

**Degradation of parathyroid hormone and fragment production by the isolated perfused dog kidney. The effect of glomerular filtration rate and perfusate  $Ca^{++}$  concentrations.**

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**Research Article**

The renal degradation of intact bovine parathyroid hormone (b-PTH 1-84) was studied with the isolated perfused dog kidney. Disappearance of b-PTH 1-84 from the perfusate occurred concomitantly with the appearance of smaller molecular weight forms of immunoreactive parathyroid hormone (PTH). These smaller molecular weight PTH fragments included both carboxyl and amino terminal regions of the PTH peptide. Perfusate from kidneys with lower glomerular filtration rates (GFR) contained b-PTH 1-84 for longer periods of time than kidneys with higher GFRs, and perfusate from kidneys with lower GFRs demonstrated greater accumulation of carboxyl terminal PTH fragments. Perfusate containing high  $Ca^{++}$  concentrations retarded, and perfusate with low  $Ca^{++}$  concentrations accelerated the rate of degradation of b-PTH 1-84 by the kidney. These studies, therefore document the production of PTH fragments during the course of intact hormone degradation by the kidney. They also demonstrate renal clearance of the PTH fragments produced and define the effects of glomerular filtration rate and calcium concentrations on degradation of intact hormone and the clearance of PTH fragments.

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# Degradation of Parathyroid Hormone and Fragment Production by the Isolated Perfused Dog Kidney

## THE EFFECT OF GLOMERULAR FILTRATION RATE AND PERFUSATE $Ca^{++}$ CONCENTRATIONS

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**ABSTRACT** The renal degradation of intact bovine parathyroid hormone (b-PTH 1-84) was studied with the isolated perfused dog kidney. Disappearance of b-PTH 1-84 from the perfusate occurred concomitantly with the appearance of smaller molecular weight forms of immunoreactive parathyroid hormone (PTH). These smaller molecular weight PTH fragments included both carboxyl and amino terminal regions of the PTH peptide. Perfusate from kidneys with lower glomerular filtration rates (GFR) contained b-PTH 1-84 for longer periods of time than kidneys with higher GFRs, and perfusate from kidneys with lower GFRs demonstrated greater accumulation of carboxyl terminal PTH fragments. Perfusate containing high  $Ca^{++}$  concentrations retarded, and perfusate with low  $Ca^{++}$  concentrations accelerated the rate of degradation of b-PTH 1-84 by the kidney. These studies, therefore, document the production of PTH fragments during the course of intact hormone degradation by the kidney. They also demonstrate renal clearance of the PTH fragments produced and define the effects of glomerular filtration rate and calcium concentrations on degradation of intact hormone and the clearance of PTH fragments.

### INTRODUCTION

The heterogenous nature of circulating parathyroid hormone (PTH)<sup>1</sup> is well recognized (1-6). However,

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<sup>1</sup>Abbreviations used in this paper: COOH terminal, carboxy terminal; GFR, glomerular filtration rate;  $NH_2$  terminal, amino terminal; PTH, parathyroid hormone; b-PTH 1-84,

the origin and biological significance of the various hormonal fragments found in the circulation remains to be determined. Evidence for a role of the liver and the kidney in the degradation of PTH and the production of PTH fragments has been obtained. Studies with the isolated perfused rat liver demonstrated degradation of intact bovine PTH (b-PTH 1-84) and appearance of PTH fragments in the perfusion fluid (7). Studies in awake dogs with indwelling hepatic vein catheters demonstrated selective uptake of intact b-PTH 1-84 by the liver and production of carboxyl terminal (COOH terminal) fragments (8). No hepatic uptake of PTH fragments, either carboxyl or amino terminal ( $NH_2$  terminal) could be demonstrated.

The kidney can extract intact PTH and both COOH- and  $NH_2$ -terminal fragments from the circulation (9). The uptake mechanism for intact PTH and the biologically active  $NH_2$ -terminal fragment (syn b-PTH 1-34) seems to involve both glomerular filtration and peritubular uptake (10). In contrast, renal uptake of COOH-terminal fragments could not be demonstrated in the absence of glomerular filtration suggesting that filtration with subsequent tubular reabsorption and degradation is the major disposal route of COOH-terminal PTH fragments in the organism.

Direct evidence for production of PTH fragments by the kidney was not obtained during the in vivo studies on the renal uptake of PTH. In addition, a clear effect of changes in plasma calcium concentrations on the disappearance of immunoreactive PTH

intact bovine PTH; i-PTH, immunoreactive PTH; syn b-PTH 1-34, biologically active amino terminal fraction of bovine PTH; RIA, radioimmunoassay; TRP, fractional phosphate reabsorption.

(i-PTH) or its renal uptake, after b-PTH 1-84 injections *in vivo*, could not be demonstrated. The present studies, with the isolated perfused kidney of the dog, were therefore designed to examine directly the role of the kidney in the degradation of b-PTH 1-84 and the production of PTH fragments; to determine the relationship between glomerular filtration rate and the production and disappearance rate of PTH fragments; and to evaluate the effect of calcium concentrations on the renal degradation of PTH.

## METHODS

Kidneys from mongrel dogs, heparinized several minutes prior to nephrectomy, were perfused on Waters MOX 100 perfusion apparatus (Waters Instrument Co., Rochester, Minn). From the time of severing the renal artery to institution of perfusate flow, 1–3 min of warm ischemia time elapsed. This was compatible with subsequent normal function of the perfused kidney. Perfusate consisted of heparinized autologous dog blood obtained from a different donor animal and diluted 5:1 with 0.9% NaCl containing potassium, phosphate, and calcium. Perfusion conditions were maintained as close to normal *in situ* values as possible. Perfusate temperature was 37–38°C; the pH was kept constant at 7.4; systolic perfusion pressure was maintained between 115–130 mm Hg; and a gas mixture of 95% O<sub>2</sub>-5% CO<sub>2</sub> was bubbled into the venous drainage reservoir at 0.5 liters/min. Perfusate hematocrits ranged from 20 to 30% and the concentrations of sodium, potassium, phosphorus, magnesium, and bicarbonate were maintained at physiological levels. After the institution of perfusate flow, there was an initial period of vasoconstriction lasting 10–15 min during which time renal blood flow was relatively low (100–150 ml/min), urine flow rates were usually less than 1.0 ml/min, and glomerular filtration rate (GFR) values were less than 10–15 ml/min. Subsequently, renal blood flow increased spontaneously, requiring increased perfusion rates to maintain normal perfusate systolic pressures. After the necessary adjustments, as vasoconstriction disappeared, renal blood flow, urine flow rates, GFR, and electrolyte excretion rates were found to be constant for approximately 2 h. Deterioration of renal function was heralded by a water diuresis with urine flow rates reaching 2–5 ml/min just before a decrease in GFR was detected.

**Study protocol.** During the period of stable renal function, 100 µg of b-PTH 1-84 was injected rapidly through an arterial injection port into the kidney perfusate. Sequential blood and urine samples were then collected every 5–10 min for determination of renal function and characterization of circulating i-PTH. Perfusate ionized calcium concentrations were maintained at 4.0–4.9 mg/dl in most experiments. In those experiments in which the effect of calcium on PTH degradation rates was studied, calcium concentrations were either lowered to 1–2 mg/dl by the addition of EDTA to one-half of the collected perfusate before its addition to the perfusion apparatus or increased to 5.6–7.0 mg/dl by the addition of calcium gluconate to the other half of the collected perfusate.

**Perfusate sample handling.** Perfusate samples collected before and after the addition of b-PTH 1-84 to the renal perfusion system were divided in two portions. One portion was centrifuged immediately, and the chemical determinations described below were performed. The other portion of the perfusate sample was placed immediately in ice and

centrifuged at 4°C, and the plasma was separated from the erythrocytes and frozen until gel filtration was performed.

**Gel filtration.** Samples of renal perfusate (1.5 ml), collected at varying times after the addition of b-PTH 1-84 to the perfusion system, were filtered in 1.5 × 50–60-cm columns of Sephadex G-50 fine (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden). The gel columns were characterized by the elution positions of dextran blue (void volume), <sup>125</sup>I (salt volume), <sup>125</sup>I-b-PTH 1-84, and <sup>125</sup>I-syn b-PTH 1-34. The elution buffer was 0.15 M ammonium acetate with 1% human plasma added.<sup>2</sup> The pH of the final column buffer was adjusted to 5.5. Sephadex gels were prepared by swelling dry beads in water and heating to 90°C for 1 h. Swollen beads were then placed in the cold (4°C) for 24 h before degassing for 2 h also at 4°C. The beads were then added to the columns and equilibrated with the ammonium acetate-1% plasma buffer for 72 h by constant running with gravity-driven flow with a pressure of 9–10 cm H<sub>2</sub>O. Column flow rate was 7–8 ml/h. All gel filtration studies were performed at 4°C, and column eluate fractions of 0.5 ml were collected. Both COOH- and NH<sub>2</sub>-terminal i-PTH were determined in the eluate fractions as described below. Recoveries of <sup>125</sup>I-b-PTH 1-84 and <sup>125</sup>I-syn b-PTH 1-34 ranged from 90 to 95%.

**Radioimmunoassay methods.** Samples of column eluates were assayed for both COOH- and NH<sub>2</sub>-terminal i-PTH by radioimmunoassay (RIA) techniques previously described (8, 9). In the present studies, the CH9 antiserum was used in RIAs for COOH-terminal immunoreactivity in a final dilution of 1:80,000. This antiserum is predominantly COOH-terminal specific (9), and its preincubation with excess syn b-PTH 1-34 did not change the elution profile of i-PTH in column eluates assayed with this antiserum. NH<sub>2</sub>-terminal i-PTH was determined utilizing the CH9N antiserum produced against syn b-PTH 1-34 and specific for NH<sub>2</sub>-terminal PTH binding sites in a final dilution of 1:8,000. 50- to 200-µl samples of each column eluate fraction were incubated with the appropriate antisera at 4°C with constant shaking for 72 h before the addition of <sup>125</sup>I-syn b-PTH 1-34. Iodination of peptides with <sup>125</sup>I was by the method of Greenwood et al. (11) as previously reported (9). The specific activities of these tracer peptides ranged from 250 to 450 µCi/µg for <sup>125</sup>I-b-PTH 1-84 and from 150 to 250 µCi/µg for <sup>125</sup>I-syn b-PTH 1-34. Separation of free from bound counts by charcoal-coated dextran, radioactivity counting, and calculations were performed as described previously (8). Corrections for the effect of column buffer on the antigen-antibody reaction were performed in each assay by adding an appropriate volume of column buffer, equal to the volume of the column fraction being assayed, to control tubes for nonspecific binding, tracer binding tubes, and standard curve tubes. The effect of these buffer additions compared to assay tubes without column buffer added was insignificant. The pH of the assay mixture with 200 µl of column buffer was 8.2 compared to 8.6 for the standard assay mixture.

**Chemical determinations.** Creatinine, phosphorus, and para-aminohippurate were determined by methods described previously (12–14). Magnesium and calcium concentrations were determined by atomic absorption spectrophotometry and ionized calcium with a calcium specific electrode (Orion Research Inc., Cambridge, Mass.).

**Sources of PTH.** "Highly purified" b-PTH 1-84 for injection into the renal perfusate, standard curves and iodination was purchased from Inolex Corp., Biomedical Division (Glenwood, Ill.). The b-PTH 1-84 had a specific biological activity of 1,000–1,500 U/mg in a rat bioassay. syn b-

<sup>2</sup> i-PTH was not detectable at this dilution of plasma.

PTH 1-34 with a specific activity of 3,400–3,700 IU/mg in the renal adenylate cyclase system was obtained from Beckman Instruments, Inc., Fullerton, Calif. Additional experiments were performed with purified b-PTH, a generous gift of Dr. Howard Rasmussen. In these experiments, this PTH served as the labeled tracer, standard curve preparation, and as the PTH for injection into the renal perfusate.

## RESULTS

*Functional parameters of the isolated perfused kidneys.* The electrolyte composition of the renal perfusate and the functional characteristics of 11 isolated perfused kidneys are presented in Table I. Experiments 1 through 5 were single kidney experiments, while numbers 6 through 11 were paired experiments. In these latter experiments, both kidneys were removed from the donor dog and perfused on separate perfusion apparatus with different perfusate calcium concentrations. Perfusion fluid sodium was 145–153 meq/liter, potassium 3.0–5.1 meq/liter, and phosphorus 3.2–5.7 mg/dl. The perfusate Ca<sup>++</sup> ranged from 4.0 to 4.9 mg/dl in the normocalcemic perfusate (normal serum Ca<sup>++</sup> in the dog in our laboratory is 4.0–5.0 mg/dl); from 5.6 to 7.0 mg/dl in the hypercalcemic perfusate; and from 1.0 to 2.4 mg/dl in the hypocalcemic perfusate.

GFR ranged from 13 to 40 ml/min. Renal plasma flow ranged from 117 to 254 ml/min and filtration fraction from 0.07 to 0.20. The low filtration fraction values observed are similar to those described by others under similar experimental conditions in perfused kidney studies (15). Base-line fractional phosphate reabsorption (TRP) ranged from 70 to 96% in these kid-

neys obtained from nonfasted dogs. After PTH administration TRP values fell and ranged from 38 to 62%, demonstrating a clear phosphaturic response to PTH.

*Control experiments.* The elution pattern of the <sup>125</sup>I-b-PTH 1-84 tracer peptide used in the RIAs described in the present studies is shown in Fig. 1. This tracer material was prepared as described above, and previously (9), and separated from free iodine and iodination damage products by absorption onto and elution from Quso G-32 (Philadelphia Quartz Co., Philadelphia, Pa.) followed by gel filtration on 1 × 20-cm columns of Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, Calif.). Only a single peak of radioactivity was observed in the column fractions. Also shown in Fig. 1 is the elution pattern of nonradioactive b-PTH 1-84 determined by COOH-terminal RIA of column eluate fractions. This b-PTH 1-84 was used as standard in the RIA and for administration to all the isolated perfused kidneys except numbers 8 and 9. There were minor radioimmunoassayable contaminants in this peptide, purchased from Inolex Corp., that eluted as a small trailing edge of the peak when compared to the elution pattern of <sup>125</sup>I-b-PTH 1-84. The elution pattern of b-PTH 1-84 incubated at 37°C for 15 min in perfusate fluid obtained after 30 min of renal perfusion is shown as the solid triangles in Fig. 1. When this experiment was repeated with an N-terminal RIA of the column eluate fractions instead of the C-terminal RIA, the elution pattern of b-PTH 1-84 again was not altered by incubation with the kidney perfusate. These experiments indicate that perfusion of isolated kidneys for 30 min did not result in the release into the perfusate of

TABLE I  
Electrolyte Composition of Renal Perfusate and Renal Function in 11 Isolated Perfused Dog Kidneys

Exp. no.	Electrolyte composition				Renal function				
	Na <sup>+</sup> meq/liter	K <sup>+</sup> meq/liter	PO <sub>4</sub> <sup>-</sup> mg/dl	Ca <sup>++</sup> mg/dl	GFR ml/min	RPF ml/min	FF	TRP	
								Cont	Exp
1	150	4.0	5.1	4.0	20	105	0.19	73	52
2	151	4.0	5.2	4.9	40	253	0.16	75	38
3	145	4.5	4.0	4.6	38	237	0.16	91	65
4	148	5.1	3.2	4.5	13	180	0.07	80	62
5	143	3.7	5.7	4.8	11	170	0.06	70	63
6	147	3.6	4.9	6.5	28	117	0.17	89	40
7	153	3.0	3.7	1.0	35	204	0.17	70	65
8	150	3.2	3.4	5.6	28	215	0.13	80	52
9	151	3.0	3.2	2.4	28	254	0.11	73	45
10	145	3.4	4.4	7.0	14	71	0.20	96	72
11	149	3.7	4.3	1.0	24	184	0.14	82	55

Brackets indicate kidney pairs. RPF, renal plasma flow; FF, filtration fraction; TRP, tubular reabsorption of phosphate; Cont, control; Exp, experimental.

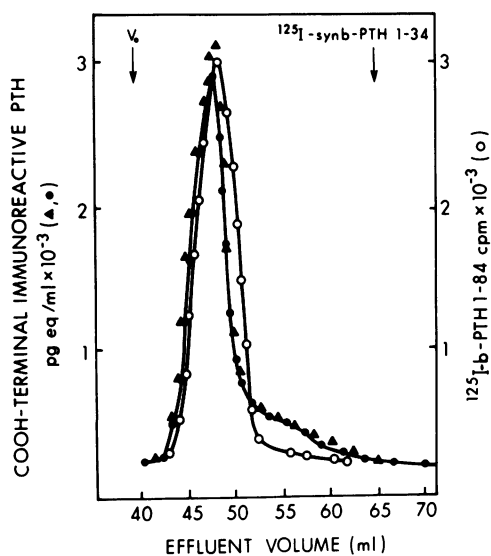


FIGURE 1 Elution patterns of  $^{125}\text{I}$ -b-PTH 1-84 ( $\circ$  —  $\circ$ ), COOH-terminal immunoreactive b-PTH 1-84 from Inolex Corp. ( $\bullet$  —  $\bullet$ ), and b-PTH 1-84 incubated with renal perfusate at  $37^\circ\text{C}$  for 15 min ( $\blacktriangle$  —  $\blacktriangle$ ). Gel filtration was performed through a  $1.5 \times 60$ -cm column of Sephadex G-50 fine. Void volume ( $V_0$ ) and the elution position of  $^{125}\text{I}$ -syn b-PTH 1-34 are marked by arrows. Column salt volume (not shown) was 102 ml. The left ordinate scale is COOH-terminal i-PTH in picogram b-PTH 1-84 equivalents per milliliter and the right is radioactivity in counts per minute  $\times 10^{-3}$ .

substances which could subsequently result in degradation of intact PTH. This experiment also indicates that contaminants in the b-PTH 1-84 preparation did not account for the appearance of smaller molecular weight forms of i-PTH that are shown below.

**Gel filtration of kidney perfusates after PTH administration.** Fig. 2 shows the elution pattern of i-PTH in a perfusate sample obtained 15 min after the addition of  $100 \mu\text{g}$  b-PTH 1-84 to the isolated perfused kidney, listed as no. 1 in Table I. The predominant peak of both COOH- and  $\text{NH}_2$ -terminal i-PTH eluted from the column in the same effluent volume as  $^{125}\text{I}$ -b-PTH 1-84. However, significant peaks of both COOH and  $\text{NH}_2$  i-PTH eluted after  $^{125}\text{I}$ -b-PTH 1-84, indicating the appearance in the renal perfusate of smaller molecular weight fragments of PTH. The smaller peak of  $\text{NH}_2$ -terminal i-PTH eluting as intact hormone compared to the COOH-terminal intact hormone peak indicates that the  $^{125}\text{I}$ -syn b-PTH 1-34 tracer binding was not displaced by intact hormone as easily as intact hormone displaced  $^{125}\text{I}$ -b-PTH 1-84 binding in the COOH-terminal assay. The elution pattern of COOH-terminal i-PTH in Fig. 2 is similar to that seen after injection of b-PTH 1-84 to dogs in vivo (8, 9). The gel filtration characteristics of the peaks of COOH-terminal i-PTH eluting just

after the intact hormone are similar to the major form of COOH-terminal i-PTH found in the human circulation (2, 4, 5). The elution pattern of  $\text{NH}_2$ -terminal i-PTH demonstrated a large portion of the detectable immunoreactivity eluting with the  $^{125}\text{I}$ -b-PTH 1-84 marker, but there was also the appearance of a peak of N-terminal immunoreactivity in the area of the  $^{125}\text{I}$ -syn b-PTH 1-34 marker that was not present in control experiments. These elution patterns indicate degradation of the b-PTH 1-84 added to the renal perfusion circuit and the appearance of at least three smaller molecular weight fragments of b-PTH 1-84.

**Effect of renal function on degradation and renal clearance of PTH.** To characterize further the disappearance of administered b-PTH 1-84 and the appearance of later eluting forms of i-PTH during renal perfusion, samples taken at different times after addition of PTH to the perfusate were gel filtered, and the eluates were assayed as above. In addition, the

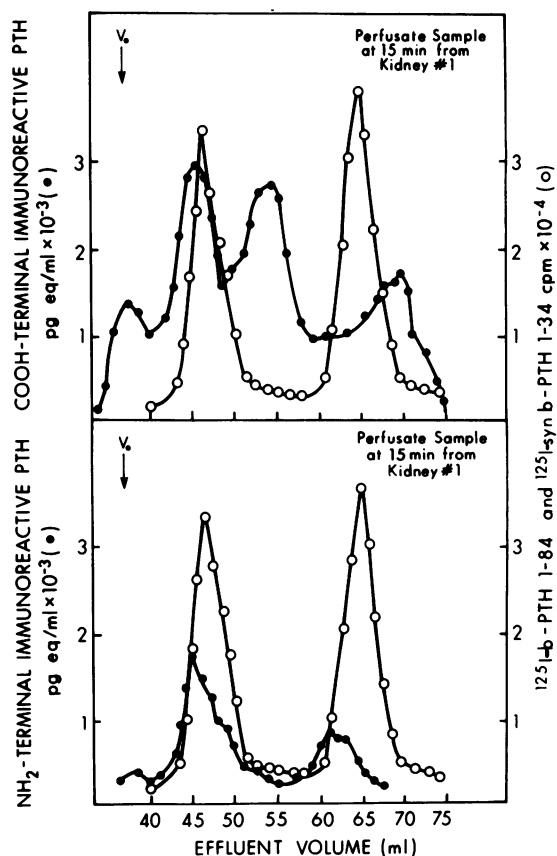


FIGURE 2 Elution patterns of COOH (top panel) and  $\text{NH}_2$ -terminal (bottom panel) i-PTH ( $\bullet$  —  $\bullet$ ) after the addition of b-PTH 1-84 to kidney 1. The elution patterns of  $^{125}\text{I}$ -b-PTH 1-84 and  $^{125}\text{I}$ -syn b-PTH 1-34 ( $\circ$  —  $\circ$ ) are portrayed for comparison. Gel filtration was through a  $1.5 \times 60$ -cm column of Sephadex G-50 fine.

relationship between GFR and degradation of b-PTH 1-84 and the disappearance of the produced fragments was studied by comparing the elution patterns of i-PTH in perfusates obtained from kidneys with varying GFRs. The i-PTH elution patterns in Fig. 3 on the left were obtained after 15 and 30 min of renal perfusion after the addition of 100  $\mu$ g b-PTH 1-84 to kidney 2, GFR 40 ml/min. The i-PTH elution patterns on the right of the figure were obtained from kidney 4, GFR 13 ml/min, in experiments performed under identical conditions. The amount of i-PTH eluting with the  $^{125}$ I-b-PTH marker at 30 min was less in the perfusate of kidney 2. This indicates a greater renal clearance for b-PTH 1-84 in the kidney with the higher GFR. The qualitative appearance of the later eluting peaks of i-PTH were similar in that after 15 min of perfusion both COOH- and NH<sub>2</sub>-terminal fragments of PTH were present in the perfusate of both kidneys. However, the disappearance of the fragments was much more rapid in

the kidney with the higher GFR. No significant amounts of late eluting COOH- or NH<sub>2</sub>-terminal degradation products were detectable in perfusate obtained 30 min after addition of PTH to the kidney with the higher GFR. In contrast, both at 15 and 30 min after addition of PTH there were greater quantities of C-terminal fragments present in the perfusate of kidneys with low GFRs as compared to kidneys with higher GFRs. These results suggest that the disappearance rate of COOH-terminal fragments is dependent upon the level of GFR of the perfused kidneys. These data are in agreement with the results obtained in dog studies in vivo (10). Results similar to those shown for the comparison of perfusate i-PTH from kidneys 2 and 4 were seen for kidneys 3 and 5.

*Effects of calcium on PTH clearance and fragment production by the kidney.* To study the effects of different calcium concentrations on the metabolism of PTH, both kidneys were removed from a donor dog

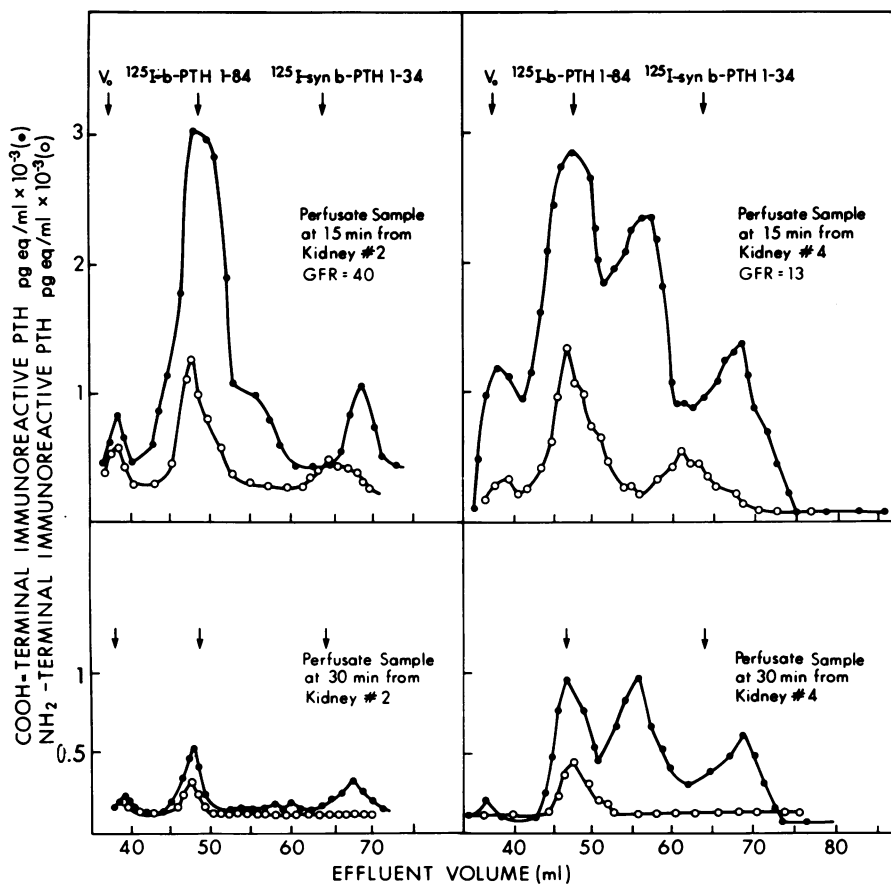


FIGURE 3 Effect of GFR. Elution patterns of COOH- and NH<sub>2</sub>-terminal i-PTH in perfusate samples obtained at 15 min (top panels) and 30 min (bottom panels) after b-PTH 1-84 addition from kidney 2 (left panels) with a GFR of 40 ml/min and kidney 4 (right panels) with a GFR of 13 ml/min are shown. Gel filtration performed through columns similar to those used in Figs. 1 and 2.

and perfused on separate perfusion apparatus. The ionized calcium in the perfusate of one of the paired kidneys was decreased to 1–2.4 mg/dl by the addition of sufficient EDTA (kidneys 7, 9, and 11 in Table I). Calcium gluconate was added to the perfusate of the other kidney in the pair sufficient to raise ionized calcium to 5.6–7.0 mg/dl (kidneys 6, 8, and 10 in Table I). Only mild degrees of hypercalcemia were employed in an attempt to avoid any effects of hypercalcemia on the GFR and renal plasma flow of the perfused kidney.

Fig. 4 shows the elution patterns of COOH- and NH<sub>2</sub>-terminal i-PTH from perfusate samples collected from kidneys 7 and 6 and gel filtered as described before. The i-PTH eluting with the <sup>125</sup>I-b-PTH 1-84 marker disappeared rapidly from the perfusate of the hypocalcemic kidney (left panels). When the elution patterns on the top left and top right of Fig. 4 were compared, there was less i-PTH eluting in the area of intact hormone at 15 min in the low Ca<sup>++</sup> perfusate than in the high Ca<sup>++</sup> perfusate. 30 min after addition of PTH to the hypocalcemic perfusate

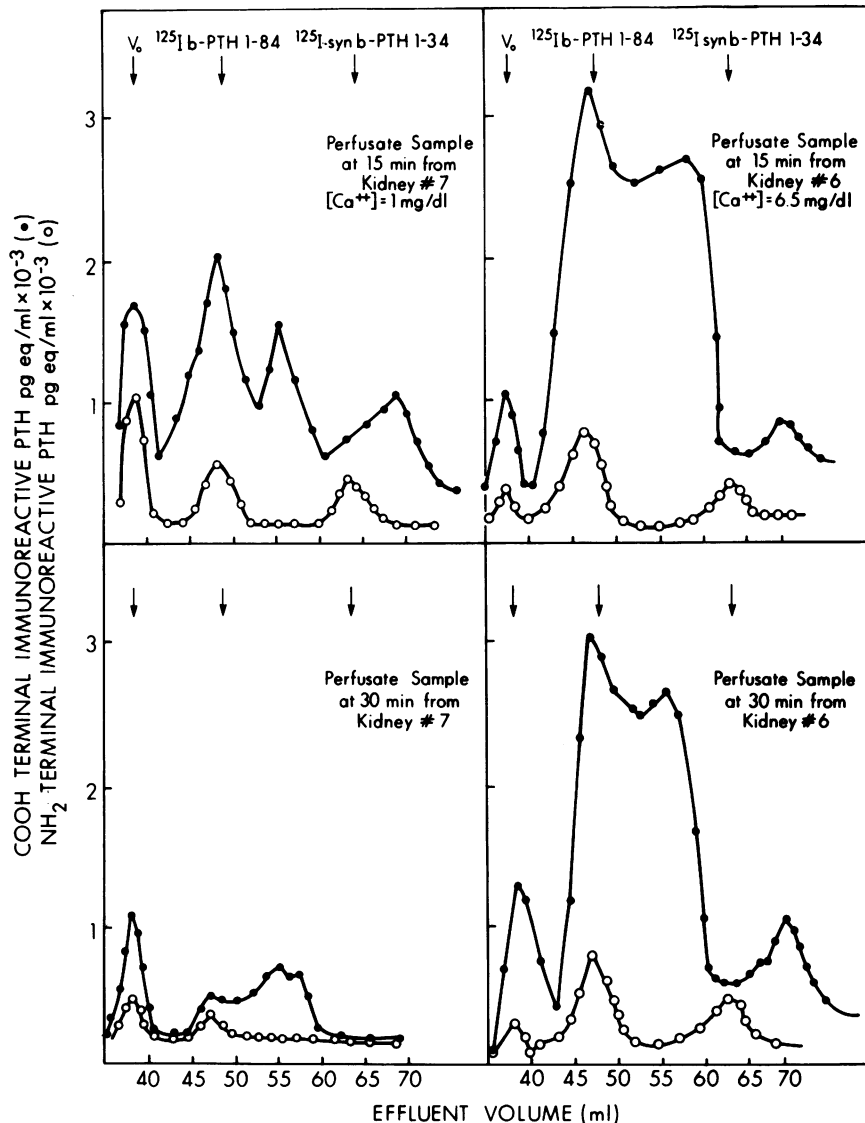


FIGURE 4 Effect of perfusate Ca<sup>++</sup> concentration. Elution patterns of COOH- and NH<sub>2</sub>-terminal i-PTH in perfusate samples obtained at 15 min (top panels) and 30 min (bottom panels) after addition of b-PTH 1-84 to kidney 7 (left panels) with a perfusate Ca<sup>++</sup> concentration of 1 mg/dl and to kidney 6 (right panels) with a perfusate Ca<sup>++</sup> concentration of 6.4 mg/dl are shown. GFR of kidney 6 was 28 ml/min and GFR of 7 was 35 ml/min. Gel filtration was through a 1.5 × 60-cm column of Sephadex G-50 Fine.

there was almost no detectable i-PTH eluting in the area of intact hormone. In contrast, the disappearance of i-PTH eluting in the area of intact hormone was markedly prolonged in the hypercalcemic perfusate. When the i-PTH elution pattern from the 30-min sample of hypercalcemic perfusate (bottom right Fig. 4) is compared to the 30-min sample of the perfusate from the low  $\text{Ca}^{++}$  kidney, an obvious delay in i-PTH disappearance eluting in the area of intact hormone can be appreciated. In addition, an  $\text{NH}_2$ -terminal fragment eluting in the area of  $^{125}\text{I}$ -syn b-PTH 1-34 is still present at 30 min in the hypercalcemic perfusate. This was the longest time at which this fragment was still detectable in all of the perfusates studied. Thus, these studies demonstrate that alterations in  $\text{Ca}^{++}$  perfusing the kidney affect the disappearance of i-PTH eluting with intact hormone and the production of PTH fragments.

Because the GFR of the hypercalcemic kidney shown in Fig. 4 was somewhat less than that of the hypocalcemic kidney, milder extremes of altered  $\text{Ca}^{++}$  concentrations were studied to further analyze the role of  $\text{Ca}^{++}$  in the degradation of b-PTH 1-84. Also, a different source of PTH was used in these experiments to further exclude any possibility that the minor contaminants found in the PTH preparation used in the studies shown in Figs. 1-4 contributed to the observed appearance of i-PTH fragments in the renal perfusate. An experiment similar to that portrayed in Fig. 1 is shown on the top left panel of Fig. 5. The  $^{125}\text{I}$ -b-PTH 1-84 (○ — ○) tracer eluted as a single peak. The Rasmussen PTH also eluted as a single peak, demonstrating absence of the trace contaminants from the descending slope of the elution pattern that were shown in Fig. 1. Incubation of b-PTH 1-84 with a renal perfusate sample obtained after 30 min of perfusion at  $37^\circ\text{C}$  for 20 min again failed to result in any degradation of the hormone. The middle and bottom panels of Fig. 5 show experiments similar to those shown in Fig. 4 except that the changes in  $\text{Ca}^{++}$  were less extreme. The effect of the milder hypercalcemia on retarding the degradation of b-PTH 1-84 was less and (or) conversely, the effect of milder hypocalcemia on enhancing the degradation of b-PTH 1-84 was less than shown in the experiments in Fig. 4 and others not shown. Nevertheless, a faster disappearance of i-PTH eluting with the  $^{125}\text{I}$ -intact hormone is shown when the hypocalcemic perfusate was used. Thus, this experiment again demonstrates degradation of b-PTH 1-84 by the perfused isolated dog kidney, and the appearance and disappearance of smaller molecular weight forms of i-PTH utilizing a second and purer PTH preparation. Also, low perfusate calcium concentrations were again associated with more rapid intact hormone renal clearance and degradation. The effect of hypercalcemia

and hypocalcemia appears to be directly related to the degree of alterations in the  $\text{Ca}^{++}$  concentration, the greater the alteration the greater retardative (hypercalcemia) or stimulatory (hypocalcemia) effect on b-PTH 1-84 disappearance rate.

## DISCUSSION

The results obtained demonstrate that the isolated perfused dog kidney can degrade b-PTH 1-84 with eventual disappearance of the peptide from the perfusion fluid. The lack of degradation of b-PTH 1-84 incubated with fluid from perfused kidneys suggest that release of nonspecific degradative enzymes into the perfusion circulation from the kidney was not responsible for the results obtained. The similar gel filtration elution profiles of b-PTH 1-84 and samples of b-PTH 1-84 incubated with fluid used to perfuse a kidney for 15 min (Fig. 1) also excluded the possibility that small amounts of late eluting contaminants in the original PTH preparation accounted for any of the observed changes in the elution profile of renal perfusate, especially the appearance of any PTH degradation products.

The disappearance of intact b-PTH 1-84 from the kidney perfusate was associated with the appearance of late eluting fragments of i-PTH with both COOH- and  $\text{NH}_2$ -terminal binding specificities. These fragments were cleared from the circulation by the perfused kidney as shown by comparing the elution patterns of renal perfusate obtained at 10 or 15 min vs. 30 min after addition of PTH to the perfusion circuit.

The late eluting i-PTH degradation products described in the present studies have the gel filtration characteristics of the circulating i-PTH fragments found *in vivo* after the injection of b-PTH 1-84 into dogs (8, 9). They are also compatible with the i-PTH fragments reported to be produced by the isolated rat liver (7) and those found in the plasma of hyperparathyroid subjects (2). Specifically, a small amount of circulating  $\text{NH}_2$ -terminal i-PTH, that eluted in the area of the  $^{125}\text{I}$ -syn b-PTH 1-34 marker, was evident in the perfusate of the isolated kidneys shortly after addition of b-PTH 1-84. This demonstrates that the kidney could potentially produce an  $\text{NH}_2$ -terminal fragment in the process of PTH degradation with similar gel filtration characteristics to the  $\text{NH}_2$ -terminal fragment shown in dogs *in vivo* (9).

At least two peaks of C-terminal i-PTH appeared in the elution profiles after the peak of intact hormone. Again, these results are similar to COOH-terminal elution patterns found by gel filtration of dog plasma obtained after injection of b-PTH 1-84 *in vivo* (8, 9). The origin of the smallest detected COOH-terminal fragment, the peak of COOH-terminal immunoreactivity eluting after the  $^{125}\text{I}$ -syn b-PTH 1-34, may be a



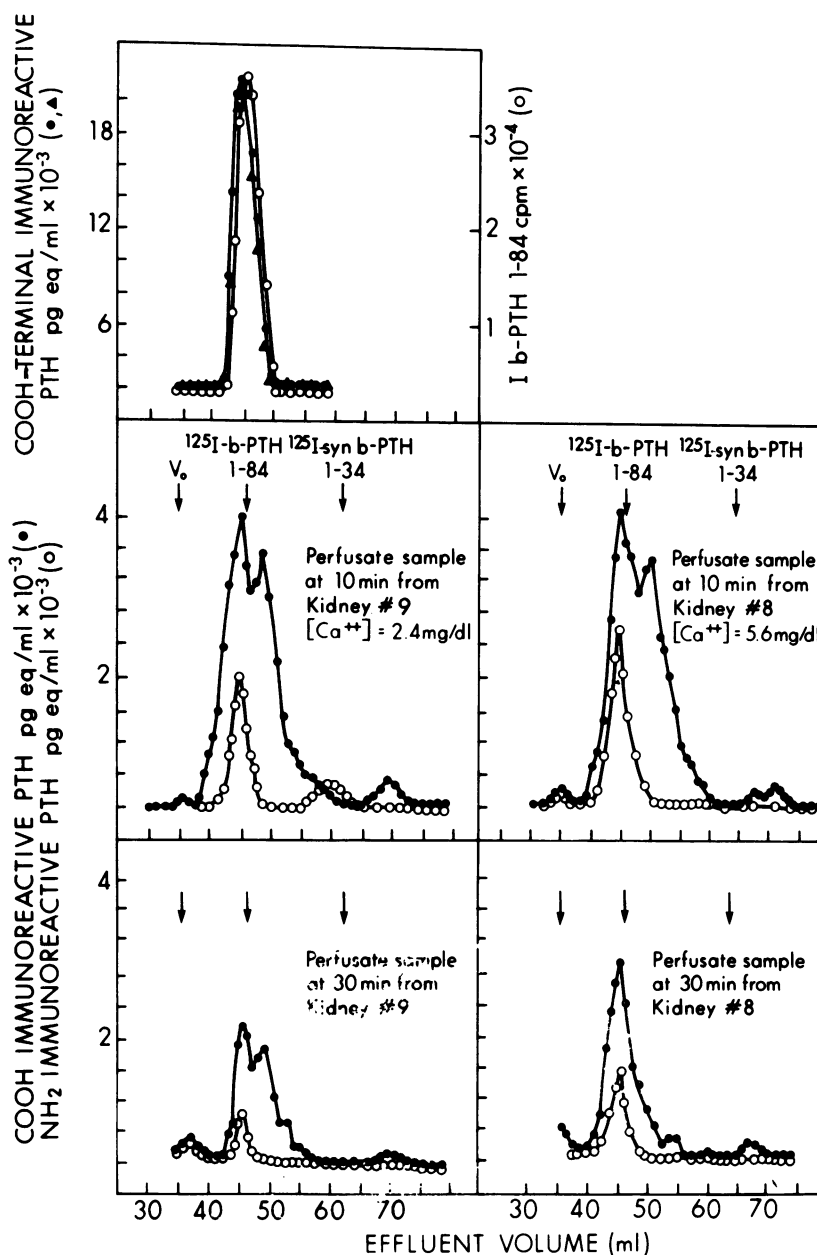


FIGURE 5 Rasmussen PTH and effect of perfusate  $\text{Ca}^{++}$  concentration. In the top left panel are elution patterns of  $^{125}\text{I}$ -b-PTH 1-84 ( $\circ$  —  $\circ$ ), COOH-terminal immunoreactive b-PTH 1-84 from H. Rasmussen ( $\bullet$  —  $\bullet$ ), and b-PTH 1-84 incubated with renal perfusate ( $\blacktriangle$  —  $\blacktriangle$ ). Elution patterns of perfusate samples obtained 10 min (middle panels) and 30 min (bottom panels) after addition of b-PTH 1-84 to kidney 9 with perfusate  $\text{Ca}^{++}$  concentration of 2.4 mg/dl (left panels) and to kidney 8 with perfusate  $\text{Ca}^{++}$  concentration of 5.6 mg/dl (right panels). GFR of kidneys 8 and 9 was 28 ml/min. Gel filtration was through a  $1.5 \times 50$ -cm column of Sephadex G-50 Fine.

product of further cleavage of the initial COOH-terminal degradation product that eluted just after the  $^{125}\text{I}$ -b-PTH 1-84 marker. This was suggested as the origin of a fragment with similar gel filtration characteristics by Canterbury et al. (7).

Previous studies from this laboratory have demonstrated that the kidney may remove PTH from the circulation by two mechanisms (10). The first involves glomerular filtration and tubular reabsorption and the second involves uptake at the antiluminal side of

tubular cells. The renal uptake of i-PTH during a constant infusion of b-PTH 1-84 or syn b-PTH 1-34 was reduced by 20–30% in dogs with nonfiltering kidneys (acute ureteral obstruction). This indicates that most of the renal uptake of intact hormone and the NH<sub>2</sub>-terminal fragment is from the peritubular blood and probably represents binding to receptors located on the antiluminal membrane of tubular epithelial cells. However, renal uptake of COOH-terminal i-PTH fragments could not be demonstrated after injections of b-PTH 1-84 in dogs with acute ureteral obstruction at a time when the circulating forms of i-PTH were comprised essentially of COOH-terminal PTH fragments. This suggests that glomerular filtration may be the sole means for the renal removal of these COOH-terminal fragments.

The results of the present studies with isolated perfused dog kidneys support and extend the observations obtained in the dog in vivo. The elution patterns of i-PTH in renal perfusate samples from kidneys with different levels of GFR demonstrate that the disappearance of i-PTH eluting in the area of intact hormone is only moderately greater in a kidney with a GFR of 40 than in one with a GFR of 13. This is compatible with the concept that most of the renal uptake of intact hormone is independent of glomerular filtration as shown in the nonfiltering kidney in vivo.

The present studies also demonstrate that there is greater accumulation of COOH-terminal i-PTH fragments in the perfusate of kidneys with low GFR. This is compatible with the concept that glomerular filtration is the major means of renal uptake for C-terminal fragments as demonstrated by the lack of COOH-terminal fragment uptake in nonfiltering kidneys in vivo. Thus, the absence of accumulation of C-terminal fragments in the perfusate of kidneys with high GFR (right side panels, Fig. 3) indicates rapid clearance of these peptides by glomerular filtration.

The effect of calcium on the renal degradation of b-PTH 1-84 was also shown. Calcium concentrations in the perfusate of 5.5 mg/dl or greater were associated with a slower disappearance of b-PTH 1-84, such that at 30 min postinjection intact PTH was still the predominant i-PTH species in the perfusate. In contrast, Ca<sup>++</sup> concentrations below 2.4 mg/dl were associated with rapid disappearance of b-PTH from perfusate samples. This effect of calcium concentration differed from that of GFR in that the rapid disappearance of intact hormone observed with very low perfusate calcium concentrations was associated with the accumulation of COOH i-PTH fragments. In contrast, high rates of GFR predominantly increased the disappearance rate of COOH-terminal fragments. Thus, the differences in GFR between the kidney with high and low calcium perfusates in Fig. 4 could not account for both rapid disappearance of intact

hormone and accumulation of COOH-terminal i-PTH fragments. These results are in agreement with the effects of calcium on PTH degradation by isolated rat liver (7) and by peptidases in vitro (16).

The present studies demonstrate production of smaller molecular weight i-PTH fragments during the degradation of intact b-PTH 1-84. The liver (7, 8) and kidney have been shown to produce PTH fragments and to degrade all of the intact hormone added to these isolated organ perfusion circuits within a short time. These studies do not exclude the possibility that circulating PTH fragments in vivo can originate from direct secretion by the parathyroid glands as suggested by other authors (6, 17, 18). They do suggest that significant fractions of the circulating fragment pool may result from degradation of intact hormone by the kidney.

The present studies also do not elucidate the biological significance of the fragments produced in the various experimental settings discussed above and in vivo. They do support the observation that COOH-terminal fragments are dependent upon glomerular filtration and tubular reabsorption for their degradation. This finding may explain the marked retention of COOH-terminal fragments observed in chronic renal failure as GFR falls.

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