JCI The Journal of Clinical Investigation

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J Clin Invest. 1954;33(3):337-341. https://doi.org/10.1172/JCI102903.

Research Article

OBSERVATIONS ON THE VISCERAL REMOVAL OF PLASMA PEPTIDASE ACTIVITY [1]

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(Submitted for publication August 7, 1953; accepted November 4, 1953)

Fluctuation in activity of plasma peptidases has been noted as a concomitant of a wide variety of clinical disorders. These proteolytic enzymes exhibit enhanced activity in the plasma of man or experimental animals subjected to any severe tissue damage, and there is convincing evidence, discussed elsewhere (1, 2), that these enzymes gain entrance to the circulation from injured or destroyed cellular components. With healing (3) or clinical remission (4, 5), elevated plasma peptidase values return to the normal range, but the mechanisms involved in the removal of peptidase activity from the plasma have not been systematically investigated. The present study was undertaken in an attempt to determine the fate of these circulating peptidases.

MATERIALS AND METHODS

A. Nephrectomy in rats

After an 18-hour fast, male rats of the Vanderbilt strain weighing 200 to 250 Gm. were bilaterally nephrectomized using pentobarbital anesthesia and a lumbodorsal operative approach. Twenty-four hours later the animals were reanesthetized, and heparinized blood was collected from the abdominal aorta. Control blood samples were similarly obtained from rats not subjected to nephrectomy.

B. Studies of splanchnic removal of plasma peptidase in man

Accepted techniques were employed for collecting simultaneous femoral arterial and hepatic venous blood samples and determining hepatic blood flow, BSP clearance, net splanchnic oxygen consumption and net splanchnic glucose production (6–8). Peripheral venous blood was obtained from the antecubital vein.

The product of the estimated hepatic blood flow and the corresponding femoral arterial-hepatic venous difference of a metabolite gives the splanchnic balance of that material. Although it is probable that hepatic activity determines the balance in greatest part, it must be remembered that those viscera drained by the portal vein (gut, spleen, pancreas, etc.) play some role in the determination of splanchnic balance. Accordingly, the term splanchnic, meaning the domain of the liver plus the various viscera drained by the portal vein, is adhered to throughout this paper.

C. Determination of peptidase activity

In all instances, peptidase activity was measured by a photometric ninhydrin technique (9), using *non-hemolyzed* heparinized plasma. Two synthetic tripeptides, glycylglycylglycine (GGG) and L-leucylglycylglycine (LGG), were used as substrates, the former for studies on rats and the latter for measurement of splanchnic A–V differences.

Enzymic hydrolysis proceeded under conditions previously described (4, 9). Samples were withdrawn after one and two hours of incubation and the rate of hydrolysis taken as the mean of the two determinations, since the reaction, initially, appeared to follow zero order kinetics. The assumption that there is cleavage of only one peptide bond was fortified by the finding that, in a third sample, routinely taken eighteen hours after the initiation of incubation, the degree of hydrolysis calculated on this basis often equaled, but never exceeded, the 100 per cent value for one-bond cleavage. In order to quantitate changes in LGG-splitting in terms of hepatic blood flow, it was decided, somewhat reluctantly, to establish a *unit* of peptidase activity which is here defined as that *amount of enzyme activity in 1 ml. of plasma which hydrolyzes 1 μM of substrate per hour* under the conditions detailed above.

RESULTS

The kidney, as a site of removal of plasma peptidase, was investigated using bilaterally nephrectomized rats. Twenty-four hours after nephrectomy, in five animals, plasma GGG-cleaving activity was found to be 7.8 ± 1.1 units,[3] which differs insignificantly ($p < 0.2$) from the mean of 9.0 ± 0.1 units in twenty-nine normal animals. These findings, together with the report (1) of normal peptidase activity in patients in advanced uremia, make it seem quite unlikely that the kidney serves as an important avenue of excretion for these enzymes.

[1] This work was supported, in part, by grants from the National Institutes of Health, United States Public Health Service, the Duke University Research Council, and the Life Insurance Medical Research Fund.

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[3] Mean ± standard error.

TABLE I

Splanchnic uptake of plasma peptidase activity

Patient	Diagnosis	Procedure	Arterial plasma LGGase activity (*units*)	BSP clearance (*% of normal*)	Splanchnic plasma flow (*ml./min./sq. M*)	Splanchnic peptidase A-V difference (*units*)	Splanchnic LGGase uptake (*units per min. per sq. M*)	Net splanchnic oxygen consumption (*ml./min./sq. M*)	Net splanchnic glucose production (*mg./min. per sq. M*)
			Group A—Normal Arterial Plasma LGGase Activity						
O. F.	Psychoneurosis	Control	5.46	87	390	−0.24	− 94	34	56
M. B.	Psychoneurosis Obesity	Cortisone 200 mg.	3.43	91	580	0.25	145	51	115
M. H.	Psychoneurosis	Cortisone 200 mg.	5.04	106	680	−0.43	−292	50	94
J. A.	Psychoneurosis	Cortisone 200 mg.	5.36	92	550	0.17	94	32	51
J. R.	Hysteria	Pressure study	4.26			−0.28			
A. G.	Cirrhosis	Pressure study	4.33			0.06			
	Mean ± S.E.		4.65± 0.319	94± 4.1	550± 60.1	−0.01± 0.109 0.4<p<0.5	− 37± 62.9 p>0.5	42± 5.1	79± 15.4
			Group B—Elevated Arterial Plasma LGGase Activity						
J. S.	Peptic ulcer	Control	6.23	96	440	0.89	393	30	50
H. M.	Peptic ulcer	Oxypolygelatin infusion	7.86	128	790	1.46	1153	45	
J. H.	Optic neuritis	Typhoid vaccine	9.60	54	880	0.55	484	53	103
S. D.	Psychoneurosis	Cortisone 200 mg.	8.55	75	430	0.70	301	37	54
R. E.	Psychoneurosis	Cortisone 200 mg.	10.18	79	640	0.69	442	36	48
L. N.	Psychoneurosis	Cortisone 200 mg.	11.28	108	490	1.66	813	38	46
E. R.	Infectious hepatitis, convalescent	Pressure study	6.82			0.02			
G. W.	Infectious hepatitis	Pressure study	16.42			1.16			
W. F.	Homologous serum jaundice	Pressure study	17.99			0.91			
	Mean ± S.E.		10.55± 1.370	90± 10.7	612± 77.9	0.89± 0.164 p<0.01	598± 132 p<0.01	40± 3.3	60± 8.8

Attention was next directed to the role of the splanchnic area in the extraction of peptidases from the plasma. LGG-hydrolyzing activity was measured in simultaneously obtained samples of plasma from the femoral artery and hepatic vein of fifteen patients. In the eight instances in which replicate samples were collected, mean values were used. Since in these individuals hepatic vein catheterizations were performed primarily for other purposes, as indicated in Table I, the diverse nature of the procedures which were carried out in some instances undoubtedly influenced some of the measures of splanchnic circulatory and metabolic activity and conceivably could have provoked changes in peptidase levels as well. However, it was possible to obtain control blood samples in all but the cortisone studies (10), and there is now ample evidence (4) that plasma peptidase levels are not *directly* affected by changes in adrenal cortical activity. These facts may offer some reassurance regarding the validity of absolute values of plasma peptidase activity obtained; but, in actuality, there would appear to be little need to provide justification for the procedures used since the primary aim was to determine quantitatively whether the splanchnic area extracted peptidases from the plasma, regardless of any disease or experimental manipulation which might have produced changes in arterial peptidase levels.

Pertinent data are presented in Table I. The patients are divided into two groups, one consisting of those with normal arterial LGG-splitting activity (Group A) and the other of individuals with elevated plasma peptidase levels (Group B). This selection was based on the determination of plasma LGG-hydrolyzing activity in fourteen "normal" subjects. The mean value in this group was 4.47 ± 0.73 units,[4] so arterial plasma enzyme levels above 5.93 units (mean plus 2 S. D.) were considered to be increased beyond the normal range.

In twelve of the fifteen patients, arterial-hepatic venous peptidase differences were positive, suggesting that enzyme was being "extracted" from the plasma in the splanchnic area. Although it is apparent that in all of the patients these differences were of a rather low magnitude, a comparison of

[4] Mean ± standard deviation.

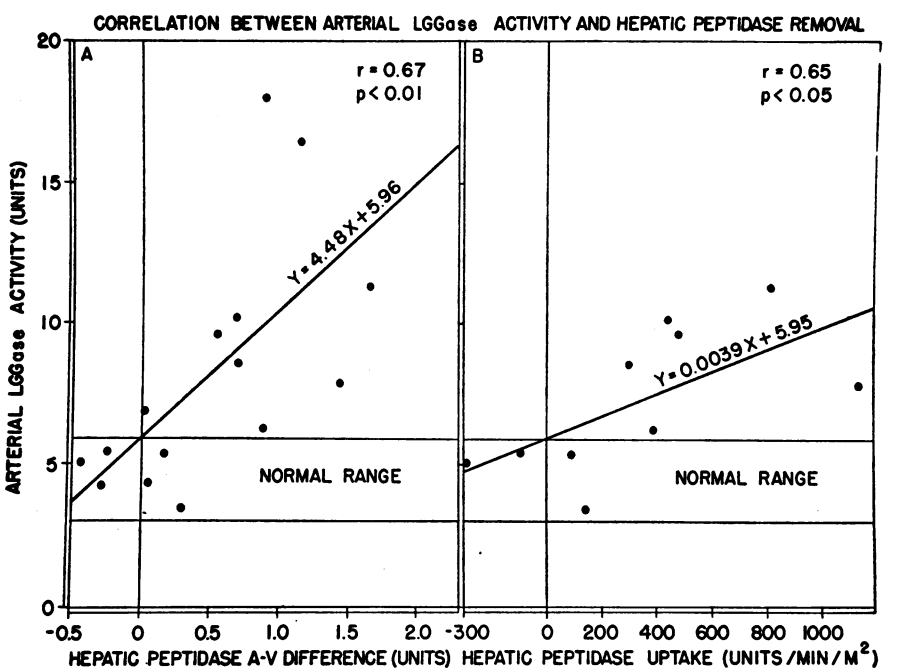

FIG. 1. THE STATISTICALLY SIGNIFICANT CORRELATION THAT EXISTS BETWEEN ARTERIAL PEPTIDASE ACTIVITY AND BOTH SPLANCHNIC PEPTIDASE ARTERIOVENOUS DIFFERENCE AND SPLANCHNIC UPTAKE IS SHOWN

The normal range of plasma LGG-splitting activity is defined by the mean value ± 2 standard deviations (4.47 ± 1.46) in fourteen normal subjects.

the two groups as indicated in Table I proved to be of interest. In Group A the hepatic A–V differences and the splanchnic uptakes were small or negative, so that mean values did not differ appreciably from zero ($p < 0.4$ and $p < 0.5$, respectively). In striking contrast, in Group B mean values for hepatic A–V differences and splanchnic uptakes were significantly increased above both zero and the appropriate mean values of Group A ($p < 0.01$ in all instances). Thus, it seems clear that in most patients with high plasma peptidase activity, significant quantities of enzymic activity are removed from the circulation by the liver and associated portal viscera, while no consistent change is detectable in normal plasma peptidase activity during passage through the splanchnic circulation.

Groups A and B are not distinguishable on the basis of differences in BSP clearance, hepatic plasma flow, net splanchnic oxygen consumption, or net splanchnic glucose production. The individual variations that occur are explicable in terms of the patient's illness or the procedure to which he was subjected.

The highest plasma peptidase levels in this series were found in patients G. W. and W. F., both ill with acute inflammatory liver disease. Indeed, the highest human plasma peptidase activity observed in this laboratory, 20.8 units, occurred in a patient in hepatic coma. This is in contrast to the slightly elevated value in E. R., who was convalescing from an attack of infectious hepatitis, and A. G., who had compensated Laennec's cirrhosis.

Viewed in another context, the observations recorded in Table I provide additional information. When, as in Figure 1, arterial LGG-hydrolyzing is plotted against hepatic peptidase A–V differences and uptakes, the relationships are seen to be roughly linear and the calculated positive correlation coefficients are significant statistically. It seems evident, then, that the higher the arterial peptidase activity, the greater the removal of enzyme in the splanchnic area and presumably the liver, a finding which is reminiscent of other known homeostatic functions of the latter organ.

DISCUSSION

It should be emphasized that the rate at which a specific peptide substrate is hydrolyzed by crude peptidase preparations, such as plasma, cannot be considered a simple function of the enzyme concentration alone. Enzymic hydrolysis is known to be strongly affected by other influences, such as temperature, pH, substrate concentration, and the presence or absence of activators or inhibitors. The maintenance of constant temperature and pH and the presence of excess substrate minimize these factors as variables, but with the procedures used in this laboratory the relative influences of enzyme concentration and activators and inhibitors remain undetermined. It is for this reason that emphasis is laid on the less specific term "peptidase *activity*," and changes in activity must be interpreted with caution. In the present instance, for example, it would be hazardous to accept the data as evidence that enzyme protein is actually removed from the plasma during its passage through the splanchnic area. The splanchnic A–V differences in peptidase activity which were observed could be due to any one or combination of the following: (1) Actual removal of enzyme protein; (2) intravascular denaturation of enzyme protein; (3) addition of an enzyme inhibitor; (4) removal of an enzyme activator.

The present observations may be interpreted to indicate that the kidney is not a major source of extraction of peptidase activity from plasma. It seems clear, however, that the splanchnic area and presumably the liver does play a role, at least in those individuals with increased plasma LGG-splitting ability, in the removal of this enzymic activity. The latter observations complement those of Fleisher and Butt (11), who found that plasma tripeptidase activity was usually elevated in patients with hepatic disease, especially in the presence of impaired biliary flow. In addition, a high concentration of tripeptidase activity was present in bile obtained after the release of biliary obstruction.

Although the liver is evidently involved in the removal, and perhaps even in the regulation of plasma peptidase activity, a definitive evaluation of this hepatic function will probably require the measurement of hepatic uptake in a normal animal subjected to an intravenous infusion of a concentrated non-toxic peptidase preparation. In these circumstances, the rate of entrance to the plasma would be a known constant and the rate of disappearance the only variable.

SUMMARY AND CONCLUSIONS

1. From acute observations in bilaterally nephrectomized rats, it is concluded that the kidney does not remove appreciable quantities of peptidase activity from the plasma.

2. Peptidase activity is removed from the plasma during its passage through the splanchnic area of patients in whom plasma enzyme levels were found to be elevated. The significant positive correlation obtained between the plasma level and the splanchnic uptake of enzymic activity suggests that the liver is intimately involved in the homeostatic control of these plasma constituents.

ACKNOWLEDGMENT

We wish to thank Drs. W. J. Taylor, R. Kibler, and L. Hamrick for supplying hepatic vein blood samples and Mrs. Rose Boyarsky for valuable technical assistance.

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