediate the biological response to ANF through cGMP as second messenger [27, 30]. Since no second messenger has been unequivocally identified when ANF binds to the C receptor, Fuller and co-workers [31] suggest that it is a 'silent' clearance receptor with internalization of the peptide and subsequent degradation within lysosomes as the outcome [31]. The relative abundance of the two receptors varies widely in the different tissues. Endopeptidase 24.11 and ANF receptors have been identified in most vascular endothelial cells, including those of the hepato-splanchnic bed [32–36]. The high ANF extraction ratio in azygos plasma, as found in the present study, is similar to extractions described in most other tissues [9–12]. Therefore, our findings may suggest that the degradation of ANF in blood drained through the azygos venous bed is mediated by the same mechanism as those described for other tissues, i.e. related to vascular endothelial cells.

Propranolol produced a substantial reduction in the blood flow rates. None the less, extraction ratios and clearances exhibited only small changes, which indicates that the disposal of ANF only manifests a minor dependence on a reduction in blood flow. No previous report has addressed this question. Peripheral plasma ANF does not change during beta-adrenergic blockade in normal humans [37, 38].

Modulation of ANF kinetics by adrenergic agonists is controversial [39, 40]. Propranolol, in itself, might alter the kinetics of ANF. However, this point of view was not supported by a recent report by Keller *et al.* [41], who did not find any change in plasma ANF during infusion of beta-adrenergic agonists or antagonists to normal humans. In the present study, ANF was directly correlated to right atrial pressure, when the values, before and after propranolol ingestion were compared. This is probably because of the small, but significant, increase in right atrial pressure during beta-adrenergic blockade [2, 38], and may thus be a consequence of a somewhat augmented release of ANF rather than of a change in disposal. ANF was directly correlated to cardiac output, a finding that probably reflects a change in the release of ANF which could be caused by the hyperdynamic circulation in the presence of cirrhosis, rather than to a change in disposal [41, 42].

To summarize, our results in patients with cirrhosis show a substantial extraction of ANF in the kidney, in the splanchnic bed drained through superior porto-systemic collaterals, and in the hepato-intestinal bed. Only minor effects on ANF extraction were observed after reduction of the blood flow with propranolol.

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