

Metabolism of Immunoreactive Parathyroid Hormone in the Dog

THE ROLE OF THE KIDNEY AND THE EFFECTS OF CHRONIC RENAL DISEASE

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ABSTRACT The role of the kidney in the metabolism of parathyroid hormone (PTH) was examined in the dog. Studies were performed in awake normal and uremic dogs after administration of bovine parathyroid hormone (b-PTH) or synthetic amino terminal tetratricontapeptide of b-PTH (syn b-PTH 1-34). The renal clearance of immunoreactive PTH was determined from the product of renal plasma flow and the percent extraction of PTH immunoreactivity by the kidney. Blood levels of circulating immunoreactive PTH were determined by radioimmunoassay.

The normal dog kidney extracted $20 \pm 1\%$ of the immunoreactive b-PTH delivered to it, and renal clearance (RC) of immunoreactivity was 60 ml/min. When RC was compared to an estimate of total metabolic clearance (MCR) of immunoreactivity, it accounted for 61% of the total. Both MCR and RC were markedly decreased in dogs with chronic renal disease. However, the percent extraction of immunoreactive PTH was unchanged in chronic renal disease, and the observed decrease in RC was due to changes in renal plasma flow. The largest portion of the reduction in total MCR was accounted for by the decrease in RC, and there was no compensation for the decrease in RC by extrarenal sites of PTH metabolism.

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Gel filtration of pooled serum samples from normal and chronic renal disease dogs demonstrated a rapid conversion of injected intact b-PTH into immunologically detectable peptide fragments of both the carboxyl (C) and amino (N) terminal portion of the PTH molecule. The elution pattern of samples from dogs with renal disease demonstrated higher levels of each immunologically detected peptide and especially C-terminal fragments throughout the time of study.

Studies with the biologically active N-terminal PTH fragment (syn b-PTH 1-34) demonstrated a higher renal extraction of $37 \pm 3\%$ and a more rapid total MCR than was seen with b-PTH. The effects of renal disease on the clearance rates of syn b-PTH 1-34 were similar to those seen with b-PTH except that there was a more marked decrease in extrarenal clearance of syn b-PTH 1-34. Extrarenal syn b-PTH 1-34 clearance was 133 ± 9 ml/min in normals and 58 ± 14 ml/min in dogs with renal disease.

These studies indicate that the kidney plays an important role in the metabolism of PTH in vivo. They describe the impaired degradation of PTH seen in uremia, and they suggest that this may be a factor that contributes to the high levels of circulating immunoreactive PTH seen in chronic renal disease. They also suggest an effect of renal disease on PTH metabolism at sites other than the kidney.

INTRODUCTION

Secondary hyperparathyroidism is a nearly universal complication of chronic renal disease. Hypocalcemia re-

sulting from phosphate retention and calcium malabsorption has been shown to cause overproduction of parathyroid hormone (PTH)¹ in advancing renal disease (1-3). The possibility that impaired degradation of PTH also contributes to the high blood levels of the hormone in chronic renal disease has not been adequately examined.

Previous studies have demonstrated that the kidney is able to degrade PTH in vitro (4, 5). Studies after nephrectomy or the development of end-stage renal disease have shown a prolonged half-time of disappearance for administered radiolabeled PTH suggesting that the kidney plays an important role in PTH metabolism in vivo (6, 7). However, the renal contribution to PTH metabolism under normal conditions has not been quantified, and the effects of uremia on the renal handling of PTH have not been investigated.

PTH has recently been shown to circulate not only as the intact hormone but also as carboxy terminal (C-terminal) (8, 9) and amino terminal (N-terminal) fragments of the molecule (10). The half-lives of the fragments in the circulation are widely divergent. Synthetic bovine N-terminal is very short lived with a half-life of approximately 5 min in the rabbit (11), while C-terminal fragments devoid of biologic activity have longer half-lives in the range of hours (9, 11). Since the rate of degradation of intact PTH and its fragments appear to differ widely, any description of PTH metabolism must address itself to the complex problem of separating and describing the rate of metabolism of each species in the circulation.

The studies reported herein were designed to examine the contribution of the dog kidney in vivo to the metabolism of PTH and its fragments. The renal contribution to the total metabolic clearance rate (MCR) of immunoreactive PTH was determined in normal and uremic dogs. The results of this study indicate that the kidney plays an important role in the metabolism of PTH and its fragments in vivo and suggest that decreased degradation of the hormone may contribute to the elevated PTH levels observed in renal insufficiency.

METHODS

Preparation of dogs. Studies were performed in female mongrel dogs weighing 12-24 kg who had free access to water and were fed standard Purina dog chow. Before study, the left kidney of each dog was explanted to a subcutaneous position on the left flank by a modification of previously described techniques (12). Through a flank incision the renal vein was traced from the renal hilum to

the inferior vena cava, and all veins of nonrenal origin connecting with the renal vein were ligated. The proximal renal vein was placed in semicircular Teflon sheath 2-3 cm long. The sheath was immobilized by suturing to the surrounding connective tissue and then was sutured to subcutaneous tissue. Fixation of the semicircular sheath subcutaneously allowed localization and percutaneous catheterization of the renal vein but did not affect glomerular filtration rate or renal plasma flow.

Chronic renal disease was induced in dogs with explanted kidneys by two methods. In the first, most of the branches of the left renal artery were ligated during surgery before explantation. The second method utilized the induction of pyelonephritis in a previously explanted kidney (13). Both methods employed contralateral nephrectomy as a final step, leaving only the diseased kidney as a source of renal function. After the final surgical procedure and 2-3 wk of recovery, the dogs were studied in the awake resting state.

Study protocol. Food was removed 12 h before study, but access to water was continued. A femoral arterial catheter for blood sampling was placed by means of percutaneous puncture of the artery with a needle through which a guide wire was directed. Then a polyethylene catheter was directed over the guide wire into the vessel lumen to the approximate level of the renal artery. The left renal vein was catheterized percutaneously. A hind leg vein catheter was placed for infusion of solutions and a bladder catheter was placed for collection of urine. After priming doses of 50 mg/kg creatinine and 4-5 mg/kg para-aminohippurate, a sustaining solution of normal saline with creatinine and para-aminohippurate was administered to maintain creatinine at the level of 10 mg/100 ml and para-aminohippurate at a concentration of 1-2 mg/100 ml. Sustaining solutions were administered through a Holter constant infusion pump, Model RL 175 (Extracorporeal Medical Specialties, Inc., King of Prussia, Penn.), at rates from 1 to 2.5 ml/min.

After an equilibration period, metabolic clearance rates of immunoreactivity were determined by either single injection or constant infusion techniques. In the single injection protocol, arterial samples were obtained at 1-10-min intervals for 3-4 h after the rapid injection (10 s) of 25-100 μ g purified bovine PTH (b-PTH) or 50-100 μ g synthetic amino terminal tetratricontapeptide of b-PTH (syn b-PTH 1-34) into a hind leg vein. Renal vein samples were collected during the disappearance curve simultaneous to various arterial samples for the calculation of A-V differences. In the constant infusion protocol, the b-PTH or syn b-PTH 1-34 was added in varying amounts to a saline infusion solution under ice so that after 2 h of equilibration the level of immunoreactive PTH or syn b-PTH 1-34 was constant, between 2-10 ng/ml. Dog hypoparathyroid serum was also added as a protein carrier to the constant infusion solution to obtain a protein concentration of 1-2 g/100 ml. No priming dose of PTH was administered in the constant infusion studies. Arterial blood levels with less than 5% variance from each other were used to ascertain the constant level of PTH.

In both experimental protocols, cross-reactivity of endogenous canine PTH with the antiserum to b-PTH, (CH-9), was subtracted as a background from all samples. In normal dogs the level of immunoreactive PTH detected by the antiserum to b-PTH was less than 300 pg/ml using a b-PTH standard curve as a reference. In uremic dogs, immunoreactive PTH ranged from 1-4 ng/ml. During experiments in which the dogs were infused as above, except that PTH was not administered and blood was taken, serum PTH levels did not change significantly. The ade-

¹Abbreviations used in this paper: b-PTH, bovine parathyroid hormone; C-terminal, carboxyl terminal; MCR, metabolic clearance; N-terminal, amino terminal; PAH, para-aminohippurate; PTH, parathyroid hormone; RC, renal clearance; RIA, radioimmunoassay; syn b-PTH 1-34, synthetic amino terminal tetratricontapeptide.

quacy of renal vein sampling was evaluated by para-amino-hippurate (PAH) extraction and only samples with 70–80% PAH extraction were considered to be without contamination of inferior vena caval blood in normal dogs. In uremic dogs, PAH extraction determined by renal vein catheterization under direct vision at laparotomy was compared with the extractions obtained in the studies with percutaneous catheterization. The results were the same in both settings.

Source of PTH. Highly purified b-PTH for administration and for the radioimmunoassay (RIA) standard curve was obtained from Inolex Pharmaceuticals, Glenwood, Ill. The specific activity of the purified PTH as determined by the Wilson Company in the Munson bioassay was 900 U/mg. Syn b-PTH 1-34 for administration and RIA standard was obtained from the Beckman Instruments, Inc., Palo Alto, Calif. The specific activity stated by the Beckman Instruments, Inc. in the adenyl cyclase system varied from 800–2,700 U/mg. Both b-PTH and syn 1-34 were reconstituted in 0.2% acetic acid, pH 3.5, and added to dog hypoparathyroid serum for injection. On gel filtration, both preparations of PTH demonstrated single peak elution patterns of immunoreactivity and for the purposes of the present studies were considered to be pure hormones although the specific activities, as reported by the manufacturers, were less than those reported for purified preparations from other laboratories (14).

Chemical determinations. Creatinine in serum and urine was determined by the Jaffe reaction as described by Folin (15) and adapted for the Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.). PAH was determined by the method of Harvey and Brothers (16) and phosphate by the methods of Kraml and Hurst (17, 18). Both PAH and phosphate methods were also adapted for the Technicon Autoanalyzer.

Radioimmunoassay methods. Serum levels of both b-PTH and syn b-PTH 1-34 were determined by RIA techniques according to methods described by Reiss and Canterbury and Arnaud, Tsao, and Littledike (19, 20). The antiserum (CH9) was obtained from a cockerel immunized with a preparation of 6 mg b-PTH (TCA precipitate, Wilson Company) in 0.5 ml complete Freund's adjuvant. The immunogen was administered into the foot pad and booster injections were given 14, 30, and every 30 days after the initial injection. The antiserum used in the present studies was obtained after 10 injections and was used in a 1:15,000 final dilution. At this dilution, there was significant displacement of tracer binding with 10–20 pg of purified b-PTH.

The regional specificity characteristics of the binding of the CH9 antiserum to PTH are portrayed in Fig. 1. As shown, binding of [¹²⁵I]b-PTH to the CH9 antiserum was displaced by increasing amounts of noniodinated b-PTH with less than 10% tracer binding present after the addition of 300 pg of purified b-PTH (●—●). When the CH9 antiserum was preadsorbed with syn b-PTH 1-34, 5 ng/100 μl of an antiserum preparation 1:3,000 dilution for 24–48 h before use at 4°C, the standard curve of tracer binding displacement was essentially unchanged (▲—▲). When syn b-PTH 1-34 was used as the noniodinated ligand, there was only a small amount of displacement of the percent [¹²⁵I]b-PTH bound with the antiserum (○—○). This displacement was incomplete even when 800 ng/ml of syn b-PTH 1-34 was used as the unlabeled ligand. Furthermore, when the CH9 antiserum was preadsorbed with syn b-PTH 1-34, there was no displacement of antiserum binding with

[¹²⁵I]b-PTH by increasing amounts of syn b-PTH 1-34 including very high concentrations (Δ—Δ). These studies indicated that the great majority of the antigenic binding sites in the CH9 antiserum were specific for amino acid sequences C-terminal to position 34 in the b-PTH molecule, and that after preadsorption with syn b-PTH 1-34 the antiserum was C-terminal specific.

The antiserum (CH9N) used in the RIA in studies after the administration of syn b-PTH 1-34 and for the determination of N-terminal immunoreactivity in gel filtration studies was obtained from a cockrel immunized with 400 μg of syn b-PTH 1-34 (Beckman Instruments Inc.) in 0.5 ml of complete Freund's adjuvant. Injections were repeated at days 14, 30, and every 30 days after the initial injection. The antiserum used in the present studies was obtained after seven injections and used at a 1:8,000 final dilution. The standard curve for displacement of [¹²⁵I]syn b-PTH 1-34 bound to antiserum by increasing amounts of syn b-PTH 1-34 is portrayed in Fig. 2. There was significant displacement of iodinated ligand by 10–15 pg of noniodinated syn b-PTH 1-34 and almost complete displacement by 1.25 ng (●—●). When b-PTH was used to displace bound [¹²⁵I]syn b-PTH 1-34 (○—○), more than 10-fold greater quantities of hormone were required to obtain the same degree of depression in percent binding demonstrated when syn b-PTH 1-34 was employed.

In addition, when the CH9N antiserum was preadsorbed with syn b-PTH 1-34, as described above, there was no detectable binding with [¹²⁵I]b-PTH. These results indicated the specificity of this antiserum for antigenic determinants on the N-terminal portion of the PTH molecule. Also the affinity of the antiserum for binding sites on the N-

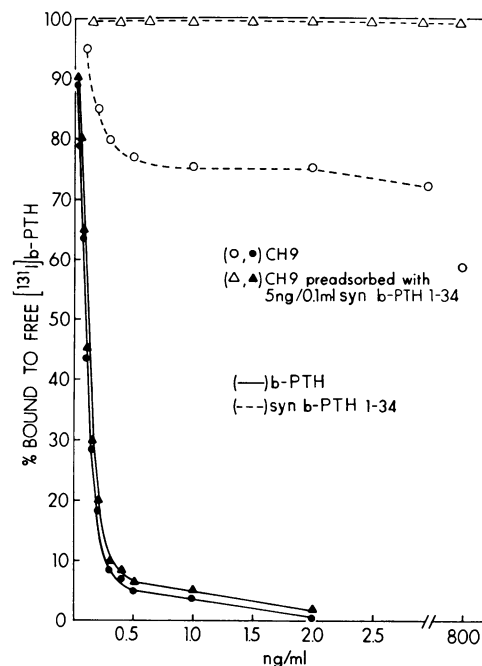


FIGURE 1 Displacement of [¹²⁵I]b-PTH tracer binding to the CH9 antiserum with increasing amounts of unlabeled b-PTH (●—●) and syn b-PTH 1-34 (○—○) and displacement of tracer binding to the CH9 antiserum preadsorbed with syn b-PTH 1-34 with increasing amounts of b-PTH (▲—▲) and syn b-PTH (Δ—Δ).

terminal portion of PTH was 10-fold less when these sites were present as a portion of the intact molecule rather than the 1-34 fragment itself.

The iodination of b-PTH and syn b-PTH 1-34 for use in the RIA was performed by the chloramine T method of Greenwood, Hunter, and Glover (21) using a ratio of 1.3 μg of b-PTH and 2.9 μg of syn b-PTH 1-34 per mCi of iodine and an 8:1 ratio of chloramine T to PTH in the reaction mixture. Iodinated hormones were separated from free ^{131}I and damaged peptides by adsorption onto and elution from Quso (22) followed by elution from a $1 \times 30\text{-cm}$ column of Bio-Gel P-10 with 10% human plasma in 0.1 M barbital buffer, pH 8.5, as the column vehicle. The specific activities of ^{131}I -b-PTH obtained by this iodination procedure ranged between 250–450 $\mu\text{Ci}/\mu\text{g}$ and for syn b-PTH 1-34 were 100–250 $\mu\text{Ci}/\mu\text{g}$.

RIA was performed by adding serum samples, standards, and 100 μl of the antiserum in the dilution noted below and incubating at 4°C for 3–4 days with constant agitation before adding 10–12,000 cpm of labeled hormone and re-incubating for an additional 36–48 h in the cold. After incubation, charcoal dextran separation was used to separate bound from free hormone (23). The charcoal dextran suspension contained 6.3 g/liter of neutralized activated charcoal and 0.63 g/liter Dextran T-70 (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) dissolved and mixed at 4°C in a 0.85% NaCl, sodium barbital 0.007 M, and sodium acetate 0.014 M buffer, pH 7.4, for 1 h and was centrifuged at 2,000 rpm for 15 min, decanted, then reconstituted in the suspension buffer with 250 mg/liter of human albumin added. The suspension was mixed at 4°C overnight and added to assay tubes in the cold. After addition of the charcoal-dextran solution assay tubes were vortexed, centrifuged, and supernate carefully decanted into clean test tubes. ^{131}I radioactivity in both bound and free fractions was counted in a gamma well spectrophotometer (Packard Instruments, Inc., Downer's Grove, Ill.).

All serum samples were tested in multiple dilutions in duplicate. Appropriate controls without added antiserum

were used in every assay to correct for nonspecific binding and/or effects due to incubation damage of radioiodinated hormone. Also, controls for antibody binding to labeled hormone in the absence of unlabeled hormone or sample (trace binding) at several different serum protein concentrations were included in each assay. Each test sample was related to the control and tracer binding with the same protein concentration.

The antiserum dilution of 1:15,000 was used for CH9 and routinely gave a B/F (bound to free) ratio of 0.7–0.9 when incubated with labeled hormone in absence of unlabeled hormone. The CH9 antiserum was used in the determination of the clearance rates for b-PTH and thus these determinations reflect the metabolism of both intact b-PTH and the major circulating PTH fragments. The CH9 antiserum preadsorbed with syn b-PTH 1-34 was used in gel filtration studies, and binding to the antiserum was thus limited to C-terminal binding sites. Analysis of variance between sample replicates was less than 10% at all times. The antiserum dilution of 1:8,000 used for CH9N routinely gave B/F ratios of 0.4–0.6 when reacted with ^{131}I -syn b-PTH 1-34 in the absence of unlabeled hormone or sample. The CH9N antiserum was used in gel filtration studies to determine the elution pattern of N-terminal immunoreactivity and in the clearance studies for syn b-PTH 1-34. The standard used in these assays was syn b-PTH 1-34.

Gel filtration of serum pools. Arterial serum samples obtained during set intervals (0–15 min, 15–50 min, and 50–180 min) of the PTH disappearance curve in normal and uremic dogs were pooled and in addition the 50–180-min pools from normal dogs were concentrated according to methods described by Canterbury, Levey, and Reiss (10). Samples of serum pools were then placed on $1.5 \times 60\text{-cm}$ columns of Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, Calif.) and filtered along with column markers of ^{131}I -b-PTH (Inolex Pharmaceuticals) and ^{131}I or ^{125}I -syn b-PTH 1-34 (Beckman Instruments, Inc.). Column eluant consisted of outdated blood bank plasma diluted 1:10 with 0.1 M sodium barbital, pH 8.5. Void volume was determined by the elution position of blue dextran, mol wt 2 million, and the salt volume was determined by the elution of ^{131}I . Column eluant was collected in 1-ml fraction volumes. The immunoreactive PTH in each fraction was determined in assays employing the CH9 antiserum preadsorbed with syn b-PTH 1-34 and the CH9N antiserum. Thus, the elution pattern of both C-terminal and N-terminal immunoreactivity was determined.

Calculations. Creatinine clearance served as an index of glomerular filtration rate. Renal plasma flow was determined employing para-aminohippurate extraction across the kidney and the Wolff modification of the Fick principle (24). The renal extraction of immunoreactive PTH and syn b-PTH 1-34 was determined from A-V differences across the kidney and the renal clearance of both substances was determined by the product of their respective extraction rates and the renal plasma flow. The result was expressed in volume of serum cleared of PTH by the kidney per unit time (milliliters per minute). MCR rates were determined by both single injection and constant infusion techniques according to methods described by Tait (25). In the single injection technique, the disappearance curve was multiexponential. A three exponential system routinely demonstrated the best curve fitting by the least squares method and was used for the determination of MCR. Analysis of the disappearance curve was performed on an IBM S/360 computer employing the SAAM program of

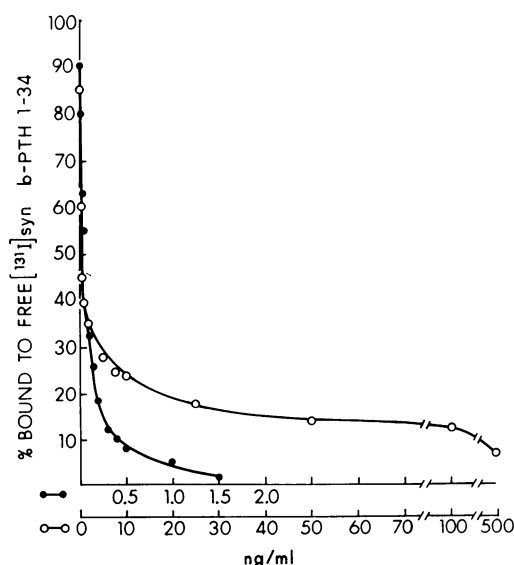


FIGURE 2 Displacement of ^{131}I -syn b-PTH tracer binding to the CH9N antiserum with increasing amounts of unlabeled syn b-PTH 1-34 (●—●) and b-PTH (○—○).

TABLE I
Studies in Normal and Uremic Dogs with b-PTH

Dog no.	b-PTH injected	GFR	RPF	PAH ext	PTH ext	RC	MCR	RC/MCR	MCR-RC
	μg	ml/min	ml/min	%	%	ml/min	ml/min	%	ml/min
1	50	66	259	77	21	54	90	60	36
2	45	89	361	82	22	79	134	49	55
3	100	57	232	75	15	35	59	59	24
4	100	90	402	81	23	93	147	63	54
5	100	86	256	87	24	61	105	48	44
6	27	73	295	81	16	47	67	70	20
	100	13	96	43	12	12	30	40	18
7	50	76	304	79	17	52	87	60	35
	50	12	93	50	15	14	48	29	34
8	50	6	35	65	28	10	38	26	28
9	50	21	114	60	16	18	33	55	15
10	30	8	81	40	20	16	30	53	14
11	30	4	93	50	15	14	48	29	34
Mean (Normal)		77	301	80	20	60	98	61	38
$\pm\text{SEM}$		5	23	2	1	8	12	2	5
Mean (CRD)		11	77	51	19	13	36	38	23
$\pm\text{SEM}$		3	13	4	2	1	3	6	3

CRD, chronic renal disease; GFR, glomerular filtration rate; RPF, renal plasma flow; PAH ext, para-aminohippurate extraction; PTH ext, parathyroid hormone extraction; RC/MCR, renal clearance divided by total MCR, i.e., the percent contribution of the kidney to total MCR; MCR-RC, total metabolic clearance minus renal clearance, i.e., the extrarenal clearance of immunoreactive PTH.

Berman, Shahn, and Weiss (26). MCR was calculated from the area under the disappearance curve employing the appropriate integration formula for a three exponential system:

$$\text{MCR} = R \cdot \frac{1}{\frac{A}{\alpha} + \frac{B}{\beta} + \frac{C}{\gamma}}$$

where R represents total dose of PTH administered and A, B, and C, are values for the y intercept of each exponent and α , β , and γ are values for the slope of each exponent. Calculation of MCR in constant infusion studies was performed by the formula: $\text{MCR} = \text{infusion rate (ng/min)}/\text{arterial concentration (ng/ml)}$. Results from both single injection and constant infusion techniques were similar and, therefore, most studies were performed by the single injection technique. Where both types of studies were performed the data portrayed for MCR represents a mean of the results in both single injection and constant infusion studies.

The data for MCR and renal clearance (RC) rates represent clearances of immunoreactivity. In the case of studies involving the administration of b-PTH, they represent the combined disappearance rate of intact b-PTH and its C-terminal fragments, as determined in assays with the CH9 antiserum. The CH9 antiserum binds intact hormone through mainly C-terminal binding sites and with nearly equal affinity for these sites as either intact hormone or as C-terminal fragments. Thus, the injected b-PTH was recognized similarly throughout its disappearance curve. However, because of the change from intact hormone to C-

terminal fragments, the calculation of MCR on the basis of immunoreactivity determined in assays with the CH9 antiserum can only be considered an estimate of overall turnover rate of immunoreactivity for comparison to the renal turnover rate and not as the metabolic turnover rate for intact PTH.

In the case of the studies after the administration of syn b-PTH 1-34, the CH9N antiserum did not detectably bind with products of degradation as determined by gel filtration studies. Therefore, the disappearance rate of immunoreactive syn b-PTH 1-34, as determined by RIA using the CH9N antiserum, represents determination of the MCR rate of this hormonal species.

RESULTS

Studies with b-PTH. The results of experiments performed in seven normal dogs and six dogs with chronic renal insufficiency during the administration of b-PTH are shown in Table I. Two dogs (6 and 7) were studied as normal and with chronic renal disease. Mean GFR was 77 ± 5 ml/min in normal dogs with a range of 57–90 ml/min. The GFR and RPF in the uremic animals was 11 ± 3 ml/min and 77 ± 13 ml/min, respectively. PAH extraction was consistently between 75–85% in the normal dogs but varied to a greater extent in the uremic animals.

The renal extraction of immunoreactivity after the injection of b-PTH appeared to be constant at widely varying PTH concentrations in both uremic and normal

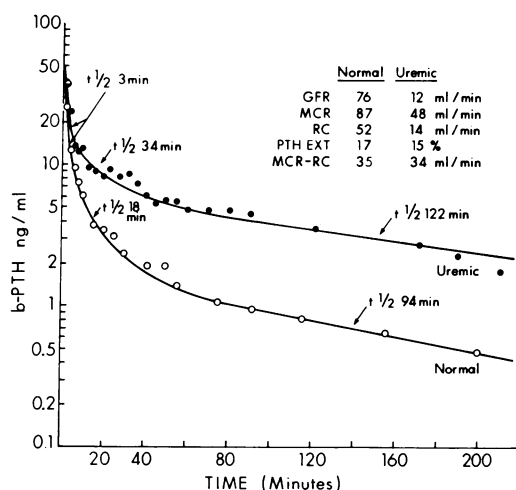


FIGURE 3 Disappearance curves of immunoreactive PTH after injection of b-PTH as measured by the CH9 antiserum in a dog studied when normal (○—○) and after the induction of uremia (●—●). $T_{1/2}$ refers to half-time of disappearance during each of three exponents in the disappearance curve. See text for additional details.

dogs, and in single injection studies percent extraction was constant throughout the entire disappearance curve. Renal extraction was $20 \pm 1\%$ (range 15–24%) in normal dogs, as determined in assays with the CH9 antiserum, and was $19 \pm 2\%$ (range 12–28%) in uremic dogs. Despite the fact that the percent of extraction of delivered immunoreactive PTH was not affected by the induction of uremia, the RC of immunoreactivity was markedly diminished from a mean of 60 ± 8 ml/min in normal dogs to 13 ± 1 ml/min in uremic animals. Urinary excretion of immunoreactive PTH accounted for less than 1% of the RC of PTH in both normal and uremic animals, ranging from 0.1 to 0.5% of the injected dose. The decrease in RC of immunoreactive b-PTH seen in dogs with reduced renal function was directly related to a decrease in renal plasma flow.

Estimations of the total MCR of immunoreactivity also decreased with the induction of chronic renal disease from 98 ± 12 ml/min in normal dogs to 36 ± 3 ml/min in uremic dogs. Most of the decrease in total MCR was accounted for by the decrease in RC (74%), as demonstrated by the significant drop in the renal contribution (RC/MCR) to the total MCR from 61% in normals to 38% in uremic dogs. There was also a small decrease in mean extrarenal immunoreactive PTH clearance (MCR-RC) despite overlap between the normal and uremic dogs in individual instances.

Fig. 3 portrays the disappearance curve of immunoreactive b-PTH in a dog that was studied in both normal and uremic states. The renal function and PTH clearance data are represented in the upper right corner. The disappearance curve for PTH is clearly multiex-

ponential in both normal and uremic states. The initial rapid disappearance with a half-time of 2–3 min appears similar in both normal and chronic renal disease states, but after this initial rapid disappearance rate the curves diverge with the uremic curve having a much longer half-time of disappearance.

To follow the alterations in the circulating form of PTH during the time of the disappearance curve and to detect the appearance and disappearance of PTH fragments, serum samples collected during set intervals of the disappearance curve (0–15 min, 15–50 min, and 50–180 min) were pooled and filtered through Bio-Gel P-10. The elution pattern of C-terminal immunoreactivity was determined by RIA of the column fractions employing the CH9 antiserum preadsorbed with syn b-PTH 1–34, and the elution pattern of N-terminal was determined by RIA using the CH9N antiserum. Fig. 4 portrays a representative elution pattern of the three serum pools from a normal dog. Immunoreactivity in the area of the b-PTH marker accounted for the

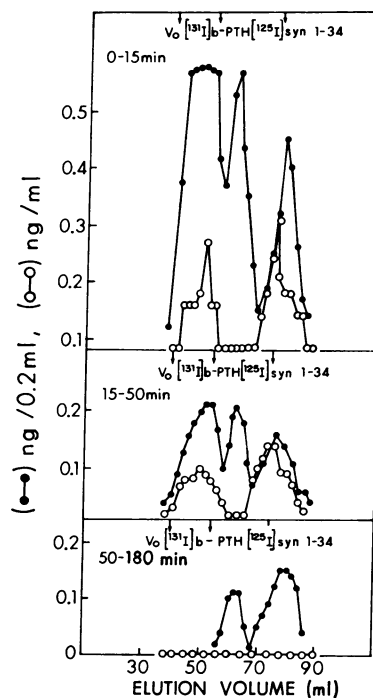


FIGURE 4 Elution patterns of immunoreactive PTH as measured in three serum pools collected during the intervals 0–15 min, 15–50 min, and 50–180 min after administration of $100 \mu\text{g}$ b-PTH to a normal dog. C-terminal (●—●) immunoreactivity was determined in assays with the CH9 antiserum preadsorbed with syn b-PTH 1–34. N-terminal immunoreactivity (○—○) was determined in assays with the CH9N antiserum. Gel filtration was performed through a $1.5 \times 60\text{-cm}$ column of Bio-Gel P-10. Not shown is the elution position of ^{125}I (salt volume) which was 180 ml in these experiments. V_0 , void volume.

TABLE II
Studies in Normal and Uremic Dogs with syn b-PTH 1-34

Dog no.	syn b-PTH 1-34 injected	GFR	RPF	PAH ext	syn b-PTH 1-34 ext	RC	MCR	RC/MCR	MCR-RC
	μg	ml/min	ml/min	%	%	ml/min	ml/min	%	ml/min
1	95.2	82	347	76	45	156	299	52	143
2	100	73	276	70	41	113	248	46	135
3	100	64	248	70	38	94	192	49	98
4	100	57	261	80	33	86	223	39	137
	100	23	106	50	34	36	57	63	21
5	50	66	333	70	28	93	247	38	154
	50	5	56	31	25	14	87	16	73
6	95.2	17	83	76	19	16	129	12	113
7	50	20	88	67	50	44	105	42	61
8	50	16	88	67	26	23	45	51	22
9	95.2	10	59	44	20	12	72	17	60
Mean (Normal)		68	293	73	37	108	241	45	133
$\pm\text{SEM}$		4	20		3	13	18	3	9
Mean (CRD)		15	80	56	29	24	83	34	58
$\pm\text{SEM}$		3	8	7	5	5	13	9	14

syn b-PTH 1-34 ext, the extraction of bovine synthetic N-terminal tetratricontapeptide.
See Table I for other abbreviations.

great portion of immunoreactive PTH eluting from the column in the first pool (0-15 min) but rapidly disappeared so that it was undetectable in the pool of samples from 50-180 min. There was early production of two smaller molecular weight fragments detected in assays with the CH9 antiserum representing fragments with C-terminal binding sites; the first eluted between the b-PTH 9,500 mol wt marker and the syn b-PTH 1-34 4,500 mol wt marker, and the second eluted at the same point as the 4,500 mol wt marker. These fragments represented the bulk of the circulating PTH after 50 min. Peaks of N-terminal immunoreactivity eluting in the same area as the b-PTH and syn b-PTH 1-34 markers were demonstrated by the CH9N antiserum. The peak of N-terminal immunoreactivity in the area of the syn b-PTH 1-34 marker was present in the largest amount in the 0-15-min pool but was undetectable in concentrated serum pools past 50 min. Pooling of the serum samples from the three periods of the disappearance curve and gel filtration of the pools was performed on samples from three normal dogs, and all patterns were similar to those portrayed in Fig. 4.

Fig. 5 portrays the elution pattern of immunoreactive PTH for both C- and N-terminal regions in three serum pools from a dog with chronic renal disease. The PTH fragments detected in these gel filtration studies were qualitatively similar to the fragments seen in the pools from normal dogs; i.e., a C-terminal fragment eluting

between the 9,500 and 4,500 mol wt markers, a C-terminal fragment eluting with the 4,500 mol wt marker, and an N-terminal fragment eluting with the 4,500 mol wt marker. The amount of immunoreactivity in each pool from the chronic renal disease dogs was greater than the amount present in corresponding pools from normal dogs. This was especially the case for C-terminal fragments eluting with the 4,500 mol wt marker. The gel filtration of pooled serum samples was performed on samples from two dogs with chronic renal disease each giving similar results.

Studies with syn b-PTH 1-34. To examine further the metabolism of the N-terminal fragment, studies were performed in five normal and five uremic dogs after injection of the biologically active syn b-PTH 1-34. Studies were performed in the same manner as the studies above with intact b-PTH. Table II shows the results of renal function and PTH clearances in normal (no. 1-5) and uremic (no. 4-9) dogs. Glomerular filtration rate, renal plasma flow, and PAH extraction were comparable to the studies portrayed in Table I.

The renal extraction of immunoreactive syn b-PTH 1-34 was higher than it was for administered b-PTH, $37\pm3\%$ vs. $20\pm1\%$, respectively, in normal dogs. The extraction of syn b-PTH 1-34 was not significantly decreased by the induction of uremia; $37\pm3\%$ of delivered syn b-PTH 1-34 was extracted in normal dogs and $29\pm5\%$ in uremic animals ($P > 0.1$). RC of immuno-

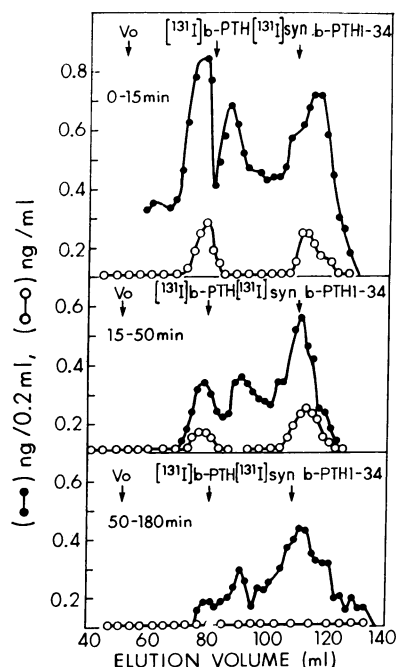


FIGURE 5 Elution patterns of immunoreactive PTH in a dog with chronic renal disease determined as in Fig. 4, (C-terminal immunoreactivity (●—●), N-terminal immunoreactivity (○—○).) Gel filtration performed through 1.5×90 -cm columns of Bio-Gel P-10. Not shown is the elution position of ^{125}I (salt volume) which was 225 ml in these experiments.

reactive syn b-PTH 1-34 decreased significantly from 108 ± 13 ml/min in normal dogs to 24 ± 5 ml/min in uremia. This change was related to the fall in renal plasma flow as in the studies with b-PTH. The mean total MCR for immunoreactive syn b-PTH 1-34 was 241 ± 18 ml/min in normal dogs and decreased to 83 ± 13 ml/min in the uremic animals. While the decrease in RC contributed to the decrease in the total MCR, there was also a marked decrease in extrarenal clearance of syn b-PTH 1-34. RC accounted for 45% of the total MCR in normals and this contribution was decreased by uremia (34%). Thus, for syn b-PTH 1-34 the decrease in total MCR was accounted for by decreases in both renal and extrarenal clearances.

The disappearance curves for syn b-PTH 1-34 in a dog studied both in the normal and uremic state are portrayed in Fig. 6. The renal function and PTH clearances are listed in the upper right corner of the figure. As was the case for the disappearance curves of intact PTH, the uremic and normal disappearance curves for syn b-PTH 1-34 appear not to differ in the first 1-4 min but then diverge widely with slower rates of decay in the uremic state. The disappearance curves for syn b-PTH 1-34 are multiexponential, as they were for intact PTH, but the rate of disappearance was much

more rapid, so that after the injection of $100 \mu\text{g}$ of syn b-PTH 1-34, the blood level was less than 0.2 ng/ml by 90 min in normal dogs.

DISCUSSION

The present studies describe the role of the canine kidney in the metabolism of PTH *in vivo*. The normal dog kidney extracted approximately 20% of the immunoreactive b-PTH delivered to it. The RC of immunoreactive PTH was 60 ml/min. When the RC of immunoreactivity was compared to the calculated MCR, it accounted for 61% of the total. In chronic renal disease, the percent extraction of delivered immunoreactive PTH was unchanged from the normal state, but RC was markedly diminished due to the decrease in renal plasma flow in the diseased kidney. Total MCR rate of immunoreactivity was also severely depressed in uremia, and the decrease in RC accounted for a large portion (circa 74%) of the decrease in total MCR. The data suggest that the renal removal of PTH from the circulation is related to functioning renal mass. In uremia, the functioning mass is decreased, thus the decrease in RPF, MCR, and RC, but the remaining mass appears to function normally judging from the unimpaired extraction rate for PTH.

The term MCR in the studies after the administration of b-PTH represents a turnover rate of immunoreactivity. Because the CH9 antiserum binds with equal affinity to intact hormone and C-terminal fragments, the MCR term is a combination of the disappearance of intact b-PTH and C-terminal fragments. The term was used to relate the RC as determined in assays with the CH9 antiserum to a total turnover rate. The usefulness of the MCR for b-PTH as a measure of the turnover rate of PTH, as determined through RIA's using

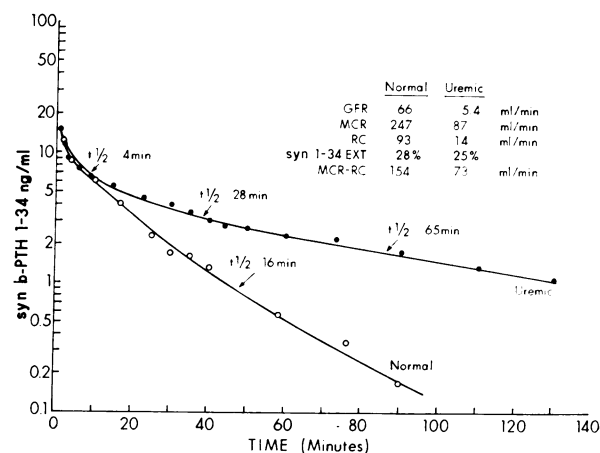


FIGURE 6 Disappearance curves of immunoreactive PTH after injection of syn b-PTH 1-34 as measured by the CH9N antiserum in a dog studied when normal (○—○) and after induction of chronic renal disease (●—●).

the CH9 antiserum, is restricted since it would vary with the use of different antisera in the RIA.

Studies performed after administration of syn b-PTH 1-34 demonstrated results similar to those obtained with intact b-PTH. Uremia did not affect extraction of syn b-PTH 1-34 significantly, but again RC was markedly diminished because of decreased blood flow to the diseased organ. However, the studies performed with syn b-PTH 1-34 in uremic dogs demonstrated a marked decrease in extrarenal clearance of immunoreactive N-terminal fragment as compared to normal dogs, more clearly demonstrating the effects of uremia on extrarenal sites of PTH degradation than was seen in the studies with intact b-PTH. The effect of uremia on extrarenal PTH metabolism has not been previously described, and the studies reported here do not indicate the site of the defective extrarenal clearance. Previous studies indicating that the kidney and the liver (27, 28) are the main sites of peripheral PTH metabolism would suggest that the liver may be the extrarenal site of degradation affected by uremia. There is no available experimental data relating the metabolism of the synthetic N-terminal of PTH used in these studies to the metabolism of the native N-terminal of PTH; and thus, the results of the studies of the metabolism of syn b-PTH 1-34 do not of necessity reflect the metabolism of native N-terminal. Some of the limitations of the MCR as described above for b-PTH do not hold for the MCR of syn b-PTH 1-34 since products of degradation did not appear to bind significantly with the CH9N antiserum.

The disappearance curves for both intact b-PTH and syn b-PTH 1-34 were multiexponential in the single injection studies reported herein. There was an initial rapid phase in both b-PTH and syn b-PTH 1-34 disappearance curves, and chronic renal disease did not appear to have a detectable effect on this early phase of PTH disappearance. However, after the early rapid phase, normal and uremic PTH disappearance curves diverged with longer half-times of PTH removal in the uremic state.

Gel filtration of the circulating PTH in pooled arterial serum samples from normal dogs, taken at set intervals during the disappearance curve of b-PTH, indicate rapid disappearance of the intact molecule and appearance of both C- and N-terminal PTH fragments. The C-terminal fragments tended to persist in the circulation and 50 min after the injection of PTH represented the major species in the circulation. The elution patterns of gel filtration studies on pooled arterial serum from chronic renal disease dogs were similar to those of normal dogs, except that the amount of immunoreactivity was greater in each pool. This was especially prominent for C-terminal immunoreactivity eluting after the intact PTH marker.

The amount of N-terminal immunoreactivity that chromatographed with the b-PTH marker can be accounted for by the ability of the CH9N antiserum to bind with N-terminal sites on the intact b-PTH molecule. The peak of N-terminal immunoreactivity occurring with the syn b-PTH 1-34 marker indicates a circulating N-terminal fragment present in low concentrations and detectable for a short time after the injection of a large amount of b-PTH. Immunoreactive PTH eluting with the syn b-PTH 1-34 marker that also demonstrated biological activity has been described in pooled concentrated plasma samples from patients with primary hyperparathyroidism (10), but N-terminal immunoreactivity in this area was not detected in studies from another laboratory (29). The reasons for these disparate results are not indicated by the present studies.

The studies described above demonstrate a marked decrease in the renal and total turnover rates of immunoreactive b-PTH and syn b-PTH 1-34 induced by chronic renal disease. They indicate that impaired degradation may be another factor, in addition to increased production rates, contributing to the high levels of PTH routinely seen in patients with chronic renal disease. Thus, in patients with renal failure the RIA for PTH may reflect not only overproduction of PTH, as it does for hyperparathyroid states without renal disease, but both overproduction and impaired degradation. Inasmuch as C-terminal fragments are the major detectable PTH species in the circulation and since their half-life is much longer than that of intact hormone or N-terminal fragments, impaired degradation may be a significant factor in the levels of C-terminal fragments seen in chronic renal disease. Thus, the use of C-terminal assays as estimates of only increased PTH production rates in chronic renal disease may result in an overestimation.

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