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Plasma β endorphin in cirrhosis and renal failure

J R Thornton, M S Losowsky

Abstract

Whether the plasma concentration of β endorphin was increased in hepatic cirrhosis like that of smaller opioid peptides methionine enkephalin and leucine enkephalin was determined. Its concentration in chronic renal failure was also measured. Plasma β endorphin was not significantly raised in cirrhotic patients with or without ascites (medians 5·2 pmol/l and 4·7 pmol/l respectively) compared with disease control subjects (4·9 pmol/l) and healthy control subjects (4·9 pmol/l). In contrast, the peptide was increased 2·5 fold ($p<0·001$) in chronic renal failure (12·4 pmol/l) and was found in many of these patients' urine. The data are compatible with the hypothesis that the liver may play an important role in the elimination of opioid peptides of octapeptide size or less but not the larger peptides such as β endorphin.

The plasma concentrations of the pentapeptides methionine enkephalin[1,2] and leucine enkephalin[3] are increased in patients with liver disease. These findings are consistent with the hypothesis that the liver may play a major role in the elimination of blood borne opioid peptides of octapeptide size or less.[1] We investigated whether the plasma concentration of a much larger opioid peptide, β endorphin, is increased in patients with hepatic cirrhosis or chronic renal failure, or both.

Methods

SUBJECTS

The following groups of subjects were studied:

(1) Fifteen patients with hepatic cirrhosis which was decompensated as judged by the presence of ascites. As assessed by Pugh's modification of Child's classification,[4] six of these patients were grade C and nine grade B.

(2) Fifteen patients with hepatic cirrhosis without ascites. All these patients were Pugh's grade A.

(3) Fifteen patients with chronic renal failure, eight of whom were having haemodialysis and four peritoneal dialysis.

(4) Fifteen disease controls who were hospital inpatients with a wide variety of illnesses (three congestive cardiac failure, three acute exacerbations of asthma, three acute on chronic pancreatitis, two untreated coeliac disease, two insulin dependent diabetes mellitus, one chronic intestinal pseudo-obstruction, and one pneumococcal pneumonia complicating bronchial carcinoma).

(5) Fifteen healthy control subjects.

As haemorrhage is a stimulus to β endorphin secretion,[5] only subjects who had not bled within the previous 10 days and were haemodynamically stable were studied.

SAMPLE COLLECTION

Venepuncture was performed with the subjects at rest – a heparinised cannula having been inserted 20 minutes earlier. Because of the diurnal variation in plasma β endorphin[6,8] all blood and urine samples were taken between 9 and 11 am. Venous blood and urine samples for β endorphin measurement were collected into chilled containers, the bottles for the former being primed with ethylenediamine tetra-acetic acid. The samples were transferred immediately to a precooled centrifuge at 4°C, spun at 3000 rpm, and stored at −70°C. Blood was also obtained for measurement of plasma creatinine, bilirubin, and alanine aminotransferase by routine laboratory techniques.

ASSAY

β endorphin was measured using a widely used, commercially available assay (ImmunoNuclear, Stillwater, Minnesota, USA).[7–11] The peptide was extracted from plasma by affinity gel chromatography. Half of 1 ml of suspended Sepharose anti-β endorphin particles were added to capped chromatography columns followed by 1·0 ml of either standards or plasma samples. Mixing was achieved by rotation for four hours at 4°C. The samples were allowed to drain, then the adsorbed β endorphin was recovered by twice eluting the columns with 0·025N hydrochloric acid.

Aliquots of 200 µl of each sample were assayed immediately and in duplicate. Altogether 100 µl of rabbit anti-β endorphin serum, 100 µl of iodine^{125} β endorphin and 50 µl of 0·1 N sodium hydroxide were mixed with each sample and then incubated at 4°C for 17 hours. The bound tracer was separated from the free tracer by incubation with 500 µl of goat anti-rabbit precipitating agent at 4°C for 20 minutes, followed by centrifugation at 1000 *g* at 20°C for 20 minutes and decanting of the supernatant. The radioactivity of the precipitates was measured in a gamma scintillation counter for five minutes each. The percentage of bound tracer divided by the tracer's total counts was calculated for the samples and the concentrations of β endorphin were determined by reference to the standard curve.

We found that the assay exhibited parallelism on serial dilution of patient samples spiked with pure β endorphin (Peninsula Laboratories). The antibody was confirmed to be highly specific as judged by its cross reactivity with pure peptides (Peninsula Laboratories). Thus it exhibited only 7% cross reactivity with β lipotropin and <0·01% with methionine enkephalin, leucine enkephalin, and peptide E. Within and between assay variations of 9% and 16% respectively were found. The minimum sensitivity of the assay was 4 pmol/l. Non-specific binding ranged from 4·5–6·3%.

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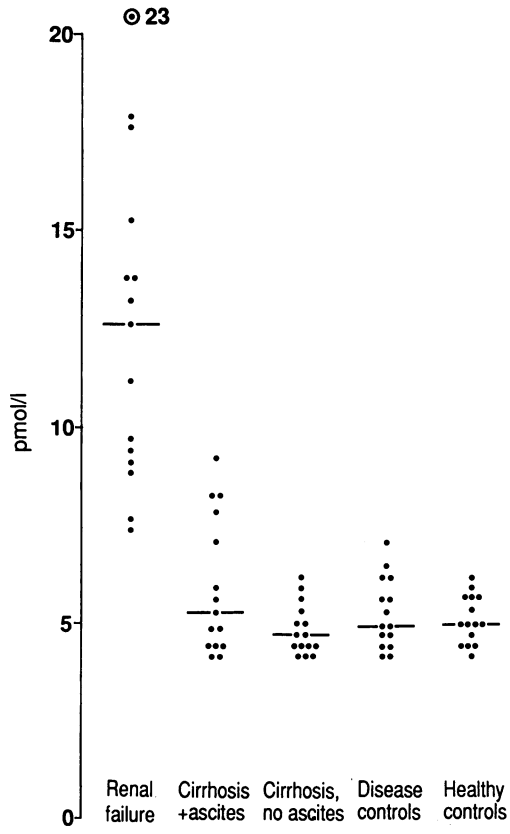

Plasma concentrations of β endorphin in the five groups of subjects.

The results are expressed as medians with ranges. The statistical significance of differences was determined by the Mann-Whitney U test or Spearman's rank correlation.

Results

The plasma concentration of β endorphin was not significantly raised in the plasma of patients with cirrhosis, either in those with ascites (median (range) 5·2 (4–9) pmol/l) or in those without ascites (4·7 (4–6) pmol/l) (Figure). In the patients with renal failure, it was increased approximately 2·5 fold (12·4 (7–23) pmol/l, $p<0·001$) compared with the value in disease control subjects (4·9 (4–7) pmol/l) and healthy control subjects (4·9 (4–6) pmol/l) and correlated significantly ($r=0·531$, $p<0·05$) with their high plasma concentration of creatinine (1045 (794–1117) μmol/l). The plasma concentration of creatinine was normal in all the subjects of the other study groups.

β endorphin was found in urine in 11 of 15 samples from the patients with renal failure (5·8 (5–8) pmol/l) but was undetectable in urine specimens from patients in the other four groups.

Discussion

Opioid peptides of octapeptide size or less are highly susceptible to enzymatic degradation[12–15] and the liver may be involved in this process.[1] Perhaps because of its much greater size, β endorphin, which comprises 31 amino acids, is highly resistant to enzymatic breakdown.[13 16 17] Its mode of elimination is uncertain. We found that the plasma concentration of β endorphin was normal in patients with cirrhosis, even when the cirrhosis was decompensated as judged by the presence of ascites, suggesting that the liver may not be important in this peptide's elimination. This finding is in contrast to the raised plasma concentrations of the much smaller opioid peptides methionine enkephalin[2] and leucine enkephalin[3] in hepatic cirrhosis.

The increase in plasma β endorphin in the patients with chronic renal failure agrees with the findings of Smith *et al*[18] but not those of Elias *et al*.[19] Peptides of similar molecular weight to β endorphin undergo glomerular filtration and catabolism in renal tubules.[20] The high plasma β endorphin concentrations in the patients with chronic renal failure may therefore indicate impaired glomerular filtration of the peptide, and its presence in many of their urine samples suggests inefficient breakdown by damaged renal tubules. Perhaps also in favour of renal catabolism of β endorphin is the peptide's trophic action on the kidney.[21] This may indicate a homeostatic mechanism whereby β endorphin is able to promote the means of its own elimination.

Besides β endorphin, other large opioid peptides which circulate in plasma[22] are resistant to peptidase degradation.[12–14] Therefore, the possibility that their plasma concentrations may also be raised in patients with renal failure deserves study. As β endorphin is capable of liberating many inflammatory mediators from mast cells, including some with pruritic actions such as histamine,[23–25] the possibility that itching in renal failure may be mediated by this and perhaps other large opioid peptides is worthy of study.

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