Role of the Liver in Splanchnic Extraction of Atrial Natriuretic Factor in the Rat

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Mesenteric, hepatic and splanchnic extraction of C-terminal and N-terminal atrial natriuretic factor was investigated in male Sprague-Dawley rats. Plasma concentrations (mean ± S.E.M.) of C-terminal atrial natriuretic factor were 55.0 ± 6.1 fmol/ml, 31.2 ± 4.0 fmol/ml and 23.5 ± 3.3 fmol/ml (n = 12) in the abdominal aorta, the portal vein and the hepatic vein, respectively. N-terminal atrial natriuretic factor plasma levels in these vessels were 3031 ± 756 fmol/ml, 2264 ± 661 fmol/ml and 1618 ± 496 fmol/ml (n = 6), respectively. Although the mesenteric extraction ratio was higher ($p < 0.05$) for C-terminal atrial natriuretic factor (42% ± 6%) than for N-terminal atrial natriuretic factor (28% ± 4%), there were no significant differences in the hepatic extraction ratio (41% ± 5% vs. 39% ± 6%) and the splanchnic extraction ratio (56% ± 5% vs. 50% ± 7%). These data suggest a major role of the liver in the splanchnic extraction of C-terminal and of N-terminal atrial natriuretic factor in the rat. (HEPATOLOGY 1992;16:790-793.)

The atrial natriuretic factor (ANF) has been shown to be involved in volume homeostasis (1-7). Furthermore, there is increasing evidence that it may play a role in immune and reproductive functions (8, 9). ANF circulates as a 28–amino acid C-terminal fragment and a 98–amino acid N-terminal fragment. Although the C-terminal fragment is known to be the bioactive compound of ANF, the biological role of the N-terminal is not yet clearly established. However, there is evidence of a vasodilatory effect of this fragment after further cleavage in plasma (10).

As yet, there is limited knowledge of the clearance of ANF from the circulation because there are only a few studies on the extraction ratios of C-terminal ANF by various organs and there is virtually no information on the clearance of the N-terminal fragment (11). In human beings and dogs, a significant extraction of ANF by the lungs, the kidney, the peripheral circulation and the splanchnic circulation has been shown (12). So far no information exists on the role of the liver in the splanchnic extraction of ANF. Although one study uses ANF infusion (13), no data are available on ANF extraction under basal conditions in the rat (14). However, these may be questions of considerable interest because there is conflicting information on ANF plasma concentrations in liver disease (7, 15-17). Therefore, to characterize the extraction of circulating ANF in more detail, we investigated the role of the liver in extraction of C-terminal and N-terminal ANF in rats.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 280 gm to 330 gm and kept under standardized conditions were anesthetized with sodium pentobarbital intraperitoneally (60 mg/kg), heparinized with 250 IU intravenously and then laparotomized. Multiple blood sampling was carried out by direct vessel puncture (abdominal aorta and portal vein) or by venous catheterization with a 20-gauge Teflon cannula (hepatic vein). Blood was immediately transferred into precooled tubes containing EDTA (1 mg/ml), centrifuged at 1500 gm and stored at −70° C until assay.

RIA for ANF. C-terminal ANF (99 to 126) was determined in the extract of 100 μl plasma by use of antibody Toni III (18, 19), as published previously. Intraassay and interassay variations were below 10% and 15%, respectively; recoveries averaged 70%. The RIA for the N-terminal fragment of pro-ANF was performed with 50 μl plasma and antibody GT 23 as described elsewhere in detail (20). The intraassay and interassay coefficients of variation were less than 12%, and recoveries were close to 100%.

Calculations and Statistical Evaluation. Extraction ratios in percentages for C-terminal and N-terminal ANF were calculated by the following formulas: Splanchnic extraction = $([C_a - C_h]/C_a) \times 100$ (%), Mesenteric extraction = $([C_a - C_p]/C_a) \times 100$ (%) and Hepatic extraction = $([\frac{2}{3}C_p + \frac{1}{3}C_a - C_h]/[\frac{2}{3}C_p + \frac{1}{3}C_a]) \times 100$ (%), where C = concentration, a = abdominal aorta, p = portal vein and h = hepatic vein. These formulas are based on the finding that blood flow through the portal vein is twice the hepatic arterial blood flow (21). Concentration gradients in femtomoles per milliliter were calculated accordingly. For these calculations values from

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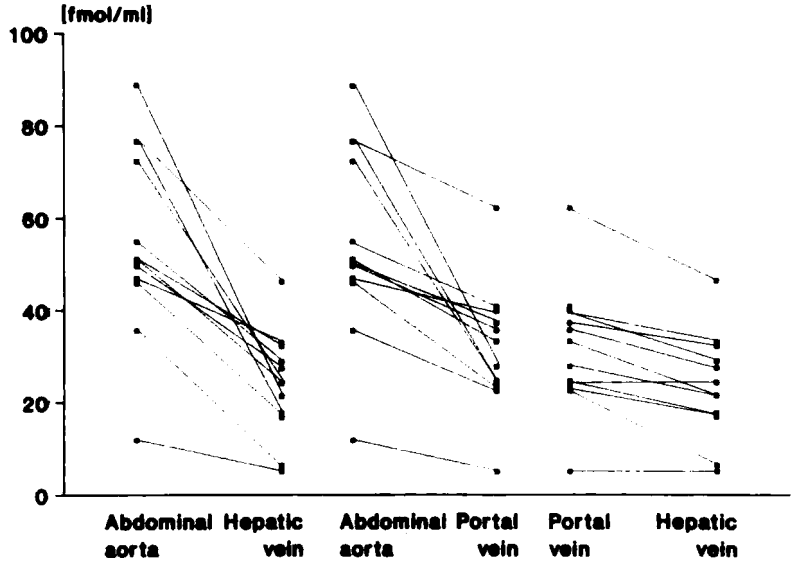

FIG. 1. Plasma concentrations of C-terminal ANF in abdominal aorta, portal vein and hepatic vein of rats. Lines connect the values obtained in the same animals.

different vascular regions of the same animal (paired samples) were used.

Differences between means were compared by paired and unpaired *t* tests where appropriate. A p value of less than 0.05 was considered statistically significant. Data are presented as mean and S.E.

RESULTS

The plasma concentrations of C-terminal ANF in the hepatic vein (23.5 ± 3.3 fmol/ml) were significantly lower ($p < 0.001$) than in the abdominal aorta (55.0 ± 6.1 fmol/ml; $n = 12$) (Fig. 1), corresponding to a splanchnic extraction ratio of 56% ± 5% (Table 1). Mesenteric ANF extraction rate was lower (42% ± 6%) (Table 1), with concentrations of C-terminal ANF in the portal vein of 31.2 ± 4.0 fmol/ml and in the abdominal aorta of 55.0 ± 6.1 fmol/ml ($n = 12$; $p < 0.001$). Plasma concentrations in the hepatic vein (23.5 ± 3.3 fmol/ml) were significantly lower ($p < 0.001$) than in the portal vein (31.2 ± 4.0 fmol/ml; $n = 12$) (Fig. 1). Taking into account the arterial part of liver perfusion, we found that 41% ± 5% of C-terminal ANF was eliminated by the liver. This figure was not significantly different from the mesenteric extraction rate of 42% ± 6% (Table 1).

Splanchnic extraction of N-terminal ANF averaged 50% ± 7% (Table 1), with concentrations of the N-terminal fragment of 3,031 ± 756 fmol/ml in the aorta and 1,618 ± 496 fmol/ml in the hepatic vein ($p < 0.005$; $n = 6$). Mesenteric extraction of the N-terminal ANF was significantly lower than that of the C-terminal ANF (28% ± 4% vs. 42% ± 6%; $p < 0.01$), with concentrations of 2,264 ± 661 fmol/ml in the portal vein and 3,031 ± 756 fmol/ml in the aorta ($p < 0.001$; $n = 6$). The concentration of N-terminal ANF was 1,618 ± 496 fmol/ml in the hepatic vein as opposed to 2,264 ± 661 fmol/ml in the portal vein ($p < 0.05$; $n = 6$) (Fig. 2), resulting in hepatic extraction of 39% ± 6% (Table 1). Hepatic extraction of N-terminal ANF was significantly higher (39% ± 6%) than mesenteric extraction (28% ± 4%; $p < 0.05$).

DISCUSSION

This study provides new results on the role of the liver in splanchnic ANF extraction and the splanchnic extraction of N-terminal ANF. The splanchnic extraction rate of C-terminal ANF in rats was found to be 56%. This rate is well in the range reported for the intestinal ANF extraction in human beings (28% to 71%) (12, 17, 22-27) and somewhat higher than the 28% to 36% reported in dogs (12, 28). The role of the liver in the splanchnic ANF extraction has not been established so far. However, there is evidence that in liver failure the clearance of ANF is reduced (29). Furthermore, a significant uptake of iodinated ANF from the circulation by the liver suggests a role of this organ in ANF clearance (30). Recently, Hollister et al. (12) observed a concentration difference corresponding to 16% of C-terminal ANF between portal and hepatic veins in dogs. In view of the much higher concentration of ANF in arterial blood and the fact that hepatic arterial blood flow constitutes about one third of total hepatic blood flow (21), this would indicate a hepatic ANF extraction of about 44%—very close to the 41% that we found in the rat. In our study the rate of hepatic extraction of C-terminal ANF (41% ± 5%) was similar to the mesenteric extraction rate (42% ± 6%).

As yet, there are no data available on extraction of the N-terminal ANF fragment. Our data show that N-terminal ANF is extracted in the splanchnic circulation and that the liver is more important in this regard than the mesenteric circulation (39% ± 6% vs. 28% ± 4% extraction; $p < 0.05$). Thus although the hepatic extraction ratio of C-terminal and N-terminal

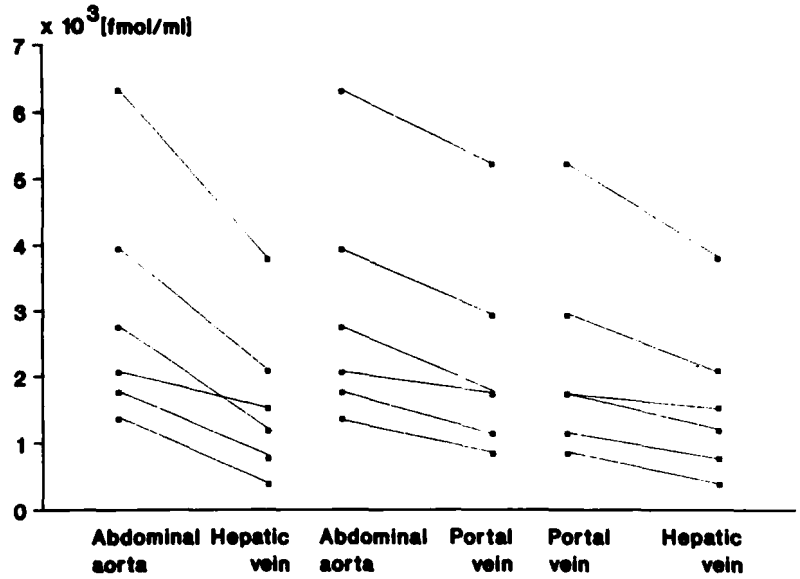

FIG. 2. Plasma concentrations of N-terminal ANF in abdominal aorta, portal vein and hepatic vein of rats. Lines connect the values obtained in the same animals.

TABLE 1. Extraction of ANF in rats

	C-terminal ANF[a]		N-terminal ANF[b]	
	Concentration gradient (fmol/ml)	Extraction (%)	Concentration gradient (fmol/ml)	Extraction (%)
Mesenteric extraction	23.8 ± 5.4[c, d]	41.6 ± 5.8[d]	766 ± 130[d, e, f]	28.3 ± 4.1[d, e, f]
Hepatic extraction	15.6 ± 2.0[d]	40.8 ± 4.6[d]	901 ± 217[d, e]	39.1 ± 6.1[d]
Splanchnic extraction	31.5 ± 1.9[f]	56.2 ± 5.0[f]	1412 ± 295[e, f]	49.9 ± 6.5[f]

[a] n = 12.
[b] n = 6.
[c] Data expressed as mean ± S.E.M.
[d] $p < 0.05$ vs. splanchnic extraction.
[e] $p < 0.05$ vs. corresponding C-terminal values.
[f] $p < 0.05$ vs. hepatic extraction.

ANF fragments by the liver is similar, the mesenteric extraction of C-terminal ANF is greater than that of the N-terminal fragment. It has been shown that clearance of the C-terminal ANF is caused by three mechanisms. The first mechanism is the binding of ANF to the C-receptor, subsequent internalization and rapid degradation in lysosomes. The second is the enzymatic degradation by endopeptidase 24.11 (11) and by a carboxypeptidase (31, 32). The third mechanism, which is rather unspecific, is glomerular filtration and renal excretion. Endopeptidase 24.11 activity has been demonstrated mainly in the kidney, but recently also in the mesentric artery of the rat by *ex vivo* perfusion and membrane preparation techniques (33). Presence of the C-receptor has been shown in various organs including the liver (34). There are no data as yet on the mechanisms of clearance of the N-terminal ANF fragment. Altogether our data suggest a major role of the liver in the splanchnic extraction of C-terminal and N-terminal ANF in the rat.

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