Splanchnic Removal of Human Alpha-Atrial Natriuretic Peptide in Humans: Enhancement After Food Intake

Jens H. Henriksen, Flemming Bendtsen, and Alexander L. Gerbes

In order to assess the effect of food ingestion on splanchnic disposal of human alpha-atrial natriuretic peptide (ANF), hepatic-intestinal removal of ANF was determined before and after a test meal. Hepatic venous and arterial plasma samples were obtained from six subjects, most of whom had only disorders of minor degree. Hepatic blood flow (HBF) increased significantly after meal ingestion (1.10 ± 0.17 [SEM] to 1.51 ± 0.26 L/min, $P < .01$). Baseline arterial ANF (10.9 ± 3.1 pmol/L) did not change significantly. In contrast, hepatic venous ANF increased after meal intake (5.7 ± 2.0 to 8.4 ± 1.9 pmol/L, $P < .05$), and accordingly the splanchnic fractional extraction decreased (0.53 ± 0.09 to 0.35 ± 0.08), although this was not statistically significant. Splanchnic clearance of ANF increased from 347 ± 90 mL/min to a maximal value of 615 ± 158 mL/min ($P < .05$). Splanchnic removal of ANF was 3.0 ± 0.5 pmol/min before and increased to a maximum value (7.1 ± 2.2 pmol/min, $P < .05$) 35 minutes after ingestion of the meal. Our results showed enhanced splanchnic removal of ANF after food intake. This is due to increased hepatic-intestinal clearance of the peptide consequent on increased splanchnic blood flow, rather than altered fractional extraction of ANF.
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ATRIAL natriuretic factor (human alpha-atrial natriuretic peptide [human ANF 99-126], abbreviation in this report: ANF) is important in the regulation of salt-water homeostasis and blood pressure.[1] Since 1981 when deBold et al[2] reported a natriuretic response to intravenous injection of atrial extracts in rats, a substantial body of reports on synthesis, release, physiological effects, and its possible role in pathophysiology and pharmacology has emerged.[1,3-5] In contrast, studies on degradation and regional removal of ANF are few. Schütten et al[6] found a significant extraction of ANF in kidney, liver-intestine, and limbs of subjects with minor disorders. Organ extraction of ANF has also been reported in patients with heart disease[7] and cirrhosis.[8,9] Recently, Vierhapper et al[10] found a basal splanchnic uptake of ANF of 8.5 pmol/min in healthy humans. However, no reports have dealt with the effect of food intake on splanchnic disposal of ANF.

The present study was undertaken to assess the effect of a meal on splanchnic removal of ANF. We studied persons undergoing a liver vein catheterization, the majority who had disorders of minor degree.

MATERIAL AND METHODS

Study Population

The study comprised three women and three men with a mean age of 62 years (range, 50 to 68 years). All subjects were referred to hepatic vein catheterization in order to exclude ischemic mesenteric artery disease. This diagnosis was absent in all, and the majority had disorders of minor degree (Table 1). The patient with chronic hepatitis was in a stable stage with normal biochemical liver function tests. Electrocardiogram (ECG), hemoglobin concentration (mean, 9.0 mmol/L; range, 8.2 to 9.5), and serum-sodium concentration (mean, 138 mmol/L; range, 136 to 141) were normal in all. The study was approved by the Ethics Committee for Medical Research in Copenhagen, and all subjects consented to participate in the following investigation.

Protocol

All subjects were studied in the morning after an overnight fast. Hepatic vein catheterization was performed from the femoral route under local anesthesia and fluoroscopic control, as described earlier.[11] An 6-Fr Ödman catheter with side holes was guided to a large right hepatic vein, the tip being approximately 7 cm from the inferior vena cava. A thin indwelling polyethylene catheter was inserted into the femoral artery by the Seldinger technique.

Baseline values of ANF concentrations and hepatic blood flow were obtained for 1 hour. Thereafter, a test meal containing 1,000 kcal (83 g carbohydrate, 83 g protein, 37 g lipid) was ingested over 10 minutes and the effect on ANF, hepatic blood flow (HBF), and splanchnic oxygen uptake was recorded for another hour.

HBF was determined by the indocyanine green (ICG) constant infusion technique.[12] In brief, after priming (2 mg), constant infusion of ICG in 5% human albumin preparation into an antecubital vein (0.2 mg/min) was maintained by a calibrated pump (Dich, Copenhagen, Denmark) for 130 minutes. After an equilibration period of 30 minutes, blood samples were collected from artery and hepatic vein at an interval of 10 minutes for 100 minutes. ICG was determined spectrophorometrically (Zeiss PMQ-II; Carl Zeiss, Oberkochen, FRG) at 800 nm and 900 nm for turbidity correction. None of the patients had lipemic plasma. Hepatic plasma flow (HPF) was determined as infusion rate, corrected for unsteady arterial concentration, divided by the arterial-hepatic venous concentration difference of ICG. HBF was obtained from HPF after correction for hematocrit.

Oxygen saturation was measured in artery and hepatic vein by OSM_2 (Radiometer, Copenhagen, Denmark), and splanchnic oxygen uptake was determined as HBF · hemoglobin concentration · oxygen difference.

Determination of ANF

Blood samples were simultaneously collected from the femoral artery and the hepatic vein after discharging catheter-dead space. Five milliliters blood were collected on ice into test tubes containing 2,500 kIU of aprotinin and 250 IU of heparin. Plasma was separated

From the Departments of Clinical Physiology and Hepatology, Hvidovre Hospital, University of Copenhagen, Copenhagen, Denmark; and Department of Medicine II, Klinikum Grosshadern, University of Munich, Munich, FRG.

Supported by the John and Birthe Meyer Foundation and the Ebba Celinder Foundation.

Address reprint requests to Jens H. Henriksen, MD, Department of Clinical Physiology and Nuclear Medicine, 239, Hvidovre Hospital, DK-2650 Hvidovre, Denmark.

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0026-0495/90/3905-0016$3.00/0

Table 1. HBF Rate and Arterial and Hepatic Venous Plasma Concentrations of ANF Before and After Intake of Test Meal in Six Subjects Undergoing Liver Vein Catheterization

			Baseline		After Intake of Meal			
						Plasma ANF pmol/L		
Subject No./Sex	Age (yr)	Diagnosis	HBF (L/min)	Plasma ANF (pmol/L; a/hv)	HBF (L/min)	a_{30}/hv_{30}*	a_{45}/hv_{45}	a_{60}/hv_{60}
1/M	68	Intercostal neuralgia	1.18	11.7/5.4	1.68	14.0/5.7	12.7/6.9	14.0/11.7
2/M	50	Dermatomyositis	0.74	23.8/12.3	0.95	23.7/12.3	18.7/10.8	19.5/12.7
3/F	61	Chronic hepatitis	1.17	14.2/10.6	1.45	17.0/11.9	24.0/13.9	30.6/12.2
4/F	62	Irritable bowel syndrome	0.96	6.0/0.7	1.44	3.2/2.9	1.9/0.6	2.4/3.8
5/F	68	Irritable bowel syndrome	0.74	6.7/4.0	0.88	8.1/2.3	6.8/3.2	9.0/7.5
6/M	62	Post cholecystectomy pain	1.83	3.1/1.2	2.64	5.6/0.6	3.2/2.5	4.3/2.3
Mean	62		1.10	10.9/5.7	1.51†	11.9/6.0	11.2/6.3	13.3/8.4‡

*Numbers denote minutes after intake of test meal.

Abbreviation: a/hv, artery/hepatic vein.

Significant difference compared with baseline value: †hepatic vein, $P < .01$; ‡$P < .05$.

from blood cells immediately by centrifugation at 4°C and frozen (−25°C) until assayed.

ANF in plasma was determined by radioimmunoassay as described earlier.[13,14] Within- and between-assay coefficients of variation were below 10% and 15%, respectively. Detection limit was 0.5 fmol/mL.

Calculations

Splanchnic removal rate of ANF (J), fractional extraction ratio (E), and clearance (Cl) were determined by the following equations, where C_a and C_{hv} are plasma concentrations of ANF in artery and hepatic vein, respectively: $J = HPF \cdot (C_a - C_{hv})$; $E = (C_a - C_{hv})/C_a$; and $Cl = (C_a - C_{hv}) \cdot HPF/C_a$.

Statistical Evaluations

Comparisons between paired and grouped differences were performed by Student's paired *t* test and ANOVA. $P < .05$ was considered significant.

RESULTS

Plasma ANF values and HBF are summarized in Table 1. HBF was 1.10 ± 0.17 L/min (mean ± SEM) and increased significantly in all subjects studied to average 1.51 ± 0.26 L/min ($P < .01$) after intake of the test meal. Likewise, splanchnic oxygen uptake increased from 64 ± 8.5 to 89 ± 12 mL O_2/min ($P < .01$). Baseline arterial concentration of ANF (10.9 ± 3.1 pmol/L) was close to the normal average, and did not change significantly after meal ingestion. Hepatic venous concentration of ANF (5.7 ± 2.0 pmol/L) increased to 8.4 ± 1.9 pmol/L ($P < .05$) 60 minutes after meal intake. Arterio-hepatic venous extraction ratios of ANF are shown in Fig 1. Baseline fractional extraction was 0.53 ± 0.09, and this value decreased somewhat after the meal, although not statistically significant. No relationship was found between splanchnic ANF and oxygen extraction. Splanchnic clearance of ANF was 347 ± 81 mL/min and increased significantly (maximum value, 615 ± 158 mL/min [$P < .05$]; average, 441 ± 90 mL/min [$P < .05$]) after food intake. Hepatic-intestinal removal of ANF was 3.0 ± 0.5 pmoL/min before, and reached a maximal value 35 ± 5 minutes after ingestion of the meal (7.1 ± 2.2 pmol/min; $P < .05$). Average time course of clearance and removal are shown in Fig 2.

DISCUSSION

Plasma disappearance of ANF after intravenous infusion is fast, indicating a substantial plasma turnover rate of this peptide.[15,16] Other peptides with a fast turnover rate, such as insulin, neurotensin, and vasoactive intestinal polypeptide, have a substantial hepatic degradation,[17-19] and this may be influenced by food intake.[20] A few reports[6,10,21] have dealt

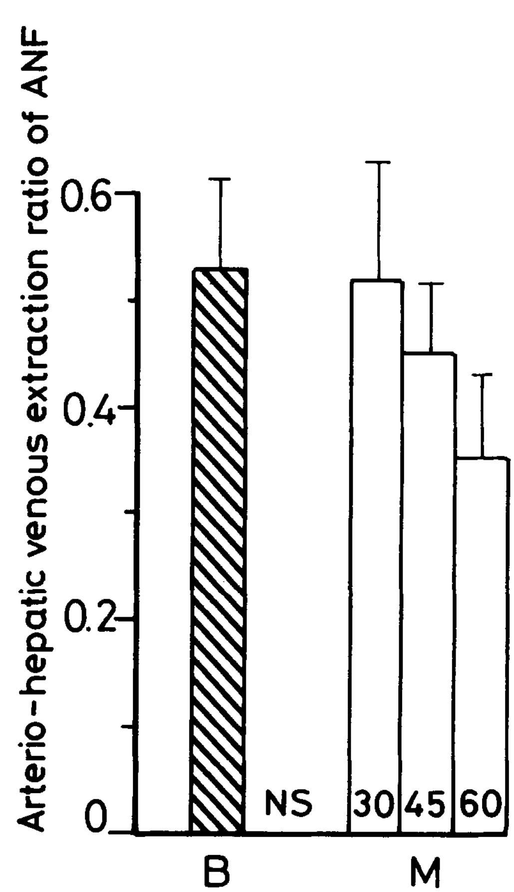

Fig 1. Splanchnic fractional extraction ratio of ANF (ordinate) before (B) and after ingestion of a test meal (M). Numbers denote minutes after finishing of meal intake. Mean + SEM.

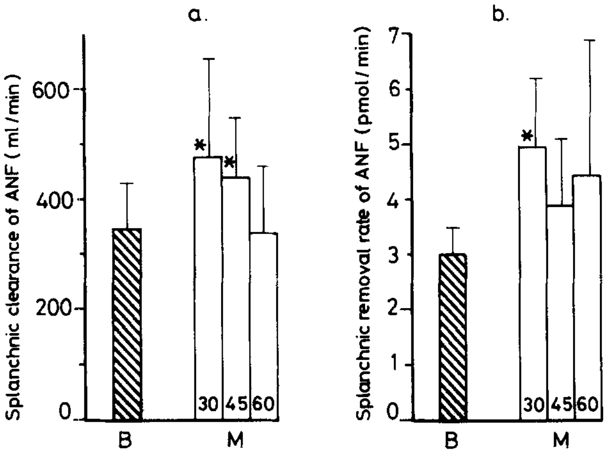

Fig 2. (a) Splanchnic clearance of ANF, and (b) splanchnic removal rate of ANF before and after ingestion of test meal (M). *Significantly different from before (B) ingestion of test meal ($P < .05$).

with the splanchnic disposal of ANF in humans, and the question of a possible effect of food intake has not been addressed.

The splanchnic uptake of ANF, as found in the present baseline period (3.0 pmol/min) is somewhat below the value (8.5 pmol/min) recently published by Vierhapper et al.[10] The reason for this it not clear, but may be due to (1) the circulating level of ANF was slightly higher in their study; (2) they found a splanchnic fractional extraction of 0.76 compared with 0.53 in our study; (3) HBF was higher in their subjects; and (4) their subjects were younger. The splanchnic fractional extraction as found in the present study is similar to that reported by Crozier et al,[7] but somewhat higher than reported by Schütten et al[6] and Hollister et al.[21]

After intake of the test meal, HBF and splanchnic oxygen uptake increased in all subjects, mean 37% and 39%, respectively. This is a normal response.[22] The splanchnic fractional extraction rate of ANF decreased somewhat, although not statistically significant. In contrast, the splanchnic clearance of ANF increased significantly after meal ingestion. Thus, the increased splanchnic ANF removal after meal ingestion is most likely due to an increased clearance secondary to the increased splanchnic blood flow observed after food intake.

Increased elimination will decrease the circulating level of ANF if the release rate is unchanged. However, we did not observe a decrease in arterial ANF. On the contrary, a slight nonsignificant increase was observed. Food intake may transitorily enhance the osmotic load of plasma and expand the plasma volume by displacement of water from the intracellular pool.[23] Plasma volume expansion may increase the release of ANF from atrial myocytes.[24,25] The increased splanchnic removal of ANF after food intake may, at least in part, counteract an increased release of ANF, leaving the arterial level of ANF relatively unchanged. In this context, however, it should be recalled that the splanchnic removal only constitutes about one fifth of overall disposal of ANF, the kidney and lung being the most prominent organs of ANF removal.[6-9,21]

In summary, ingestion of food enhances the splanchnic disposal of ANF as evaluated by the hepatic vein catheterization technique. The increased hepatic-intestinal removal of ANF is due to an increased clearance of this peptide consequent on increased splanchnic blood flow and not to increased splanchnic fractional extraction of ANF. Increased splanchnic removal may, in part, oppose any increased release of ANF after food intake.

ACKNOWLEDGMENT

R. Arendt is thanked for his support, in particular for supplying the antibody Toni III. The authors wish to express their gratitude to the technicians and nurses at the Department of Clinical Physiology, Hvidovre Hospital, and to Ruth Werner, secretary, for skillful assistance.

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