Liver and biliary

Hepatic intestinal uptake and release of catecholamines in alcoholic cirrhosis. Evidence of enhanced hepatic intestinal sympathetic nervous activity

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SUMMARY Hepatic intestinal and whole body plasma clearance and appearance of noradrenaline (NA) was quantified in patients with alcoholic cirrhosis ($n=12$) and in controls ($n=6$). As NA may be released as well as removed in the same vascular bed, infusion of tritium labelled NA ($^3\text{H-NA}$) was carried out during hepatic vein catheterisation in order to determine both flux rates. In alcoholic cirrhosis plasma concentrations of endogenous NA and adrenaline (A) were significantly above control values (NA: median 2.4 ± 1.7 nmol/l, $p<0.02$; A: 0.38 ± 0.19 nmol/l, $p<0.01$). Whole body clearance of $^3\text{H-NA}$ equal in the two groups (1.6 ± 1.7 l/min, ns), while as the overall appearance rate of NA was significantly higher in alcoholic cirrhosis (4.2 ± 2.6 nmol/min, $p<0.02$) indicating an enhanced sympathoadrenal activity in this group. The hepatic intestinal clearances of A, NA, and $^3\text{H-NA}$ were not significantly different in patients and controls, but the estimated hepatic intestinal spillover rate of NA was 0.24 nmol/min in patients as compared with 0.0 nmol/min in controls ($p<0.02$). As a result of portosystemic shunting in cirrhosis the present estimation of NA spillover represents a minimum value. Our results indicate that the augmented circulating catecholamines in cirrhosis do not result from diminished removal but are contributed to from increased sympathetic nervous activity in the hepatic intestinal area (enhanced mesenteric sympathetic nervous activity).

Noradrenaline (NA) is the neurotransmitter released from axon terminals of sympathetic postganglionic neurones which normally control cardiovascular responses during – for example, postural changes and exercise.¹ Adrenaline (A), a hormone originating from the adrenal medulla, together with NA controls several functions, including metabolism and the secretion of other hormones. The concentrations of endogenous NA and A in plasma may be accurately measured by enzymatic isotope derivative techniques.^{2,3} This is important as NA leaks from the synaptic nerve terminals into the plasma where its concentration reflects the neurotransmitter activity, provided there is normal NA-clearance from

plasma.^{4,5} Because the same vascular bed may release as well as remove NA, quantification of NA-kinetics demands a method such as the application of labelled NA,^{6,7} which is able to distinguish both flux rates.

Recent studies have shown an increased concentration of circulating NA in supine patients with decompensated cirrhosis.⁸⁻¹⁰ The kidneys have been identified as a source of increased NA release in cirrhosis, but enhanced sympathetic nervous activity in other regions may also be important.¹¹⁻¹³ The gastrointestinal tract and the liver are richly supplied with sympathetic nerve fibres, and it is conceivable that the splanchnic system, besides removing NA, also releases this amine. Therefore, the objective of the study has been to quantify and compare the hepatic intestinal and the whole body uptake and release of NA in patients with cirrhosis and in control subjects. For this purpose we used constant intra-

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venous infusion of tritium labelled noradrenaline ($^3\text{H-NA}$) during hepatic vein catheterisation.

Methods

PATIENTS

The study comprised 12 patients with biopsy verified alcoholic cirrhosis (two women, 10 men; age 42–74 years, height 155–190 cm, weight 58–95 kg). All patients were considered to be in a stable stage of their disease. All patients had abstained from alcohol for at least one week and were without signs of actual alcoholic consumption or withdrawal symptoms. None of the patients had experienced recent gastro-intestinal bleeding or hepatic encephalopathy. Ten patients had signs of fluid retention (10 ascites, six oedema of lower limbs).

Six subjects served as controls. Three were patients without abnormal findings, one had fatty liver, one had slight cholestasis on liver biopsy, and one had chronic bronchitis without liver disease. All were men, age 36–68 years, height 170–180 cm, weight 62–99 kg.

Patients and controls consented to participate in the investigation after thorough oral and written explanation. The study was approved by the Ethics Committee for Medical Research in Copenhagen, and no complications or side effects were seen during the investigative procedure.

CATHETERISATION

All subjects were studied on the morning after an overnight fast. Hepatic vein catheterisation was done under local anaesthesia in the supine subject from an antecubital or femoral vein, as previously described.^{13,14} A small indwelling polyethylene catheter was placed in the femoral artery by the Seldinger technique. Pressures were measured in the wedged and free hepatic vein, and in the femoral artery by a capacitance transducer (Simonsen & Weel, Copenhagen), the zero pressure reference level being the midaxillary line. Hepatic plasma flow (HPF) and blood flow (HBF) were determined by the indocyanine green (ICG) constant infusion technique as previously described.^{10,13} Indocyanine green clearance and the hepatic extraction ratio of the dye were measured as infusion rate divided by arterial ICG concentration and as arteriohepatic venous ICG difference divided by arterial ICG concentration, respectively.

PROTOCOL

Plasma samples for determination of endogenous NA and A were obtained simultaneously from artery and hepatic vein. Whole body and hepatic intestinal plasma clearances of noradrenaline were determined

by constant intravenous infusion of tritium-labelled L-noradrenaline ($^3\text{H-NA}$, 20 Ci/nmol (740 GBq/nmol) specific activity, New England Nuclear, Boston). The infusion rate, 0.7–1.0 $\mu\text{Ci/min}$ (26–37 KBq/min, 6–8 ng NA/min),* was maintained by a calibrated pump (Dich, Copenhagen). After 25 minutes of constant $^3\text{H-NA}$ infusion plasma samples were simultaneously obtained from artery and hepatic vein. The infusion period was chosen because of the possibility of recirculation of labelled NA. As discussed in detail elsewhere,^{15,16} steady state will be achieved within this period, and no appreciable recirculation between nerve endings and blood takes place. The calculated whole body radiation dose was less than 200 mrem (2 m Sv).

ANALYSIS

Plasma concentrations of endogenous NA and A in artery and hepatic vein were determined by an enzymatic isotope derivative technique.³ The within assay coefficients of variation were approximately 11% and 7% for A and NA, respectively. $^3\text{H-NA}$ in plasma was extracted by alumina, eluted by acid, freeze dried and counted in liquid scintillator as earlier described.⁴ Deaminated metabolites constituted less than 1% of the counts in the alumina eluates.

CALCULATIONS

Splanchnic (hepatic intestinal) extraction ratios (E_s) of A, NA and $^3\text{H-NA}$ were calculated as: $E_s = (C_a - C_{hv})/C_a$, where C_a and C_{hv} are the plasma concentrations of A, NA, and $^3\text{H-NA}$ in artery and hepatic vein, respectively. Splanchnic (hepatic intestinal) plasma clearances (Cl_s) of A, NA and $^3\text{H-NA}$ were estimated as: $Cl_s = \text{HPF} \cdot E_s$. Splanchnic (hepatic intestinal) release rate ($\dot{J}_{s,\text{release}}$) of noradrenaline was estimated from the equation:

$$\dot{J}_{s,\text{release}} = \text{HPF} \cdot C_{a,\text{NA}} \cdot [(C_{hv}/C_a)_{\text{NA}} - (C_{hv}/C_a)_{^3\text{H-NA}}]$$

Whole body clearance (Cl_{WB}) of noradrenaline was determined as $Cl_{\text{WB}} = (\text{infusion rate of } ^3\text{H-NA})/C_{a,^3\text{H-NA}}$, and whole body appearance rate was calculated as $Cl_{\text{WB}} \cdot C_{a,\text{NA}}$.

STATISTICAL ANALYSIS

The significance of differences between median values was tested by Wilcoxon's and Mann-Whitney tests for paired or grouped data, respectively. Correlations were determined by the method of least squares. $p < 0.05$ was considered significant.

* 8 ng NA/min will increase the concentration of circulating NA by less than 2% which is beyond the limit of detection.

Table 1 Biochemical and haemodynamic data in patients with alcoholic liver cirrhosis and controls

	Serum albumin conc.	Coagulation factor II, VII, X	Serum bilirubin conc.	Alanine transaminase	Alkaline phosphatase	Artery	Right atrium	Weged hepatic vein	Free hepatic vein	Hepatic blood flow
	$\mu\text{mol/l}$		$\mu\text{mol/l}$	U/l	U/l		Mean blood pressure mm Hg			l/min
	(540–800)‡	(0.7–1.3)	(2–17)	(10–40)	(50–275)		(<6)	(<15)	(<6)	(0.5–2.3)
Alcoholic cirrhosis (n=12)	461* (337–762)	0.47† (0.30–0.90)	20* (5–183)	51* (18–185)	343† (207–916)	85 (62–125)	3.0 (0.0–7.0)	25† (10–33)	8.7* (5.5–14)	1.43 (0.78–2.50)
Controls (n=6)	635 (555–716)	1.02 (0.78–1.2)	7 (2–15)	26 (13–39)	188 (61–248)	95 (83–113)	3.7 (0.5–9.5)	8.4 (7.6–11.5)	5.2 (3.5–9.5)	1.23 (1.01–1.45)

Median values, ranges in parentheses; significantly different from controls: * $p<0.05$; † $p<0.01$; ‡normal reference range (95% confidence).

Table 2 Whole body catecholamine kinetics in patients with alcoholic cirrhosis and controls

	Plasma concentration		Whole body plasma clearance of noradrenaline (Cl_{WB}) l/min	Overall appearance rate of noradrenaline nmol/min
	Noradrenaline (NA) nmol/l	Adrenaline (A) nmol/l		
Alcoholic cirrhosis (n=12)	2.4* (1.1–8.2)	0.38† (0.16–1.6)	1.57 (1.21–1.91)	4.2* (1.9–6.8)
Controls (n=6)	1.7 (0.6–3.0)	0.19 (0.09–0.23)	1.68 (0.86–2.16)	2.6 (1.5–4.7)

Median values, ranges in parentheses; significantly different from controls: * $p<0.02$; † $p<0.01$.

Results

The biochemical and haemodynamic findings are summarised in Table 1.

Endogenous arterial plasma NA and A concentrations in patients with alcoholic cirrhosis were significantly above those of the controls (Table 2). Arterial NA concentrations were positively correlated with wedged hepatic venous pressure ($r = 0.51$, $p<0.05$) but not with any other haemodynamic variable. Cl_{WB} was equal in the two groups, and consequently the overall appearance rate of noradrenaline was significantly higher in the patient group (Table 2).

The estimated splanchnic (hepatic intestinal) release rate of noradrenaline ($\dot{J}_{s, \text{release}}$) was median 0.24 nmol/min in the cirrhotic patients as compared with 0.0 nmol/min in controls ($p<0.02$, Fig. 1), and this value amounted to median 7% and 0%, respectively, of the overall appearance rate of noradrenaline. $\dot{J}_{s, \text{release}}$ was negatively correlated with mean arterial blood pressure ($r = -0.47$, $p<0.05$). No significant relationship was found between $\dot{J}_{s, \text{release}}$ and wedged-to-free hepatic vein pressure or any other haemodynamic variable.

Splanchnic (hepatic intestinal) extraction ratios of ICG, A, NA and ^3H -NA are shown in Table III. $E_{S, A}$, $E_{S, NA}$ and $E_{S, ^3\text{H-NA}}$ were positively correlated

Fig. 1 Estimated splanchnic (hepatic intestinal) release rate of noradrenaline ($\dot{J}_{s, \text{release}}$) in patients with alcoholic liver disease (LD) and controls (C).

Fig. 2 Relationship between splanchnic (hepatic intestinal) extraction ratios of indocyanine green (E_{ICG}) and adrenaline ($E_{S,A}$), noradrenaline ($E_{S,NA}$) and tritium labelled L-noradrenaline ($E_{S,3H-NA}$) in patients with alcoholic liver cirrhosis and controls. ● cirrhosis, ○ controls.

with E_{ICG} ($r = 0.66$, $p < 0.005$; $r = 0.58$, $p < 0.01$; $r = 0.40$, $p < 0.1$, respectively, Fig. 2). $Cl_{S,A}$, $Cl_{S,NA}$, and $Cl_{S,3H-NA}$ were not significantly different in patients and controls (Fig. 3). $Cl_{S,NA}$ was significantly lower than $Cl_{S,A}$ and $Cl_{S,3H-NA}$ ($p < 0.02$, and $p < 0.01$, respectively) in patients but not in controls. In patients splanchnic (hepatic intestinal) catechol-

amine clearance was significantly higher than that of ICG ($p < 0.01$). No significant difference was found in controls. $Cl_{S,3H-NA}$ was a median 35% and 25% of Cl_{WB} in patients and controls, respectively (ns).

Discussion

The present study shows that the removal of catecholamines from plasma is normal in patients with alcoholic cirrhosis, as determined from 3H -NA whole body and hepatic intestinal clearances. The raised circulating noradrenaline and adrenaline in some cirrhotic patients, therefore, reflect an increased appearance of catecholamines, indicating enhanced sympathoadrenal activity in this condition.

Enhanced sympathetic nervous activity in cirrhosis has been identified in the kidney¹¹⁻¹³ and possibly in parts of the peripheral circulation.¹⁷ The hepatic intestinal area, however, has not hitherto been evaluated with respect to sympathetic nervous activity by methods which allow estimation of unidirectional transport of catecholamines between the blood stream and the splanchnic organs.

The hepatic intestinal clearance of endogenous NA was significantly smaller than that of 3H -NA ($p < 0.01$) and A ($p < 0.02$) in patients but not in controls. This discordance between tracer and endogenous substance found in patients indicates that besides removal there is also a significant spillover of NA from the splanchnic system into the systemic circulation, as clearly shown in the present study (Fig. 1). Hepatic intestinal NA release was absent in the controls. As all splanchnic blood passes through liver veins in normal subjects the estimated value is likely to be correct in that group. Patients with cirrhosis, however, have portosystemic shunts, and the estimated release of NA only covers the amount passing through the liver veins. An unknown quantity flows through the portosystemic collaterals. Therefore, in cirrhosis the present estimation of $J_{s,release}$ represents a minimum value. If it is assumed that the average blood flow through portosystemic collaterals amounts to 0.8 l/min,¹⁸ and the content of NA in plasma from these collaterals is similar to that of the hepatic vein, net release may be underestimated by: $(0.8/1.43) \cdot 0.24 \text{ nmol/min} = 0.13 \text{ nmol/min}$. If the concentration of NA in collaterals is higher or the collaterals preferably drain – for example, spleen, reflecting local phenomena, the extent of this underestimation cannot be determined.

In the controls, hepatic intestinal extraction ratios of endogenous and tracer NA ($E_{S,NA}$ and $E_{S,3H-NA}$) were not significantly different from that of indocyanine green (E_{ICG}), but the extraction ratio of A ($E_{S,A}$) was approximately 25% higher (Table 3). In

Fig. 3 Splanchnic (hepatic intestinal) clearances (ml/min) of indocyanine green ($Cl_{s,ICG}$), adrenaline ($Cl_{s,A}$), noradrenaline ($Cl_{s,NA}$) and tritium labelled L-noradrenaline ($Cl_{s,3H-NA}$) in patients with alcoholic cirrhosis (LD) and controls (C). * $p < 0.01$ v $Cl_{s,3H-NA}$ and $p < 0.02$ v $Cl_{s,A}$. ** $p < 0.01$ v $Cl_{s,A}$, $Cl_{s,NA}$ and $Cl_{s,3H-NA}$. ns = not significantly different. Transverse bars indicate median values.

contrast, hepatic intestinal catecholamine extraction ratios in the patients were substantially higher than E_{ICG} . This may be because of the different metabolic pathways of ICG and catecholamines^{19,20} and to capillary wall tightening in cirrhosis ('capillarisation'),²¹ the latter favouring the transcapillary passage of catecholamines relative to ICG, as ICG is firmly bound to proteins.²⁰ The slight but significant direct relationship between ICG and catecholamine extraction, however, may suggest some relationship between hepatocellular function and catecholamine metabolism.

The estimated hepatic intestinal clearance of catecholamines followed the pattern of the extraction ratios. Moreover, these clearance values were so high (approximately $\frac{1}{3}$ to $\frac{1}{4}$ of Cl_{WB}) that the hepatic

intestinal system may be identified as an important site of catecholamine removal even in patients with chronic liver disease. As a result of portosystemic shunting the hepatic intestinal clearance of catecholamines may be underestimated in patients with cirrhosis in the same way as the NA release (see above).

Increasing evidence suggests that circulatory underfilling and low arterial blood pressure in patients with cirrhosis may be responsible for enhanced sympathetic nervous activity in this condition.^{9,22} In addition, a non-volume dependent hepatic baroreceptor has been suggested as a possible sympathetic afferent trigger.²³ The present findings of inverse correlation between estimated hepatic intestinal NA release and mean arterial blood pressure

Table 3 Splanchnic (hepatic intestinal) extraction ratios of indocyanine green, endogenous catecholamines, and tritium labelled L-noradrenaline

	Indocyanine green E_{ICG}	Adrenaline $E_{s,A}$	Noradrenaline $E_{s,NA}$	Tritium labelled L-noradrenaline $E_{s,3H-NA}$
Alcoholic cirrhosis (n=12)	0.22* (0.08-0.59)	0.60 (0.0-0.96)	0.37‡ (0.17-0.67)	0.56 (0.31-0.85)
Controls (n=6)	0.66† (0.33-0.82)	0.84 (0.50-1.0)	0.64 (0.31-0.72)	0.59 (0.26-0.86)
p	<0.005	NS	<0.025	NS

Median values, ranges in parentheses.

* $p < 0.001$ compared with $E_{s,A}$, $E_{s,NA}$, or $E_{s,3H-NA}$; † $p < 0.05$ compared with $E_{s,A}$; ‡ $p < 0.01$ compared with $E_{s,3H-NA}$ and $p < 0.02$ compared with $E_{s,A}$.

and the direct relationship between circulating NA and wedged hepatic vein pressure are in accordance with this view, and it would be reasonable to suppose that the catecholamine response might be inadequate to overcome the potent stimulus giving rise to the deranged systemic and splanchnic haemodynamics. Another possibility, however, is that enhanced sympathetic tone in itself may be of importance for the raised portal venous pressure found in patients with cirrhosis, as suggested by Willett *et al.*²⁴

In summary, our results indicate that the augmented circulating catecholamines in cirrhosis are not the results of diminished removal but originates from increased sympathetic nervous activity. This increased activity has previously been shown in the kidney and peripheral circulation, but the present study shows that it also occurs in the hepatic intestinal area. Because of the presence of portosystemic collaterals of different size only a minimum value of hepatic intestinal noradrenaline spillover can be determined in patients with cirrhosis.

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