Parathyroid Hormone (PTH) Metabolism in Chronic Renal Disease (CRD)

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Introduction

The basis for the extremely high levels of parathyroid hormone in patients with chronic renal disease has not been totally elucidated. Disorders in calcium and phosphate metabolism are important factors in the over-production of parathyroid hormone (Slatopolsky et al, 1971; 1972; Coburn et al, 1973), but the role of decreased degradation of the hormone in the genesis of hyperparathyroidism has not been quantified.

In vitro studies have demonstrated that the kidney is an important organ in parathyroid hormone (PTH) metabolism (Orimo et al, 1965). Previous in vivo studies have documented a prolonged half-life of PTH in the circulation of uraemic subjects (Melick & Martin, 1969). However, the renal contribution to PTH metabolism under normal conditions has not been quantified, and the effects of uraemia on the renal handling of PTH has not been investigated. The present studies were undertaken to define the role of the canine kidney in the metabolism of bovine PTH 1–84 (b-PTH 1–84), and the synthetic bovine amino terminal (N-terminal) PTH fragment (syn b-PTH 1–34); and to determine the effects of experimental renal disease on the metabolic clearance rate of both forms of the hormone.

MATERIALS AND METHODS

Studies were performed on female mongrel dogs which had free access to water and were fed standard Purina Dog Chow. Prior to study, the left kidney of each dog was explanted from its retroperitoneal location to a subcutaneous position on the left flank by a modification of the technique described by Rhoads (1934).

Uraemia was induced by stepwise reduction of functioning renal mass as
previously described from our laboratory (Bricker et al, 1964; Slatopolsky et al, 1971). Dogs were studied in the awake resting state. Renal venous blood sampling was accomplished by percutaneous catheterization of the renal vein. Catheters were also placed in the femoral artery and the urinary bladder for the collection of samples. Infusions were administered through a hind leg vein.

Glomerular filtration rate was measured as creatinine clearance, renal plasma flow was determined from renal venous and femoral arterial para-hippurate concentrations using the Wolff modification (1950) of the Fick principle.

Highly purified bovine PTH (b-PTH 1–84) and synthetic b-PTH (syn b-PTH 1–34) were reconstituted in 0.2% acetic acid and added to dog hypoparathyroid serum for injection. After an equilibration period, the metabolic clearance rate (MCR) of immunoreactivity was determined either by single injection or constant infusion techniques (Tait, 1963). The renal clearance (RC) of immunoreactive PTH and syn b-PTH 1–34 was determined by the product of their respective arteriovenous extraction rates and the renal plasma flow. The result was expressed as the volume of serum cleared of PTH by the kidney per unit of time.

Parathyroid hormone or syn b-PTH 1–34 concentrations were determined by radioimmunoassay using two different antisera produced in our laboratory. One was obtained by injecting b-PTH 1–84 (TCA Wilson) into a rooster (CH9); the second one also was produced in a rooster (CH9N) after the administration of the synthetic N-terminal portion of the molecule, syn b-PTH 1–34. The radioimmunoassay was performed according to the method described by Reiss and Canterbury (1968) and Arnaud et al (1971). Serum samples, standard, and 100 μl of antiserum were incubated at 4°C for 3–4 days with constant agitation before adding 10,000–20,000 counts/min of labelled hormone and reincubated for an additional 36–48 hours in the cold. Following incubation, a charcoal-dextran preparation was used to separate bound from free hormone (Herbert et al, 1965).

RESULTS

The standard curve for the displacement of 131I b-PTH 1–84 bound to the CH9 antiserum (dilution 1:15,000) by increasing amounts of unlabelled b-PTH 1–84 is portrayed in Figure 1. The bound to free ratios of iodinated hormone were significantly depressed by 20–30 picograms (pg) of unlabelled hormone and displacement was 90% complete with the addition of 300 pg of unlabelled hormone. This assay was used in the determination of the clearance rates for b-PTH 1–84.

In Figure 2, the standard curve for the displacement of 131I syn b-PTH 1–34 is illustrated. There was significant depression of the bound to free ratio of 131I syn b-PTH 1–34 with the addition of 15–25 pg of unlabelled hormone and displacement was nearly complete with the addition of 1,000 pg of unlabelled hormone.
Figure 1. Displacement of $^{131}$I b-PTH tracer binding to the CH9 antiserum with increasing amounts of unlabelled b-PTH 1–84

In studies following the administration of b-PTH 1–84 in varying amounts, significant reductions in total metabolic clearance rates (MCR) and renal clearance (RC) rates of immunoreactive b-PTH were observed in dogs with chronic renal disease (CRD) as compared to normal control animals (Figure 3). Mean glomerular filtration rate was decreased by 85% by the induction of renal disease. Total MCR for immunoreactive b-PTH decreased by more than 60% in CRD animals. The renal clearance of PTH was similarly affected. The decreased renal clearance in CRD was related directly to changes in renal plasma flow (Figure 4), since the arterio-venous difference of immunoreactive b-PTH was unchanged between control and CRD dogs.

The relationship between renal plasma flow and the metabolic clearance rate for b-PTH 1–84 is portrayed in Figure 4. As it is illustrated in this figure, changes in the MCR were related to changes in RPF. Dogs with CRD and reduced RPF had proportional decreases in the total MCR of b-PTH.
Figure 2. Displacement of $^{131}$I syn b-PTH 1–34 tracer binding to the CH9N antiserum with increasing amounts of unlabelled syn b-PTH 1–34.

Studies following the administration of syn b-PTH 1–34 to normal and CRD dogs are depicted in Figure 5. Significant reductions in renal function, total MCR and RC were documented in CRD dogs. The results were similar to those obtained following the administration of b-PTH 1–84. However, the decrease in renal clearance of syn b-PTH 1–34 accounted for only 53% of the decrease in total MCR. Extrarenal clearances of syn b-PTH 1–34 were also significantly decreased in CRD dogs.

**DISCUSSION**

These studies describe the role of the kidney in the metabolism of PTH in vivo. The renal arterio-venous extraction for PTH is around 20%; thus, in normal dogs approximately 2/3 of the total MCR of b-PTH 1–84 is accounted for by renal
Figure 3. Glomerular filtration rate (GFR), metabolic clearance rate (MCR) and renal clearance of PTH (RC) before and after the induction of experimental chronic renal disease.

Figure 4. Relationship between renal plasma flow (RPF) and MCR of b-PTH 1–84 in a group of dogs with different degrees of renal function.
degradation. In chronic renal disease, the percent extraction of delivered immuno-reactive PTH was unchanged from the normal state, but the renal clearance was greatly diminished due to a decrease in renal plasma flow in the diseased kidney. Similar results were obtained in the studies in which the biologically active fragment terminal 1–34 was injected. Furthermore, in spite of a decrease in renal clearance for PTH, no increase in extrarenal degradation of PTH was observed. Our studies in uraemic dogs clearly indicate that the diminution of the renal mass is responsible for the decreased degradation of PTH, adding a new contributory factor to the development of high levels of circulating parathyroid hormone in chronic renal disease.

CONCLUSIONS

1. The kidney plays an important role in the degradation of PTH in vivo.
2. In experimental renal disease in the dog there is a significant decrease in the total metabolic clearance rate of PTH. This reduction in MCR is largely accounted for by a decrease in RPF.
3. Studies with syn b-PTH 1–34 suggest that uraemia also affects the extrarenal degradation of PTH.
Acknowledgments

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References

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Open Discussion

CHAIRMAN Thank you Dr Slatopolsky: you tried successfully to complicate an already complicated subject. Are there any questions?

DR F P BRUNNER (Switzerland) Am I right in assuming the uraemic dogs were not phosphate depleted and had much higher PTH levels?

SLATOPOLSKY Yes.

DR SACHS I would like to know whether you have any information on the difference between the metabolic rates of the C terminal fragment and the whole hormone, because one might imagine that the secretion of PTH in uraemia could be normal. The high measured PTH would be explained by a low metabolic clearance rate. But the problem is more complicated by your information on the 1 to 34 metabolic rate changes. Perhaps when you measure the total hormone you are also measuring the C terminal?

SLATOPOLSKY That is correct. Our first antibody was produced by immunising a rooster with 1 to 84 antigen. Thus, the antibody would recognise both the N and C terminal, 7,000 MW fragment, and the 4,000 MW C terminal chain. There is then a combination of the whole hormone plus the two C terminals, 7,000 MW and 4,000 MW. The second antibody is specific for the N terminals since we have
immunised the animal with a 1 to 34 fragment. We have also measured a specific 
C terminal, by taking the first antibody and pre-incubating with an excess of N, 
so that the antibody can no longer recognise the N portion of the molecule, thus, 
we have been able to follow the activity of the C terminal. The half life of the 
1–34 fragment is very short, less than 30 minutes, while the whole hormone, 
1–84, is not seen after one hour.

DR BETTER This is a beautiful work but I think that Orimo and certain other 
workers from Japan tried to show the same with a less sophisticated method. Our 
group also showed that the diseased kidney is not effective in degrading PTH.

SLATOPOLSKY Sure, I do remember the work, I was inspired by it.

DR KLEEMAN Do you know whether abnormal Vit. D metabolism in azotaemic 
dogs can contribute to the renal and extra-renal metabolism of PTH, in other 
words, can Vit. D or its metabolites influence the turnover of the hormone?

SLATOPOLSKY I don't have any information on that.

KLEEMAN One other question, if you produce the same level of uraemia by say 
tying the ureter as by removing renal tissue, is the hormone turnover the same?

SLATOPOLSKY We are studying this question now. We have some preliminary 
data in our laboratories and even after infusing very large amounts of PTH into 
the rats we could not find any PTH in the proximal tubule. It is very likely that 
PTH works on the peritubular side. This is very preliminary.

CHAIRMAN It may interest you that Bowman in Dr Ulrick's Department in 
Frankfurt could clearly show in microperfusion studies that PTH acts from the 
peritubular side, and is inactive from the luminal side.