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Parathyroidectomy does not prevent the renal PTH/PTHrP receptor down-regulation in uremic rats

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Parathyroidectomy does not prevent the renal PTH/PTHrP receptor down-regulation in uremic rats. In a recent study we demonstrated that the PTH/PTHrP receptor (PTH-R) mRNA was markedly down-regulated in the remnant kidney of uremic rats with severe secondary hyperparathyroidism. Among the factors potentially implicated in this down-regulation, to date only PTH has been demonstrated to modulate PTH-R expression. Here, we examined the effect of thyroparathyroidectomy (TPTX) on the renal expression of PTH-R in rats with normal renal function or with chronic renal failure (CRF) induced by 5/6 nephrectomy. Four groups of rats were studied: control, TPTX, CRF, and CRF+TPTX. Moderate-degree renal failure was documented by mean (± SD) creatinine clearances (μl/min/100 g body wt) of 259 ± 40 and 212 ± 45 in CRF and CRF+TPTX rats, compared with 646 ± 123 and 511 ± 156 in control and TPTX rats, respectively. Plasma phosphorus, calcitriol, and ionized calcium were significantly lower in CRF and CRF+TPTX than in control animals. Plasma ionized calcium and calcitriol were also lower in TPTX than in control rats. Plasma PTH levels (pg/ml) were increased in CRF rats (41.8 ± 29.4), and markedly decreased in TPTX (10.1 ± 7.8) and CRF+TPTX (8.0 ± 3.8) rats compared with control rats (21.7 ± 7.5). Northern blot analysis showed that the level of the steady-state PTH-R mRNA in the kidney of CRF and CRF+TPTX rats was markedly decreased compared with that of control rats, the ratios of PTH-R mRNA/β-actin mRNA being 0.28 ± 0.04 and 0.27 ± 0.03 versus 0.54 ± 0.05, respectively. The PTH-R mRNA expression was also found decreased in bone tissue from two uremic animals compared with control rats: 0.59 versus 0.78, respectively. No change was observed in the renal PTH-R mRNA level in TPTX animals. There was also no change in the PTH-R mRNA expression in the liver of uremic rats. The expression of the PTHrP mRNA was comparable in the kidney of control and CRF animals. CRF and CRF+TPTX rats showed a similarly reduced PTH-sensitive adenylyl cyclase activity in crude renal membrane preparations, compared with control rats. Despite the reduction of PTH-R mRNA and PTH-sensitive adenylyl cyclase in the kidney, CRF rats with intact parathyroid glands had lower urinary calcium excretion and higher phosphate excretion than CRF-TPTX rats, suggesting that PTH was still capable of controlling mineral ion metabolism through the remaining PTH-R in the residual nephrons. In conclusion, our data demonstrate that neither an increase in plasma PTH and phosphate nor a decrease in plasma calcium are important in renal PTH-R down-regulation during chronic renal failure. It is also unlikely that an increase in the locally-produced renal PTHrP could down-regulate its own receptor.

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The mammalian kidney plays a crucial role in the homeostasis of calcium, magnesium, and phosphate. To maintain a net balance, more than 98% of the filtered load of each ion is reabsorbed along the nephron [1–5]. The distal parts of the nephron, namely, the thick ascending limb of Henle's loop, distal convoluted, and connecting tubules are the main regulatory sites for final excretion in the urine. These nephron segments have receptors for several calciotropic hormones such as parathyroid hormone (PTH), calcitonin, and calcitriol [6–9]. Theoretically, a modification in the expression of one or several of these receptors in the kidney could lead to an alteration of the mineral and bone metabolism.

In a recent study we demonstrated that the renal level of the PTH/PTH-related peptide (PTHrP) receptor (PTH-R) mRNA and the PTH-sensitive adenylyl cyclase activity were markedly down-regulated in the remnant kidney of uremic rats with severe secondary hyperparathyroidism [10]. The decrease in the renal PTH-R mRNA, with a subsequent reduction of PTH-R number, could at least in part be responsible for the receptor desensitization to PTH. Moreover, the renal PTH-R down-regulation in the setting of uremia was associated with severe hypocalcemia, hyperphosphatemia, high plasma PTH, and low plasma calcitriol concentrations. These plasma abnormalities, characteristic of uremic secondary hyperparathyroidism [11], could be partially explained by the renal PTH-R receptor down-regulation for the following reasons: firstly, because receptor down-regulation could directly lead to a decrease in PTH-stimulated tubular calcium reabsorption and a decrease in phosphate excretion resulting in phosphate retention and indirectly in hypocalcemia; secondly, because the subsequent hyperphosphatemia could interfere with the PTH-stimulated synthesis of 1,25 $(OH)_2D_3$ by the kidney, resulting in a decrease in intestinal calcium absorption and hypocalcemia.

Among the factors potentially implicated in the PTH-R mRNA down-regulation, to date only PTH has been demonstrated, in *in vitro* studies, to modulate the receptor mRNA expression [12]. In order to investigate whether circulating PTH plays an important role in the down-regulation of its own receptor in the kidney of uremic animals, we have assessed its renal expression in thyroparathyroidectomized (TPTX) uremic rats, compared with uremic rats with intact parathyroid glands. Moreover, we have examined the possible role of abnormal mineral ion metabolism in the renal PTH-R down-regulation.

The results of our study show that PTH reduction to levels

lower than normal does not prevent the PTH-R down-regulation in the kidney of uremic rats. They also show that despite the marked reduction in PTH-stimulated adenylyl cyclase activity and in PTH-R mRNA expression, the residual nephrons of uremic rats are still capable of responding to high plasma PTH concentrations and regulating mineral ion metabolism in a compensatory manner, in keeping with the "trade-off" hypothesis [13, 14].

Methods

Animals and surgical procedures

Thirty male, 12- to 14-week-old Wistar AF rats (IFFA CREDO, Lyon, France) weighing 250 to 300 g were fed *ad libitum* with a standard diet containing 0.9% P, 1.2% Ca, 0.2% Mg, 25% protein, and 2,000 UI/kg vitamin D_3. Animals were allowed free access to food and deionized water.

They were divided in four groups. A first group of seven rats underwent a sham operation (renal decapsulation) and served as control animals. A second group of six rats had 5/6 nephrectomy according to a standard two-step operation method [15] leading to chronic renal failure (CRF). A third group of eight rats underwent total thyroparathyroidectomy and 5/6 nephrectomy (CRF+TPTX). TPTX was performed 10 days before the 5/6 nephrectomy. Lastly, a fourth group of nine rats with normal renal function underwent TPTX alone (TPTX). TPTX in these animals was also performed 10 days before renal sham operation.

Four weeks after the second renal surgery, all animals were placed in metabolic cages for a three-day adaptation period followed by a three-day period of renal function assessment.

Blood and urine biochemistry

The fifth week after renal surgery, all the animals were anesthetized by injection of pentobarbital i.p. before sacrifice and a blood sample was obtained by aortic puncture. An aliquot of heparinized blood was used for ionized calcium determination (ICA1 Ionized Calcium Analyzer, Radiometer, Copenhagen, Denmark). The remaining blood was centrifuged at 3,000 g for 20 minutes. Plasma was stored at −50°C until assays were performed. Plasma total calcium, phosphorus, magnesium, total protein concentration, urea and creatinine were determined by standard laboratory methods performed on a multiparametric analyzer (Hitachi 717 analyzer, Boehringer Mannheim, Germany). For the determination of calcium, phosphate, magnesium, creatinine, and urea concentrations in urine, samples were diluted 1:5 (vol/vol) in sterile bi-distilled water.

Plasma rat immunoreactive parathyroid hormone (riPTH) was measured with a rat PTH (IRMA) kit (Immutopics, Inc., CA, USA) [10, 16] recognizing the N-terminal portion of the hormone. Plasma calcitriol was measured by radioreceptor assay (Nichols Institute, San Juan Capistrano, CA, USA) using calf thymus receptor according to the method of Hollis [17].

Preparation of renal membranes

The cortex of kidneys from control, TPTX, CRF, and CRF+TPTX rats was dissected and renal membranes were prepared by a modification of the procedure described by Marx, Fedak and Aurbach [18]. After homogenization in a 30% (vol/vol) buffer solution containing 0.25 M sucrose, 10 mM Tris and 1 mM Na_2EDTA, pH 7.5, the tissue was diluted 1:1 with buffer solution and centrifuged at 4°C in a Sorvall ultracentrifuge. When the rotor speed reached 4,500 rpm, centrifugation was stopped and the supernatant was collected. After a second identical centrifugation the supernatant was centrifuged at 4,500 rpm for 15 minutes. The supernatant was discarded and the upper portion of the resulting "double-layered" pellet was resuspended in 3 volumes of a buffer solution containing 10 mM Tris, 1 mM Na_2EDTA, pH 7.5 and stored at −80°C until used for the determination of protein concentration and adenylyl cyclase activity. Protein content was measured using the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, Illinois, USA), a modification of the original Lowry method [19].

Adenylyl cyclase assay and cAMP measurement

The adenylyl cyclase assay was a modification of the procedure described by Carnes, Anast and Forte [20]. The reaction mixture contained: renal membrane preparation containing 75 μg of protein, 0.1% bovine serum albumin, 50 mM Tris-HCl (pH 7.5), 1.7 mM $MgCl_2$, 1.2 mM ATP, 7 mM phosphocreatine, 1 mM 3-isobutyl-1-methylxanthine (IBMX), and 1.08 units of creatine kinase. This preparation was incubated for 30 minutes at 37°C with vehicle (acetic acid 10 mM), 10 mM NaF, 1 mM forskolin, or 1 μM rat [$Nle^{8,21}$,Tyr^{34}] PTH (1-34) from Bachem (CA, USA). The reaction was stopped by adding 500 μl of 95% ethanol and the denatured proteins were removed by centrifugation five minutes at 3,000 rpm. The supernatant was lyophilized and resuspended in 1 ml of 50 mM Na acetate pH 6.2. The amount of cAMP generated was measured by a radioimmunoassay method. The ^{125}I-labeled cAMP was purchased from ERIA Diagnostics Pasteur, France, and we used a rabbit polyclonal antibody raised against cAMP which was offered by Dr. P. Ronco (Paris, France).

RNA preparation and Northern blot hybridization

Total RNA from homogenized renal, liver, and femoral head tissues was prepared using the acid-guanidinium-isothiocyanate-phenol-chloroform extraction method [21]. Poly $(A)^+$ RNA was selected by oligo (dT) chromatography. RNA preparations were denatured and separated by electrophoresis (1% agarose/2.2 M formaldehyde gel), transferred to a nitrocellulose membrane (Schleicher & Schuell, Keene, NH, USA), and baked (2 hr, 80°C). The filters were hybridized with a 1.8 kb, BamHI-NotI cDNA fragment corresponding to most of the rat PTH/PTHrP receptor cDNA's coding region [22]. The probe was ^{32}P-labeled using the random-priming method to a specific activity of 1×10^9 cpm/μg. All filters were prehybridized for three to four hours at 42°C with a solution containing 50% of formamide, 6.6× standard saline citrate (SSC), 0.1% SDS, 5× Denhardt's solution, 200 μg/ml of salmon sperm DNA and 0.01 M EDTA. The labeled probe was then added to the prehybridization solution (1×10^6 cpm/ml) and the filters were incubated overnight (16 to 18 hr) at 42°C. They were then washed twice, 30 minutes at 42°C with 300 ml of 1× SSC plus 0.1% SDS, and 30 minutes at 65°C with the same solution. The filters were exposed to an autoradiographic film with an intensifying screen for seven days at −80°C. After hybridization with the PTH/PTHrP receptor probe and exposure, the filters were washed for 30 min at 95°C in 0.01% SDS, and rehybridized with either a 450 bp-Hinf I fragment of a human β-actin cDNA [23] which was also ^{32}P-labeled by random-priming or with a 30 base pairs oligomer (5′ GGG TGG AGG CCG CCG CGA GTG CAG ATC TTG 3′) complementary to the 28S ribosomal RNA which was ^{32}P end-labeled using the bacteriophage polynucleotide

Table 1. Plasma biochemistry

	Control ($N = 7$)	TPTX ($N = 9$)	CRF ($N = 6$)	CRF+TPTX ($N = 8$)
Total proteins *g/liter*	62.1 ± 1.2	67.7 ± 2.6[a]	55.3 ± 4.7	61.5 ± 3.4
Urea *mM*	6.7 ± 1.2	7.5 ± 1.2	22.7 ± 1.2[b]	25.0 ± 7.7[b]
Creatinine *μM*	35 ± 6	55 ± 4[a]	115 ± 6[b]	129 ± 37[b]
Total calcium *mM*	2.69 ± 0.11	1.85 ± 0.45[a]	2.58 ± 0.19	2.53 ± 0.18
Ionized calcium *mM*	1.30 ± 0.09	1.01 ± 0.16[a]	1.24 ± 0.07	1.10 ± 0.08[c]
Phosphate *mM*	2.82 ± 0.14[a]	3.37 ± 0.48[a]	2.23 ± 0.26	2.03 ± 0.24
Magnesium *mM*	0.75 ± 0.05	0.62 ± 0.05	0.73 ± 0.05	0.80 ± 0.05
riPTH-(1-34) *pg/ml*	21.7 ± 7.5[a]	10.1 ± 7.8	41.8 ± 29.4[a]	8.0 ± 3.8
Calcitriol *pg/ml*	73.7 ± 12.6[a]	19.3 ± 8.2	22.1 ± 13.8	4.4 ± 2.3[a]

Abbreviations are: riPTH-(1-34), rat immunoreactive parathyroid hormone fragment (1-34). Results are expressed as mean ± SD.
[a] $P < 0.01$ compared to the three other groups
[b] $P < 0.001$ compared to control and TPTX groups
[c] $P < 0.02$ compared to CRF

Table 2. Urine biochemistry

	Control ($N = 7$)	TPTX ($N = 9$)	CRF ($N = 6$)	CRF+TPTX ($N = 8$)
Urine volume *ml/24 hr/100 g body wt*	5 ± 1	5 ± 2	14 ± 3[b]	10 ± 3[b]
Creatinine *μmol/24 hr/100 g body wt*	37.3 ± 7.4	40.7 ± 14.8	42.6 ± 6.2	37.8 ± 5.7
Urea *mmol/24 hr/100 g body wt*	4.2 ± 1.1	4.6 ± 1.7	5.0 ± 2.1	5.0 ± 1.4
Calcium *μmol/24 hr/100 g body wt*	27 ± 10	23 ± 18	29 ± 12	63 ± 26[a]
Phosphate *μmol/24 hr/100 g body wt*	20 ± 10	10 ± 7	100 ± 50[a]	40 ± 10[a]
Magnesium *μmol/24 hr/100 g body wt*	3 ± 2[a]	21 ± 13[a]	41 ± 12	55 ± 18

Results are expressed as mean ± SD.
[a] $P < 0.01$, compared to three groups
[b] $P < 0.001$, compared to control and TPTX groups

Table 3. Calculations

	Control ($N = 7$)	TPTX ($N = 9$)	CRF ($N = 6$)	CRF+TPTX ($N = 8$)
Creatinine *μl/min/100 g body wt*	646 ± 123[a]	511 ± 156[a]	259 ± 40	212 ± 45
FE calcium $C_{ca}/C_{Cr} \times 100$	1.0 ± 0.3	1.7 ± 0.9	3.1 ± 1.1[a]	8.1 ± 2.3[a]
FE phosphate $C_{PO_4}/C_{Cr} \times 100$	0.8 ± 0.5[a]	0.2 ± 0.1	13.8 ± 7.2[a]	6.9 ± 2.2[a]
FE magnesium $C_{Mg}/C_{Cr} \times 100$	0.3 ± 0.2[a]	6.9 ± 2.8[a]	16.3 ± 11.2	21.4 ± 7.1

Abbreviations are: FE, fractional excretion; C_{Ca}, calcium clearance; C_{Cr}, creatinine clearance; C_{PO_4}, phosphate clearance; C_{Mg}, magnesium clearence.
[a] $P < 0.01$, compared to three groups. Results are expressed as mean ± SD.

kinase [24]. The abundance of PTH-R, β-actin, and 28S RNAs was quantified by densitometric analysis (Scan Analysis, Biosoft, UK). The PTH-R mRNA expression was normalized to the densitometric values obtained for the β-actin probe and for the 28S ribosomal RNA. Results are expressed as the ratio PTH-R/β-actin mRNA, and PTH-R/28S ribosomal RNA.

Slot blot hybridization

Total RNA prepared from the kidney of control and CRF animals was loaded on each slot, denatured and fixed on a nitrocellulose membrane as described by Kafatos, Jones and Efstratiadis [25]. The filter was prehybridized for three to four hours as described above and then hybridized with a 1,087 bp fragment of the rat PTHrP cDNA provided by Dr. A.C. Karaplis (McGill University, Canada) [26], and with the previously described 28S ribosomal RNA probes [24], respectively. The autoradiograph was developed after a 10-day exposure with an intensifying screen at −80°C for the PTHrP mRNA and only 15 minutes for the 28S ribosomal RNA. The PTHrP mRNA expression was normalized to the densitometric values obtained for the 28S ribosomal RNA. Results are expressed as the ratio PTHrP/28S ribosomal RNA.

Statistical analysis

Results have been expressed as means ± SD and statistical significance determined by the unpaired Student's *t*-test or ANOVA test, as appropriate. *P* values less than 0.05 were considered significant.

Results

Plasma biochemistry

Table 1 shows the results of plasma biochemistry. Mean (± SD) plasma creatinine levels were higher in CRF and CRF+TPTX than in control and TPTX rats even though their body weight remained comparable. Mean blood ionized calcium was significantly lower in TPTX, CRF, and CRF+TPTX than in control animals. At this degree of renal insufficiency, plasma phosphorus was lower in CRF and CRF+TPTX than in control rats. TPTX animals had the highest plasma phosphorus levels. There was no significant difference in total plasma magnesium in the four groups. Plasma riPTH levels were significantly higher in CRF than in rats with normal renal function and significantly lower in TPTX and CRF+TPTX rats than in rats with intact parathyroid glands. Mean plasma calcitriol concentration was significantly lower in CRF, TPTX, and CRF+TPTX than in control rats. Even though there was no significant difference in the calculated protein intake among the four groups, total plasma protein concentration was higher in TPTX rats than in other groups.

Urine biochemistry and calculations

Twenty-four-hour urine volume was two to three times higher in CRF and CRF+TPTX, than in control and TPTX animals, respectively (Table 2). Daily urinary excretion of creatinine and urea were comparable in four groups (Table 2). Mean creatinine clearances were significantly lower in CRF and CRF+TPTX rats than in control and TPTX rats, respectively (Table 3). Total and fractional urinary calcium excretion were significantly higher in CRF+TPTX rats than in control, TPTX, and CRF rats (Tables 2, 3 and Fig. 1). Total and fractional urinary phosphate excretion were significantly higher in CRF rats than in control, TPTX, and CRF+TPTX rats (Tables 2, 3 and Fig. 1). Total and fractional urinary magnesium excretion were significantly higher in CRF+TPTX rats than in control, TPTX, and CRF rats (Tables 2, 3 and Fig. 1).

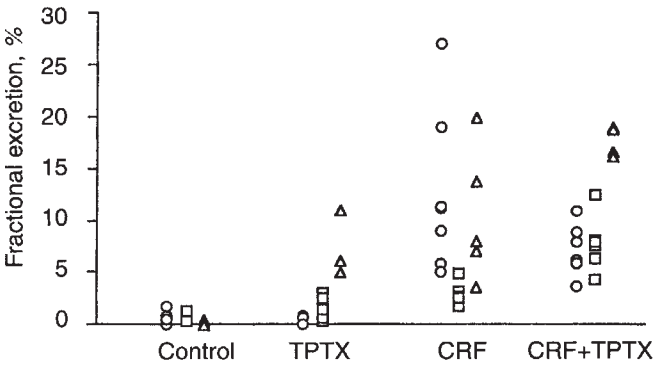

Fig. 1. *The fractional excretion (× 100) of calcium (□), phosphate (○), and magnesium (△) in the four groups of rats.*

Adenylyl cyclase activity

Basal adenylyl cyclase activities in crude renal membrane preparations were not significantly different in the four groups of animals (Table 4). However, the adenylyl cyclase activity was reduced in rats with CRF and CRF+TPTX as compared with normal and TPTX rats after stimulation by NaF, forskolin, and PTH, respectively. TPTX rats had a significantly higher PTH-stimulated adenylyl cyclase activity than the other three groups of animals (Table 4).

PTH-R mRNA

The level of the steady-state PTH-R mRNA in the kidney of CRF and CRF+TPTX rats was markedly decreased compared with that of control rats, the ratio PTH-R mRNA/β-actin mRNA being 0.28 ± 0.04, and 0.27 ± 0.03 versus 0.54 ± 0.05, respectively, and the ratio PTH-R mRNA/28S being 0.30 ± 0.05 and 0.33 ± 0.09 versus 0.57 ± 0.05, respectively (Fig. 3). No change was observed in the renal level of PTH-R mRNA in TPTX animals (Fig. 2). The ratio PTH-R mRNA/β-actin mRNA in control rats was 0.90 ± 0.04 and in TPTX rats 0.90 ± 0.05 (Fig. 2). There was a significant reduction in the PTH-R mRNA expression in the bone tissue of two uremic rats: the ratio PTH-R mRNA/28S was 0.78 in control animals versus 0.59 in CRF animals (Fig. 4). This PTH-R down-regulation seems to be specific for the remnant kidney and for the bone since no significant change was observed in the PTH-R mRNA expression in other organs including the heart (data not shown) and the liver. In the liver the ratio PTH-R mRNA/β-actin mRNA was 3.06 in a control rats versus 2.99 in uremic rats (Fig. 5).

Slot blot hybridization

Figure 6 shows slot blot analysis of renal tissue from control (1 to 4) and uremic animals (CRF, 5 to 8). No significant difference was found in the renal expression of PTHrP mRNA between control and uremic rats. The ratio PTHrP mRNA/28S ribosomal RNA was 0.50 ± 0.06 versus 0.41 ± 0.02 in control and CRF rats, respectively (Fig. 6).

Discussion

The present study demonstrates that the reduction of plasma PTH concentration after TPTX to levels near zero did not prevent the decrease in the renal PTH-R expression induced by chronic renal failure. It also shows that despite reduced PTH-R expression, uremic rats with intact parathyroid glands and secondary hyperparathyroidism had lower urinary calcium and magnesium excretion and higher phosphate excretion than uremic TPTX animals. This suggests that at this stage of moderate-degree uremia PTH still exerts a powerful action regulating mineral ion metabolism through the remaining PTH-R in the kidney.

Previous *in vitro* studies [12, 27–29] and *in vivo* studies in rats with normal renal function [30, 31] have illustrated that excess of endogenous plasma PTH or pre-treatment with PTH reduced the PTH-R number, as assessed by radioreceptor assays, and partially desensitized the PTH-stimulated adenylyl cyclase activity and the calcium messenger system [29]. However, it is still ill understood whether the desensitization to PTH is due to decreased PTH-R number and/or to post-receptor events, such as receptor internalization, sequestration, degradation, and phosphorylation or uncoupling from transducing G proteins. Neither is it known whether the reduced PTH-R number in these studies was the consequence of pre- or post-transcriptional PTH-R gene regulation. There are several observations supporting a negative regulatory effect of PTH on the PTH-R mRNA. First, when cultured rat (ROS 17/2.8) or human osteoblastic-like cells (SaOS-2) were treated with PTH, a moderate reduction in the level of the steady-state PTH-R mRNA has been observed after two days of treatment [12, 32]. However, its expression tended to normalize toward the sixth day, despite the continuous treatment with PTH. Second, PTH blocked the increase in PTH-R mRNA induced by dexamethasone in ROS 17/2.8 cells [12]. Third, we have observed only a transient decrease in the renal PTH-R mRNA in an *in vivo* model of phosphate-induced acute hypocalcemia and increased plasma PTH levels (personal unpublished results).

In spite of all this evidence, the results obtained in the present study indicate that the renal PTH-R down-regulation during chronic renal failure requires other factors than solely high plasma PTH levels. This is in accord with the recent observation

Table 4. Adenylyl cyclase activity expressed as absolute values (pmol of cAMP/30 min at 37°C/μg of protein) and values relative to the basal activity

	Control ($N = 7$)	TPTX ($N = 6$)	CRF ($N = 6$)	CRF+TPTX ($N = 6$)
Absolute values *pmol of cAMP/ 30 min at 37° C/μg of protein*				
Basal	18.1 ± 2.0	13.2 ± 3.0	18.3 ± 4.6	14.9 ± 4.4
NaF	192.0 ± 56.7	163.5 ± 53.3	107.8 ± 11.3[a]	111.7 ± 8.7[a]
Forskolin	139.5 ± 31.3	113.7 ± 30.4	90.4 ± 26.4[a]	70.6 ± 12.6[a]
PTH	81.7 ± 9.0	98.5 ± 7.9	61.7 ± 8.6[a]	48.4 ± 22.2[a]
Relative values				
Basal	1	1	1	1
NaF	10.6 ± 3.1	9.2 ± 6.5	5.8 ± 6.2[a]	7.1 ± 1.1[a]
Forskolin	7.7 ± 1.7	8.6 ± 2.3	4.9 ± 1.4[a]	4.7 ± 0.8[a]
PTH	4.2 ± 0.4	7.4 ± 0.6	3.6 ± 1.0[a]	3.2 ± 1.4[a]

Seventy-five micrograms of protein from the renal cortex membrane preparation was incubated for 30 min at 37°C with vehicle (acetic acid 10 mM), 10 mM NaF, 1 mM forskolin, or 1 μM (1-34) rat PTH, in presence of 1 mM IBMX. Abbreviations are: CRF, chronic renal failure; NaF, sodium fluoride; TPTX, thyroparathyroidectomy. Values are expressed as means ± SD.

[a] $P < 0.05$, compared to control and TPTX groups.

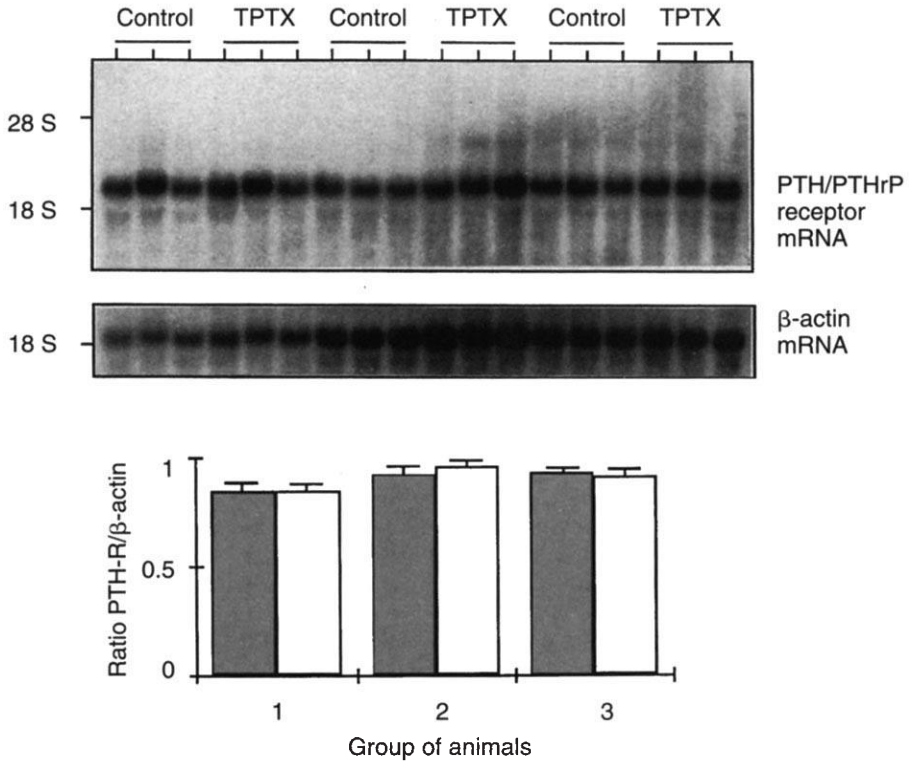

Fig. 2. *The upper panel shows Northern blot analysis of kidney tissue from 3 representative control and 3 thyroparathyroidectomized (TPTX) rats.* Twenty micrograms of total RNA were loaded in each lane, transferred to a nitrocellulose membrane and hybridized with the rat PTH/PTHrP receptor probe. The autoradiograph was developed after a 7-day exposure with an intensifying screen at −80°C. The lower panel shows the results of the hybridization of the same filter with the β-actin probe after a 2-day exposure. The bar graph shows the densitometric values obtained for the PTH/PTHrP receptor and for β-actin hybridizations, expressed as the ratio PTH/PTHrP receptor/β-actin in the three control (□) and TPTX (▨) rats.

that the reduction of increased plasma PTH to normal levels did not improve the blunted calcemic response to PTH in azotemic animals [33]. Our results also rule out a direct role of plasma calcium or magnesium concentrations in the PTH-R down-regulation. Plasma levels of these ions in uremic rats were not significantly different from those of control animals. It is also unlikely that plasma phosphate concentration plays a role in the receptor down-regulation. CRF rats of the present study had an even lower plasma phosphate level than control rats, in contrast to the marked diet-induced hyperphosphatemia in the CRF rats of our previous study [10]. Furthermore, the high plasma phosphate levels observed in the TPTX group with intact kidneys and no receptor down-regulation strengthen our conclusion that hyperphosphatemia has no influence on the renal PTH-R mRNA.

Even though no direct evidence is available, one could hypothesize that plasma calcitriol concentration is an important factor regulating the PTH-R expression in the kidney of uremic animals. A recent *in vitro* study in mouse renal distal tubular cells has shown that 1,25 $(OH)_2D_3$ up-regulated the PTH-R mRNA [34]. Moreover, it has been demonstrated that the impaired calcemic response to PTH in humans and animals could be reversed by the administration of 1,25 $(OH)_2D_3$ [35]. Nevertheless, it should be noted that the level of PTH-R mRNA was normal in the kidney of TPTX rats which had a decrease in plasma calcitriol level similar to that of CRF rats, compared with control animals. Therefore, whether the correction of the diminished plasma 1,25 $(OH)_2D_3$ concentration in uremic animals has a positive effect on renal PTH-R expression remains to be determined. At this degree of renal failure and despite the high PTH levels, CRF rats could not maintain normal plasma calcitriol concentrations, probably because of either the reduced renal mass, renal PTH-R down-regulation, or uremic substances [36, 37] inhibiting PTH-stimulated 1α hydroxylation of 25 OH D_3. Thus, it is possible that the PTH-R down-regulation in the kidney contributes to calcitriol deficiency and initiates secondary hyperparathyroidism even in the absence of hypocalcemia or hyperphosphatemia.

Local factors could also be involved in the renal receptor down-regulation. A recent observation in rats pointed out that acute renal ischemia resulted in increased local secretion of PTHrP and decreased levels of PTH-R mRNA in the kidney [38]. It has also been observed that PTHrP reduces the PTH-R number and desensitizes the PTH-stimulated adenylyl cyclase activity in ROS 17/2.8 cells [28]. Therefore, it could be possible that high concentrations of locally-produced PTHrP could favor the interaction with the PTH-R and induce its down-regulation. However, our findings demonstrating no change in the renal PTHrP mRNA expression in uremic rats did not support this hypothesis. Several cytokines have also been demonstrated to modulate the PTH-R number. For instance, TNFα and interleukin-1 down-regulated the PTH-R in osteoblast-like cells [39, 40]. Thus, it is possible that high circulating levels of cytokines, which have previously been demonstrated to occur in chronic renal failure [41], or locally produced cytokines contribute to the PTH-R down-regulation. Cytokine-induced PTHrP production may also be implicated in receptor down-regulation [42, 43]. In addition, several peptides such as angiotensin II are capable of diminishing the PTH-R mRNA level in cultured smooth muscle cells [44, 45]. High concentration of angiotensin II has been found in glomerular filtrate, star vessel plasma and the lumen of the proximal tubule of uremic animals [46, 47]. Local overproduction or deficiency of other growth factors could also be theoretically involved.

In the present study we also confirm our previous observation

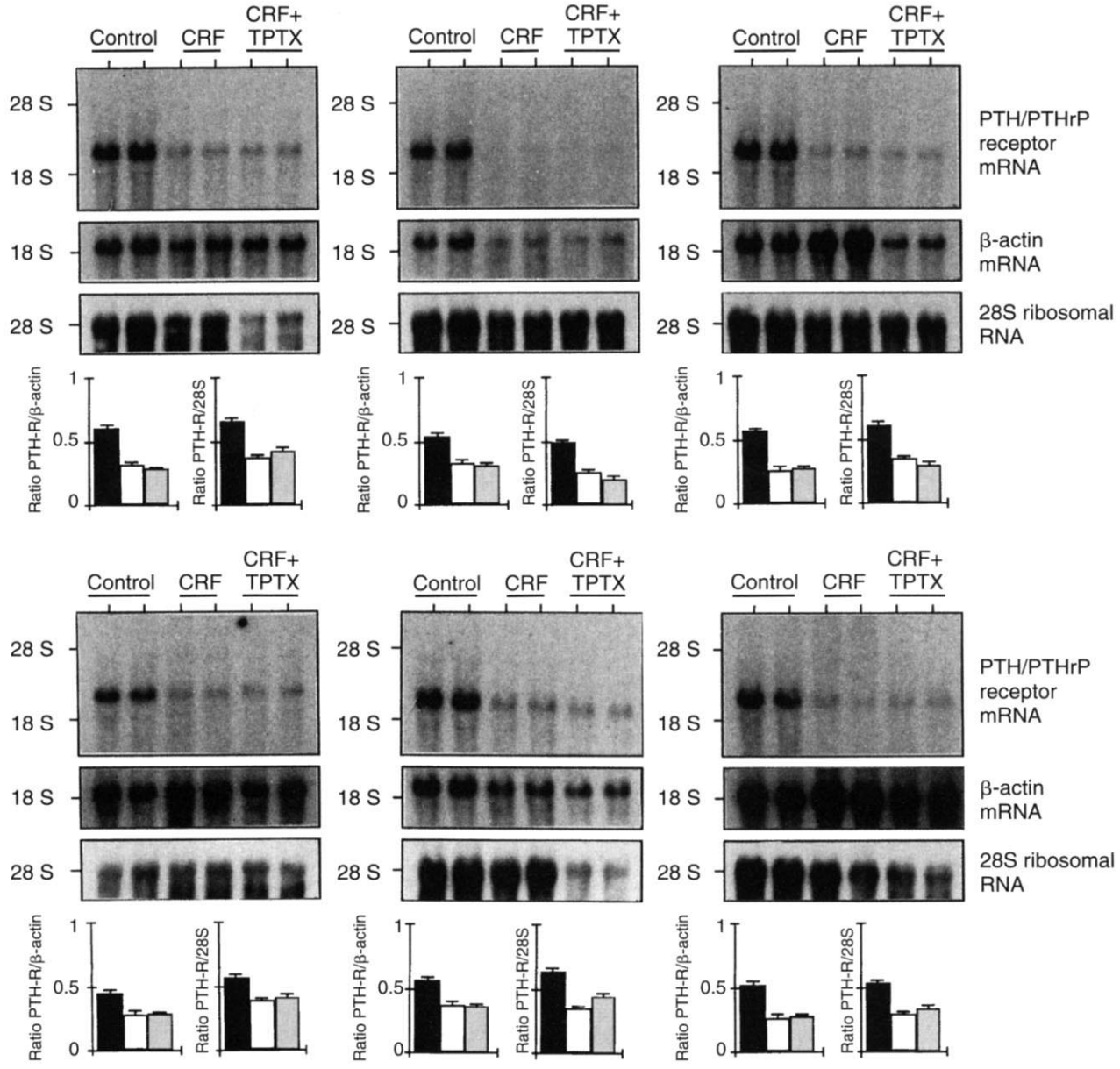

Fig. 3. *Northern blot analysis of kidney tissue from 6 pairs of control, uremic (CRF), and CRF+TPTX animals.* Twenty micrograms of total RNA were loaded in each lane, transferred to a nitrocellulose membrane and hybridized with the rat PTH/PTHrP receptor, β-actin, and 28S ribosomal RNA probes, respectively. The corresponding bar graphs show the densitometric values obtained for the PTH/PTHrP receptor, β-actin, and 28S ribosomal RNA hybridizations, expressed as the ratio PTH/PTHrP receptor/β-actin and PTH/PTHrP receptor/28S ribosomal RNA in the control (■), CRF (□), and CRF+TPTX (▨) rats.

demonstrating a decreased PTH-stimulated adenylyl cyclase activity in renal membranes of azotemic rats [10]. However, we did not observe any difference in the basal activity between normal, uremic, and TPTX animals. This may be explained either by the moderate degree of chronic renal failure or by the lower plasma concentrations of phosphorus and PTH in the present animals. Although the renal PTH-R mRNA was normally expressed, PTH-stimulated adenylyl cyclase activity was significantly higher in TPTX animals compared with normal and azotemic rats. This could be due to a phenomenon of hypersensitization following hormone depletion as described for other hormonal systems [48].

Concerning the effects of the renal PTH-R down-regulation on mineral ion metabolism and its possible role in the development of secondary hyperparathyroidism, we observed that even though the renal PTH-R in CRF rats with intact PTH glands was down-regulated, these animals were normocalcemic and capable of reabsorbing the filtered urinary calcium and magnesium to a much greater extent than CRF+TPTX rats. The hypocalcemia and the marked increase in absolute and fractional urinary calcium and magnesium excretion found in CRF+TPTX animals suggest that in rats with moderate chronic renal failure having intact PTH glands the remaining renal PTH-R still responded to high plasma PTH levels activating para- or intracellular mediators of tubular calcium and magnesium reabsorption. In contrast, the

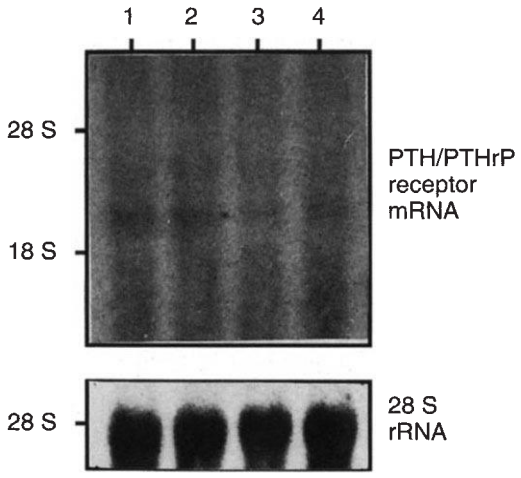

Fig. 4. *Northern blot analysis of bone tissue from control (lane 1 and 2) and from uremic (lane 3 and 4).* Twenty micrograms of total RNA, extracted from the femoral head, were loaded in each lane, transferred to a nitrocellulose membrane and hybridized with the rat PTH/PTHrP receptor, and with the 28S ribosomal RNA probes, respectively.

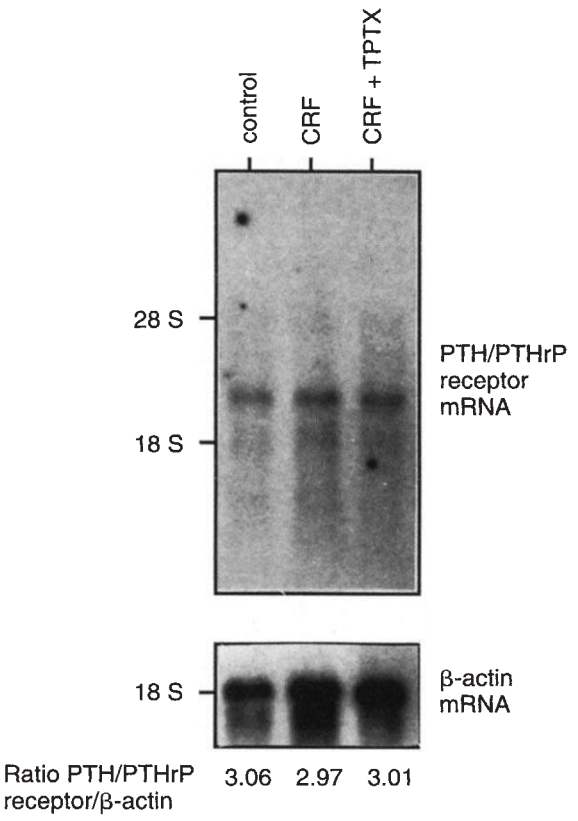

Fig. 5. *Northern blot analysis of liver tissue from control (lane 1), uremic (CRF, lane 2), and CRF+thyroparathyroidectomized (CRF+TPTX, lane 3) animals.* Five micrograms of poly $(A)^+$ RNA were loaded in each lane, transferred to a nitrocellulose membrane and hybridized with the rat PTH/PTHrP receptor, and with β-actin probes, respectively.

tubular reabsorption of these two ions, which was already altered due to CRF, was further diminished by TPTX. Interestingly, plasma magnesium levels of CRF+TPTX rats did not differ significantly from those of controls and CRF rats, while their absolute urinary magnesium excretion was twenty times higher, and their fractional excretion hundred times higher than that of control rats. This may indicate that urinary magnesium excretion is even more tightly controlled by PTH and/or vitamin D than urinary calcium excretion. The normal plasma magnesium concentration in the state of PTH and vitamin D deficiency suggests that other mechanisms are responsible for maintaining its plasma concentration within rather narrow limits.

As discussed above, hypophosphatemia was found in this model of moderate CRF in spite of PTH-R down-regulation in the kidney. It is generally accepted that the absence of hyperphosphatemia in early renal failure does not necessarily indicate an altered phosphate metabolism. Normal or even low plasma phosphorus levels are often found at this degree of renal failure, probably due to high plasma PTH concentrations [49] or to uremic phosphaturic factors [4, 50]. The presence of such uremic phosphaturic factors may be suggested by the observation that the fractional excretion of phosphate remained high in CRF+TPTX rats. Taken together, these results make the hypothesis difficult to accept that hyperphosphatemia *per se* is a major factor in the early development of uremic secondary hyperparathyroidism.

The finding of a down-regulation of the PTH-R mRNA in bone tissue and not in the liver and in the heart suggests that the PTH-R expression is regulated in a cell specific manner regardless of the uremic state. Accordingly, the *in vivo* tissue-specific regulation of the PTH-R observed in the present study supports our prior *in vitro* observation showing that glucocorticoids up-regulated the PTH-R in osteoblastic cells (ROS 17/2.8) whereas they down-regulated it in renal cells (OK) [12]. Both, the reduced PTH-R mRNA in the kidney and in bone may be the mechanisms responsible for the blunted calcemic response to PTH in azotemic animals.

In conclusion, our data demonstrate that neither elevated plasma PTH and phosphate nor decreased plasma calcium are important factors in renal PTH-R down-regulation during chronic renal failure. It is also unlikely that an increase in the locally-produced renal PTHrP could down-regulate its own receptor. They also demonstrate that despite this down-regulation, the kidney of uremic rats is still capable of responding to high plasma PTH concentrations in a compensatory manner. There is no hyperphosphatemia secondary to renal PTH-R down-regulation in the initial stage of chronic renal failure. Therefore, an extracellular phosphate retention cannot be considered as a major factor in the early development of secondary hyperparathyroidism.

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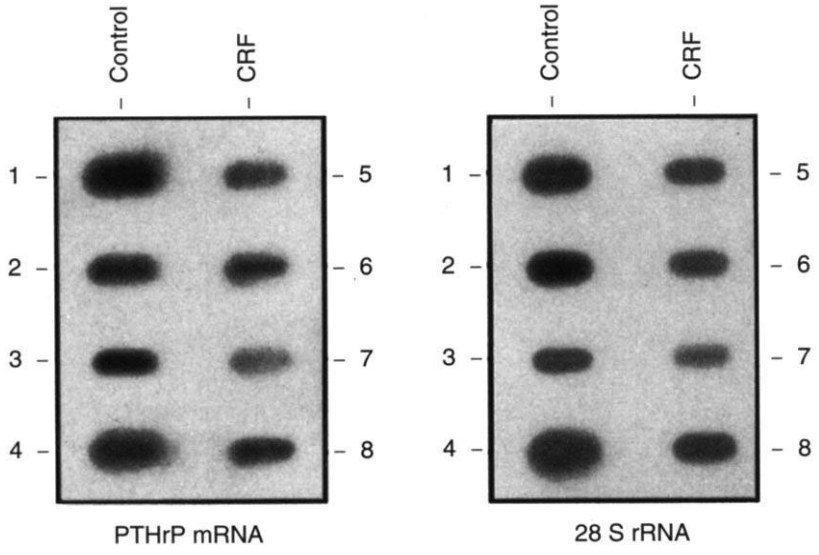

Fig. 6. *Slot blot analysis of renal tissue from control (1 to 4) and uremic animals (CRF, 5 to 8).* Sixty micrograms of total RNA were loaded on each slot, denatured and fixed on a nitrocellulose membrane. The filter was hybridized with the rat PTHrP cDNA, and with the 28S ribosomal RNA probes, respectively. The autoradiograph was developed after a 10-day exposure with an intensifying screen at −80°C for the PTHrP messenger RNA and only after 15 min for the 28S ribosomal RNA.

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