Long-Term Effects of Recombinant Human Erythropoietin Therapy on Growth Hormone Secretion in Uremic Patients Undergoing Peritoneal Dialysis

Juan J. Díez, Pedro Iglesias, Julia Sastre, Abelardo Aguilera, María A. Bajo, Javier Méndez, Antonio Gómez Pan, and Rafael Selgas

Recombinant human erythropoietin (rhEPO) is being successfully used for the treatment of uremic anemia. Short-term studies have proved that correction of anemia with rhEPO therapy is accompanied by several changes in growth hormone (GH) secretion in uremic patients. The present study aimed to assess the influence of long-term rhEPO therapy on baseline and stimulated GH concentrations in a group of uremic patients undergoing continuous ambulatory peritoneal dialysis (CAPD). Seven well-nourished and clinically stable CAPD patients were studied. Ten normal subjects were studied as controls. GH responses to direct pituitary stimulation with GH-releasing hormone (GHRH) (100 μg intravenously [IV]) and indirect hypothalamic stimulation with insulin-induced hypoglycemia (0.1 U/kg body weight IV) and clonidine (0.15 mg/m² orally), were assessed before and after 3, 6, and 12 months of subcutaneously administered rhEPO therapy. After rhEPO administration, an increase of the hemoglobin concentration was observed in all patients and maintained at about 12 g/dL throughout the study period. rhEPO therapy did not induce any significant change in baseline concentrations of GH and insulin-like growth factor I. Correction of the anemia was accompanied by a clear increase in the area under the curve (AUC) and the area above the baseline (AAB) of GH secretion in response to GHRH stimulation. These changes were statistically significant after 3 and 6 months of therapy, although at 12 months no significant differences in relation to pretreatment values could be observed. rhEPO treatment was associated with a progressive decrement in the GH AUC and AAB in response to hypoglycemic challenge, reaching statistically significant values at months 6 and 12. On the other hand, compared with the control group, GH responses to clonidine were blunted at the start of the study in CAPD patients, and rhEPO therapy was not accompanied by any modification. In conclusion, long-term treatment with rhEPO in CAPD patients is associated with complex and profound effects on somatotrope cell function, characterized by diverse effects on GH responses to stimuli that release GH through different mechanisms. Some of these rhEPO-induced alterations in somatotrope function are dependent on the duration of treatment.

SINCE THE END of the 1980s, recombinant human erythropoietin (rhEPO) has been used with unquestionable clinical success for the treatment of anemia in patients with chronic renal insufficiency.[1,2] Correction of anemia with rhEPO therapy has been accompanied not only by increasing blood hemoglobin concentrations but also by an improvement in physical and mental activity, amelioration in exercise tolerance, increased appetite, and improved sense of well-being, suggesting the existence of some extrahematologic effects of this protein.[2,3] Several lines of evidence have suggested that long-term therapy with rhEPO is associated with changes in hypothalamic-hypophyseal function in patients with end-stage renal disease. Various studies have demonstrated improvement in sexual performance,[4-6] as well as modifications in baseline and stimulated pituitary hormone concentrations. As a matter of fact, a normalization of prolactin concentrations,[4,5,7-9] an increase in testosterone in males,[8,10,11] a decrease in baseline levels of follicle-stimulating hormone (FSH) and luteinizing hormone,[8,10] a restoration of FSH responses to gonadotropin-releasing hormone,[7] an increase in total and free thyroid hormone concentrations,[12] and normalization of the response of thyrotropin to thyrotropin-releasing hormone (TRH)[7] have been reported as endocrine changes accompanying rhEPO therapy in uremic patients.

Concerning growth hormone (GH) secretion, most studies have been performed in patients treated with rhEPO for less than 6 months. Ramírez et al[13] reported that the basal elevation of GH was normalized in a group of five hemodialysis patients after correction of anemia with rhEPO therapy for 10 weeks. In these patients, the exaggerated response of GH to GH-releasing hormone (GHRH) remained after this therapy. However, a significant potentiation of the GH response to GHRH after 3 months of rhEPO administration has been found in a group of eight hemodialysis patients studied by Cremagnani et al[14] and a group of nine patients undergoing continuous ambulatory peritoneal dialysis (CAPD) studied by our group.[15] The paradoxical response of GH to TRH that is frequently associated with uremia has been reported to be abolished after correction of the anemia with rhEPO therapy.[7] Moreover, Kokot et al[16] reported a significant reduction in basal GH and the GH response to hypoglycemia in a group of five hemodialysis patients treated for 3 months with rhEPO. However, no effect of rhEPO treatment on the GH response to clonidine, an α_2-adrenergic receptor agonist, was observed in our preliminary study in CAPD patients.[15]

Long-term studies designed to evaluate the influence of long-term rhEPO therapy on somatotrope function have been few and were limited to hemodialysis patients. Carlson et al[17] found a modest decrease in basal GH concentrations in 21 hemodialyzed men treated with rhEPO for 8 months. Steffensen and Aunsholt[18] found a significant increase in GH levels in a group of 10 hemodialysis patients treated for 6 months, although another group of 10 patients did not show any significant change. In the longest study performed so far, Kokot

From the Departments of Endocrinology, Biochemistry, and Nephrology, Hospital La Paz, Madrid, Spain.

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Address reprint requests to Juan J. Díez, MD, Travesía Tellez 8, 4R, 28007 Madrid, Spain.

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et al[19] reported that basal plasma GH levels showed a significant decline from a mean value of 17.2 μg/L before therapy to 9.0 μg/L after 9 months of rhEPO therapy, although there was an increase to pretreatment values at month 12. Notwithstanding this, in none of these long-term studies were dynamic tests of GH secretion performed.

Based on this background, the present study was performed with the aim of evaluating the influence of long-term therapy with rhEPO on different parts of the GH axis in uremic patients undergoing CAPD. We chose a GHRH stimulation test to study GH responses to direct pituitary stimulation, insulin-induced hypoglycemia to study GH release after indirect stimulation by reduction of the endogenous somatostatinergic tone, and clonidine to evaluate GH secretion after increasing endogenous GHRH secretion. Therefore, GH responses to stimulation with GHRH, insulin, and clonidine were evaluated before and during a 1-year period of subcutaneously administered rhEPO treatment.

SUBJECTS AND METHODS

Patients

We studied the evolution of hematologic and GH secretion parameters over 12 months of rhEPO therapy in seven patients (six women and one man; median age, 56 years; range, 30 to 67) with chronic renal failure who were enrolled in a CAPD program at the peritoneal dialysis unit of Hospital La Paz. The median duration of CAPD was 12 months. Three patients had end-stage renal disease because of chronic glomerulonephritis, two had chronic pyelonephritis, one had hypertensive nephrosclerosis, and a further one had renal failure of unknown etiology. Ten healthy subjects (eight women and two men; median age, 56.5 years; range, 35 to 63) with normal renal function (median plasma creatinine, 0.9 mg/dL (0.8 to 1.2); median hemoglobin concentration, 13.6 g/dL (11.9 to 15.1) were studied as controls. There were no statistically significant differences between patients and control subjects in age, sex, and body mass index. The study was approved by the local ethics committee, and informed consent was obtained from all study participants before testing.

All patients were clinically stable and did not receive any medication known to affect GH secretion. They performed three or four 2-L bag exchanges per day. Dialysis solutions with 1.5% to 3 86% dextrose were used. The median weekly glucose load was 1,050 g (770 to 1,356) and this load did not show clinically important variations throughout the study period (Table 1). All patients received aluminum hydroxide and water-soluble vitamins. None had diabetes mellitus or other endocrine disorder. No patients had clinical signs of severe secondary hyperparathyroidism, and liver function tests were normal in all of them. Four patients were hypertensive and were treated with calcium channel blockers or angiotensin-converting enzyme inhibitors with adequate control of blood pressure. All patients were on a stable diet containing about 35 kcal/kg/d and about 1 g/kg high–biological value protein per day, with no important modification during the study period.

Endocrine Tests

In every patient, three endocrine studies were performed in random order 3 days apart after an overnight fast. Tests began at 8:30 AM with the subjects recumbent. An indwelling catheter was placed in a forearm vein and kept patent with a slow infusion of 0.9% NaCl. Thirty minutes later, the first blood sample was collected. Two basal blood samples were obtained at a 30-minute interval, and stimuli were applied at 0 minutes. Synthetic GHRH (GHRH 1-29; Geref, Serono, Spain) 100 μg intravenous (IV) bolus was administered to each patient. Insulin-induced hypoglycemia (Actrapid; Novo-Nordisk, Copenhagen, Denmark; 0.1 U/kg IV bolus) and clonidine (Catapresan; Boehringer, Ingelheim, Germany; 0.15 mg/m^2 orally) were used as indirect stimuli for GH release. Blood samples were collected at −30, 0, 15, 30, 60, 90, and 120 minutes. For all blood samples, plasma GH concentrations were assessed. In the insulin-induced hypoglycemia tests, plasma glucose concentrations were also determined. At the time 0 sample of one of the provocative tests, the following hormone levels were measured: insulin-like growth factor-I (IGF-I), free thyroxine, thyrotropin, and prolactin.

rhEPO Protocol

Prestudy hematocrits for all dialysis patients were less than 30%. rhEPO was administered subcutaneously two or three times per week. The dosage was titrated to the needs of each patient to maintain hematocrit values between 30% and 40%, to a maximum of 12,000 U/wk. GHRH, insulin-induced hypoglycemia, and clonidine tests were performed once in the control group, whereas dialysis patients were studied before starting rhEPO therapy and again after 3, 6, and 12 months of treatment. At the same time the endocrine tests were performed, we also assessed clinical data and analytical parameters. The following tests were used: blood hemoglobin concentration, hematocrit value, and serum concentration of urea, creatinine, albumin, total proteins, sodium, potassium, total calcium, phosphorus, cholesterol, triglycerides, ferritin, and transferrin. Urea kinetics were assessed by

Table 1. Evolution of Hematological, Nutritional, and Dialysis Parameters During rhEPO Therapy

Parameter	Basal		Month 3		Month 6		Month 12	
	Median	Range	Median	Range	Median	Range	Median	Range
rhEPO dose (U/kg/wk)		0	90	60.6-120	64.5	38.1-146.3	65.1	31.7-142
Hematocrit (%)	28	25.6-29.6	37.8†	30.2-41.4	36.7†	30.3-44.1	38.25†	33.9-40.8
Hemoglobin (g/dL)	9.0	8.3-9.9	12.7†	10.5-13.5	12.3†	10.5-14.9	12.1†	11.0-13.8
Weight (kg)	61	44-76	62	45-78	62	48-82	61.5	47.5-84
Body mass index (kg/m^2)	23.1	19.3-34.2	23.1	19.7-35.1	24.4	20.3-36.9	23.3	20.3-37.8
Albumin (g/L)	40.0	35-45	39	31-45	39	26-45	42.3	38-43
Transferrin (mg/dL)	261	197-358	260	134-318	272	175-328	270	212-371
Ferritin (ng/mL)	213	34.9-387	148	54.6-524	121	46-274	120	58-292
Cholesterol (mg/dL)	241	176-279	203*	160-233	216	119-269	201*	167-227
Triglycerides (mg/dL)	201	119-330	173	68-224	160	70-344	179	110-296
Weekly urea Kt/V	2.15	1.7-3.0	2.0	1.8-2.9	2.3	1.6-2.8	2.1	1.6-2.7
Normalized protein catabolic rate (g/kg)	1.086	0.889-1.300	1.076	0.960-1.454	1.076	0.680-1.454	1.070	0.911-1.476
Glucose load (g/wk)	1,050	770-1,356	910	576-1,340	1,120	770-1,480	1,140‡	910-1,200

*$P < .05$, †$P < .001$ *v* basal.

‡$P < .05$ *v* month 3.

the weekly urea Kt/V and the normalized protein catabolic rate. One patient did not complete this protocol because of transfer to hemodialysis therapy after month 6. In another patient, endocrine tests could not be performed at month 6, but they were performed at month 12. The insulin-induced hypoglycemia test was not performed in one of the patients; therefore, for this test, the number of evaluated patients was six at baseline and month 3 and five at months 6 and 12.

Blood Analysis and Hormone Assay

Blood samples were centrifuged immediately, and the plasma was stored at −20°C until assay. Blood hemoglobin and hematocrit values were measured in a Coulter counter (Technicon H3RTX, Bayer, Germany), and serum chemistry determinations were made using an automated multichannel analyzer. Urea kinetic parameters (urea Kt/V and normalized protein catabolic rate) were calculated according to previously described methods.[20] Human plasma GH concentrations were determined using an automated immunoenzymatic assay (AIA 1200; Tosoh, Tokyo, Japan). Maximal intraassay and interassay coefficients of variation for GH were 5.4% and 3.3%, respectively. The sensitivity of the GH assay was 0.1 μg/L. Plasma thyrotropin and prolactin concentrations were also determined using the Tosoh immunoenzymatic assay. For thyrotropin assay, the sensitivity was 0.06 μU/mL and the maximal intraassay and interassay coefficients of variation were 3.3% and 3.4%, respectively. For prolactin assay, the sensitivity and maximal intraassay and interassay coefficients of variation were, respectively, 1 μg/L, 6%, and 4.5%. IGF-I concentrations were determined using commercially available radioimmunoassay kits (Nichols Institute, San Juan Capistrano, CA) after extraction by acid-ethanol precipitation. Maximal intraassay and interassay coefficients of variation were 3.0% and 8.4%, respectively, and the sensitivity of the assay was 13.5 μg/L. A heterogeneous competitive immunoassay (Immuno 1 System; Miles, Tarrytown, NY) was used to quantify free thyroxine concentrations.

Statistical Methods

The results are expressed as the median, the most appropriate measure of the central trend considering the variability in GH responses and the small number of patients. The area under the secretory curve (AUC) and area above the baseline value (AAB) for GH secretion were calculated between 0 and 120 minutes by a trapezoidal method. Peak GH was considered in each test as the maximum level of GH regardless of the time required. For comparisons between control subjects and uremic patients before starting therapy, the Mann-Whitney test was used. For statistical evaluation of GH responses to different stimuli before and after rhEPO therapy in uremic patients, a repeated-measures ANOVA was used. Individual comparisons were performed by the Scheffe test and Fisher's least-significance difference test. The Spearman rank correlation coefficient was used to assess the association between pairs of variables. Differences were considered significant at a P level less than .05.

RESULTS

Clinical, Analytical, and Hematological Evolution

The time course for clinical and analytical parameters is shown in Table 1. All patients responded to rhEPO therapy with a prompt and clear increase in hematocrit and hemoglobin levels that reached statistical significance ($P < .001$) from the third month of therapy to the end of the study. There was no correlation between the rhEPO dose and the increase in hematocrit or hemoglobin at 3, 6, or 12 months.

We could not find any significant changes in systolic (baseline, 130 mm Hg (110 to 150); month 12, 135 mm Hg (110 to 155)) and diastolic (baseline, 80 mm Hg (70 to 90); month 12, 90 mm Hg (70 to 110)) blood pressure before and after correction of anemia with rhEPO therapy. The nutritional status of the patients did not change significantly during the study period. In fact, the body weight and body mass index were similar before and after treatment. Serum albumin concentrations did not show any significant modification after rhEPO therapy, nor did total proteins or serum transferrin. Ferritin concentrations, which showed great variability at baseline (213 ng/mL (34.9 to 387)), did not significantly change with treatment. Neither was there any significant modification in serum iron concentrations.

Serum triglyceride concentrations did not change with rhEPO therapy. Nonetheless, cholesterol levels decreased during treatment and reached statistical significance ($P < .05$) at months 3 and 12. There were no modifications in the weekly urea Kt/V and the normalized protein catabolic rate throughout the 12 months of follow-up study (Table 1). The remaining analytical parameters, ie, serum concentrations of urea, creatinine, sodium, potassium, calcium, and phosphorus, did not exhibit any significant modification after correction of anemia with rhEPO administration (data not shown).

Baseline Hormonal Concentrations

Collectively, the patients exhibited baseline GH concentrations (2 μg/L (1 to 10)) higher than the levels found in control subjects (1.2 μg/L (1 to 2)), although this difference did not reach statistical significance due to the limited number of patients and the great variability of GH values in uremic subjects. Correction of the anemia with rhEPO treatment was not accompanied by any significant change in basal GH levels (Table 2). On the contrary, IGF-I concentrations were significantly higher in patients (225 μg/L (182 to 571)) versus controls (156 μg/L (99-299), $P < .05$) at the baseline evaluation. Again,

Table 2. Evolution of Baseline Hormonal Concentrations During rhEPO Therapy

Parameter	Basal (n = 7)		Month 3 (n = 7)		Month 6 (n = 6)		Month 12 (n = 6)		Control (n = 10)	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
Mean GH (μg/L)*	2	1-10	1.5	1-9.2	1.5	1-10.3	2.3	1-7	1.2	1-2
IGF-I (μg/L)	225	182-571	254	128-394	288	157-482	297	180-450	156†	99-299
Free thyroxine (ng/dL)	1.10	0.83-1.50	1.20	1.10-1.60	1.40	0.99-1.81	1.67	1.09-1.80	1.25	0.89-1.80
Thyrotropin (μU/mL)	1.43	0.90-3.44	1.90	0.90-3.18	2.10	0.5-2.80	2.30	1.13-3.40	1.15	0.31-3.21
Prolactin (μg/L)	24	10-46	15	9-20	16	13-25	14.5	12-55	7.8‡	5-16

*Mean of 6 baseline determinations.
†$P < .05$, ‡$P < .01$, control *v* basal.

rhEPO therapy did not induce any significant modification of basal IGF-I concentrations in uremic patients.

On the other hand, all patients had normal concentrations of thyrotropin and free thyroxine at the start of the study. This situation remained unchanged for 12 months, ie, all patients were euthyroid throughout the study period. In contrast, prolactin concentrations were significantly higher in patients (24 μg/L (10 to 46)) versus controls (7.8 μg/L (5 to 15), $P < .01$) before starting rhEPO therapy. A slight (nonsignificant) decrease in prolactin concentrations was observed after correction of anemia with rhEPO treatment (Table 2).

Responses to GHRH

GH responses to GHRH were characterized by great interindividual variability in uremic patients regarding the amount and the time to the maximal peak, and this variability remained throughout the 12 months of the study period. Before therapy, GHRH administration was followed by prompt GH release that reached a maximum of 7 μg/L (4 to 41) at 30 to 90 minutes. This maximum did not differ from that found in control subjects (11.0 μg/L (6 to 18)). The AUC and AAB for GH secretion in CAPD patients were also comparable to the values found in healthy subjects (Table 3).

However, rhEPO therapy was accompanied by an increase in the AUC and AAB for GH secretion that reached statistical significance ($P < .05$) at months 3 and 6. Maximum GH concentrations also showed an increase after rhEPO therapy that did not reach statistical significance (Table 3). At month 12, neither the AUC, AAB, nor peak GH concentration exhibited significant differences with regard to values obtained before rhEPO administration.

Analysis of the curves for GH secretion after GHRH stimulation showed significant differences between basal (pretreatment) values and values obtained after 3 (minute 60, $P < .05$) and 6 (minute 60, $P < .01$; minute 90, $P < .05$; minute 120, $P < .05$) months of therapy, but there were no significant differences between basal values and the levels at month 12 of rhEPO treatment (Fig 1A).

Responses to Insulin-Induced Hypoglycemia

Fasting blood glucose concentrations were 108 mg/dL (86 to 138) in CAPD patients before therapy and 97 mg/dL (78 to 112) in control subjects (NS). Insulin administration decreased glucose levels which reached a nadir at 30 minutes in both groups. The minimum glucose concentration was 38.5 mg/dL (23 to 56) in patients and 31 mg/dL (15 to 45) in controls, thus indicating no significant differences in the hypoglycemic response in the two groups of subjects. Moreover, we could not find any significant change in fasting blood glucose levels and in the glucose nadir in response to insulin injection before and after 3, 6, and 12 months of rhEPO therapy (data not shown).

After hypoglycemic stimulation, GH levels clearly increased in uremic patients before starting rhEPO, reaching a peak of 10.5 μg/L (5 to 30) at 60 to 120 minutes. This value was not significantly different from the value in controls (13.5 μg/L (8 to 38), although most normal subjects showed a maximum at 60 minutes. Pretreatment values for the AUC and AAB of GH secretion did not show significant differences in uremic patients versus the controls (Table 3). Throughout the study period, we observed a progressive decrease both in the maximum GH level and in the AUC and AAB for GH secretion after hypoglycemic challenge, with these differences being statistically significant ($P < .05$) at months 6 and 12 (Table 3). GH levels in response to insulin injection were significantly different versus baseline (pretreatment) values at minute 120 ($P < .05$) after 3 months, at minutes 90 ($P < .05$) and 120 ($P < .01$) after 6 months, and at minutes 60 ($P < .05$), 90 ($P < .01$), and 120 ($P < .01$) after 12 months of rhEPO therapy (Fig 1B).

Responses to Clonidine

Clonidine administration was followed by an elevation in GH levels that reached a maximum of 6.5 μg/L (4 to 17) at 90 minutes in healthy subjects. However, most of the CAPD patients exhibited a blunted response to this adrenergic agonist, with the median peak obtained at 1 μg/L (1 to 8) ($P < .05$ *v* control group). In accordance with these blunted responses, the AUC and AAB for GH secretion after clonidine stimulation

Table 3. Evolution of GH Responses to GHRH, Insulin-Induced Hypoglycemia, and Clonidine Stimulation Tests During rhEPO Therapy

	Basal (n = 7)		Month 3 (n = 7)		Month 6 (n = 6)		Month 12 (n = 6)		Control (n = 10)	
Parameter	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
GHRH test										
AUC (μg · h/L)	9.5	4.5-61.9	21.9*	8.3-96.3	21.4*	7.8-76	21.4	2-37.1	11.2	6.1-21.6
AAB (μg · h/L)	3.5	1.5-37.9	16.4*	6.3-64.3	18.4*	5.8-46	15.4	0-20.4	8.2	4.1-19.6
Peak (μg/L)	7	4-41	15	9-104	15	9-49	14.5	1-28	11.0	6-18
Hypoglycemia test‖										
AUC (μg · h/L)	11.3	6.5-43.4	10.4	3.4-19	6.6*	3-11	5.5*	2-10.5	13	7-44.9
AAB (μg · h/L)	8.1	4.5-15.4	7 44	1.4-13	1.5*	−3.38-7	1.5*	−0 75-8.5	10	5-38.8
Peak (μg/L)	10.5	5-30	8.5	2-19	5*	2-10	4†	1-12	13.5	8-38
Clonidine test										
AUC (μg · h/L)	2.1	2-10 6	3.6	2-8.8	2	2-14.5	4.3	2-9	5.9‡	3.3-13.3
AAB (μg · h/L)	0	−4.5-0.6	0	−4.4-3.8	0	−1.5-0	0	−2.4-3	3.9§	−0 75-11.3
Peak (μg/L)	1	1-8	3	1-6	1	1-14	4	1-7	6.5‡	4-17

*$P < .05$, †$P < .01$ *v* basal.

‡$P < .05$, §$P < .01$, control *v* basal.

‖n = 6, basal and month 3; n = 5, months 6 and 12.

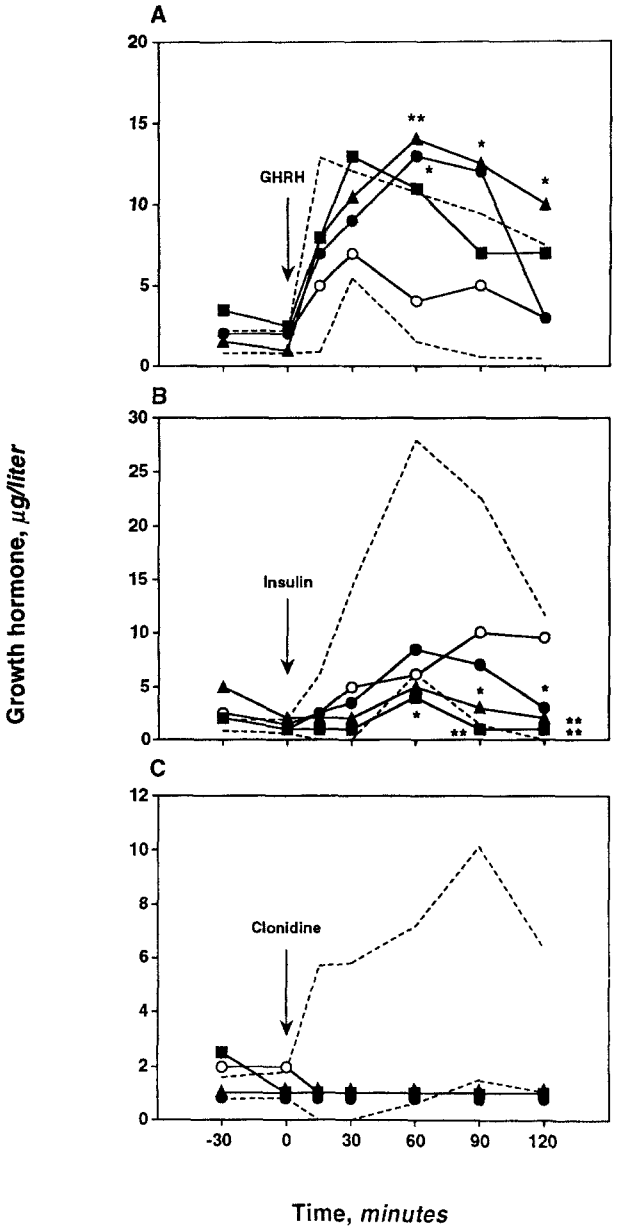

Fig 1. Plasma GH response to GHRH (A), insulin-induced hypoglycemia (B), and clonidine (C) stimulation tests in uremic patients on CAPD before (○) and 3 (●), 6 (▲), and 12 (■) months after starting therapy with rhEPO. Each point represents the median for 7 patients (basal and month 3) or 6 patients (months 6 and 12). In B, each point represents the median for 6 patients (basal and month 3) or 5 patients (months 6 and 12). (---) The mean ± 1 SD of values obtained in 10 healthy subjects. *$P < .05$, **$P < .01$ *v* values obtained before rhEPO therapy.

were significantly ($P < .05$ and $P < .01$, respectively) lower in patients versus controls (Table 3). The clonidine stimulation tests performed after starting rhEPO therapy demonstrated that these blunted responses remained unmodified during the 12 months of the study, with no significant changes both in the maximum GH concentration and in the AUC and AAB for GH secretion (Table 3 and Fig 1C).

Spearman correlations showed a significant correlation between the increment in hemoglobin and the increment in peak GH after GHRH stimulation at month 3. Nevertheless, we could not demonstrate any other significant correlation between the increment in hematocrit or the increment in hemoglobin and any of the parameters (AUC, AAB, and peak) used to evaluate GH responses to direct (GHRH) or indirect (insulin and clonidine) stimuli at any time.

DISCUSSION

To the best of our knowledge, this is the first report in which the long-term influence of rhEPO therapy on GH responses to direct and indirect stimuli has been investigated. These results show that GH responses to pituitary (GHRH) and hypothalamic (insulin-induced hypoglycemia and clonidine) stimulation in uremic patients are influenced by rhEPO in various ways. Firstly, GH responses to GHRH are significantly potentiated during the first 6 months of therapy, although this effect did not persist when patients were evaluated at month 12. Secondly, GH release after hypoglycemic challenge is progressively and significantly diminished. And thirdly, rhEPO treatment could not induce any change in the blunted GH responses to clonidine exhibited by CAPD patients at the baseline examination. These effects were accompanied by a clear-cut increase in blood hemoglobin that persisted at about 12 g/dL throughout the study period. However, contemporary to these phenomena, we could not find any significant modification in the nutritional status or urea kinetics parameters in our patients. Moreover, as reported by other investigators, correction of the anemia in our patients was not accompanied by significant changes in IGF-I levels,[13,17] thyroid function status,[17,21] or prolactin concentrations.[6,17,18,22,23]

Results of this study confirm our previous data obtained in patients on short-term rhEPO treatment[15] and are in agreement with reports by others concerning GH responses to GHRH[14] and insulin-induced hypoglycemia.[8,16] However, these observed effects are not consistent with results obtained by Watschinger et al[24] and Ramírez et al,[13] who found no significant changes in the GH response to GHRH in seven and five hemodialysis patients, respectively. With regard to baseline GH levels, which exhibited no significant changes in our patients, most investigators have reported that these are not modified by rhEPO therapy both in short-term studies[14,15,24] and in studies lasting 6 months or more.[17,18] Again, there are discrepancies, since some have reported a diminution of basal GH after 3 months[7,13] or after 3 to 9 months[19] of rhEPO administration. Discordant results reported by several groups of investigators can be explained by differences in the age of study subjects, the modality and duration of dialysis treatment, the serum creatinine level, and the use of medications that can alter hormone concentrations. The presence of chronic illness or the general health status might be a concurrent factor, since some studies in which few significant hormonal changes were found have been performed in elderly and sick patients.[17] Responsiveness to rhEPO therapy is another factor that may influence endocrine changes, as suggested by Tomoda et al.[12]

The various effects on GH release that accompany rhEPO therapy cannot be explained by a unique and simple mechanism. The increase in hemoglobin causes improved tissue

oxygenation, and this improved oxygen supply has been advocated as the main causative factor for changes in pituitary secretion.[7,13,14] However, indirect evidence also suggests the possibility of a trophic or modulatory action of rhEPO at pituitary or suprapituitary levels. This evidence comes from results obtained in acute experimental protocols in which an elevation in the hematocrit can be reasonably ruled out. In fact, rhEPO acutely administered to uremic patients has been shown to potentiate GH responses to GHRH[25] and abolish paradoxical GH release after TRH stimulation.[26] These data support the hypothesis that rhEPO by itself without correction of anemia can modulate the response of somatotropes through a mechanism different from increased oxygen supply.

According to the present results, the effect of correction of anemia on GH release after hypoglycemic challenge appears to be constant, producing a progressive blunting of GH release with almost complete blunting at 12 months. However, our data did not show any correlation between these responses and the increments in hematocrit or hemoglobin concentrations. This finding might be accounted for by an increase in somatostatin release or a reduction in GHRH secretion induced by rhEPO, although direct evidence for these actions is lacking. On the contrary, we and others[14] have found a potentiation of the GH response to GHRH accompanying correction of anemia with rhEPO therapy. However, a concordance between this potentiation of the GH response and the increase in hemoglobin could not be found. It has been speculated that chronic rhEPO exposure could diminish hypothalamic GHRH secretion and thus sensitize pituitary receptors to exogenous GHRH administration.[15] Nevertheless, this study shows that rhEPO-induced changes in the GH response to GHRH were transient and restricted to the first 6 months of therapy, similar to the effects of rhEPO on several endocrine organs described by Kokot et al[19] in hemodialysis patients. On the other hand, rhEPO therapy was unable to modify the blunted response to clonidine exhibited by CAPD patients in the baseline (pretreatment) exploration. Clonidine is an α_2-adrenergic receptor agonist that stimulates GH release through an increase in hypothalamic secretion of GHRH[27] or a decrease in somatostatinergic tone.[28] Therefore, our data suggest that long-term rhEPO administration does not alter GH secretion after pharmacological manipulation of the adrenergic pathways involved in its hypothalamic regulation.

Our patients had plasma cholesterol levels that significantly decreased after correction of the anemia. This effect was previously observed by others,[7] and a recent prospective study[29] has demonstrated that EPO treatment is associated with a significant decrease in total cholesterol and apoprotein B in hemodialysis and CAPD patients. As already mentioned, the body weight, body mass index, and biochemical markers of nutritional status did not change significantly in our patients after rhEPO therapy as shown in other reports.[7,13] On the other hand, as also reported by others,[10,16,19,30] correction of the anemia in our patients was not accompanied by an increase in blood arterial pressure or significant changes in plasma levels of electrolytes, glucose, urea, and creatinine.

The limitations of this study are derived from the restricted number of patients and from the absence, due to ethical implications, of a comparable CAPD population randomized not to receive rhEPO during the same period. On the other hand, we are aware that the effects of rhEPO therapy on growth in uremic children cannot be predicted from the present study and other studies on GH secretion in uremic adults. Interestingly, the EPO receptor belongs to the cytokine receptor superfamily, which also includes receptors for GH and prolactin.[31] Besides, EPO has been considered one of several growth factors identified in the last few years,[32] and a role for the GH–IGF-I axis in the regulation of hematopoiesis has been recently demonstrated in humans.[33] However, although an increase in appetite has been reported in children receiving rhEPO, a consistent increase in dietary intake or body weight or an improvement in growth have not been substantiated.[34]

To summarize, the present study shows that long-term treatment with rhEPO in CAPD patients is associated with complex and profound effects on somatotrope cell function. Improved oxygenation of the hypothalamo-hypophyseal areas seems to be the main causative factor, although a direct or modulatory action of rhEPO at the hypothalamic or pituitary level might also play a role in these endocrine changes. Nevertheless, the mechanisms for the GH changes are not elucidated by the present study. In vitro studies on rhEPO effects on pituitary function have been suggested,[26] but are lacking at the present time. Our results also suggest that some rhEPO-induced alterations in somatotrope function are dependent on the duration of treatment.

REFERENCES

1. Winearls CG, Oliver DO, Pippard MJ, et al: Effect of human erythropoietin derived from recombinant DNA on the anemia of patients maintained by chronic haemodialysis. Lancet 2:1175-1178, 1986

2. Eschbach JW, Egrie JC, Downing MR, et al: Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. N Engl J Med 316:73-78, 1987

3. Bommer J, Ekrion C, Müller-Bühl U, et al: Recombinant human erythropoietin therapy in haemodialysis patients—Dose determination and clinical experience. Nephrol Dial Transplant 2:238-242, 1987

4. Schaefer RM, Kokot F, Heidland A: Impact of recombinant erythropoietin on sexual function in hemodialysis patients. Contrib Nephrol 76:273-282, 1989

5. Schaefer RM, Kokot F, Wernze H, et al: Improved sexual function in hemodialysis patients on recombinant erythropoietin: A possible role of prolactin. Clin Nephrol 31:1-5, 1989

6. Bommer J, Kugel M, Schwöbel B, et al: Improved sexual function during recombinant human erythropoietin therapy. Nephrol Dial Transplant 5:204-207, 1990

7. Ramírez G, Bittle PA, Sanders H, et al: Hypothalamo-hypophyseal thyroid and gonadal function before and after erythropoietin therapy in dialysis patients. J Clin Endocrinol Metab 74:517-524, 1992

8. Kokot F, Wiecek A, Grzeszczak W, et al: Influence of erythropoietin treatment on endocrine abnormalities in hemodialyzed patients. Contrib Nephrol 76:257-272, 1989

9. Schaefer RM, Kokot F, Kürner B, et al: Normalization of elevated prolactin levels in hemodialysis patients on erythropoietin. Nephron 50:400-401, 1988

10. Kokot F, Wiecek A, Grzeszczak W, et al: Influence of erythropoietin treatment on follitropin and lutropin response to luliberin and plasma testosterone levels in haemodialyzed patients. Nephron 56:126-129, 1990

11. Haley NR, Matsumoto AM, Eschbach JW, et al: Low testosterone levels increase in male hemodialysis patients treated with recombinant human erythropoietin. Kidney Int 35:193, 1989 (abstr)

12. Tomoda F, Takata M, Izumino K, et al: Effects of erythropoietin treatment on thyroid dysfunction in hemodialysis patients with renal anemia. Nephron 66:307-311, 1994

13. Ramírez G, Bittle PA, Sanders H, et al: The effects of corticotropin and growth hormone releasing hormone on their respective secretory axes in chronic hemodialysis patients before and after correction of anemia with recombinant human erythropoietin. J Clin Endocrinol Metab 78:63-69, 1994

14. Cremagnani L, Cantalamessa L, Orsatti A, et al: Recombinant human erythropoietin (rhEPO) treatment potentiates growth hormone (GH) response to growth hormone releasing hormone (GHRH) stimulation in hemodialysis patients. Clin Nephrol 39:282-286, 1993

15. Díez JJ, Iglesias P, Sastre J, et al: Growth hormone responses to growth hormone–releasing hormone and clonidine before and after erythropoietin therapy in CAPD patients. Nephron 74:548-554, 1996

16. Kokot F, Wiecek A, Grzeszczak W, et al: Influence of erythropoietin treatment on function of the pituitary-adrenal axis and somatotropin secretion in hemodialyzed patients. Clin Nephrol 33:241-246, 1990

17. Carlson HE, Graber ML, Gelato MC, et al: Endocrine effects of erythropoietin. Int J Artif Organs 18:309-314, 1995

18. Steffensen G, Aunsholt NA: Does erythropoietin cause hormonal changes in haemodialysis patients? Nephrol Dial Transplant 8:1215-1218, 1993

19. Kokot F, Wiecek A, Schmidt-Gayk H, et al: Function of endocrine organs in hemodialyzed patients on long-term erythropoietin therapy. Artif Organs 19:428-435, 1995

20. Selgas R, Bajo MA, Fernandez-Reyes MJ, et al: An analysis of adequacy of dialysis in a selected population on CAPD for over 3 years: The influence of urea and creatinine kinetics. Nephrol Dial Transplant 8:1244-1253, 1993

21. Goffin E, Oliveira DBG, Raggatt P, et al: Assessment of the thyroid function of patients undergoing regular haemodialysis. Nephron 65:568-572, 1993

22. Schaefer F, Van Kaick B, Veldhuis JD, et al: Changes in the kinetics and biopotency of luteinizing hormone in hemodialyzed men during treatment with recombinant human erythropoietin. J Am Soc Nephrol 5:1208-1215, 1994

23. Lawrence IG, Price DE, Howlet TA, et al: Erythropoietin and sexual dysfunction. Nephrol Dial Transplant 12:741-747, 1997

24. Watschinger B, Watzinger U, Templ H, et al: Effect of recombinant human erythropoietin on anterior pituitary function in patients on chronic hemodialysis. Horm Res 36:22-26, 1991

25. Cantalamessa L, Cremagnani L, Orsatti A, et al: Increased growth hormone response to growth hormone releasing hormone induced by erythropoietin in uraemic patients. Clin Endocrinol (Oxf) 34:85-89, 1991

26. Díez JJ, Iglesias P, Sastre J, et al: Influence of erythropoietin on paradoxical responses of growth hormone to thyrotropin-releasing hormone in uremic patients. Kidney Int 46:1387-1391, 1994

27. Alba-Roth J, Losa M, Spiess Y, et al: Interaction of clonidine and GHRH on GH secretion in vivo and in vitro. Clin Endocrinol (Oxf) 30:485-491, 1989

28. Devesa J, Arce V, Lois N, et al: Alpha-2-adrenergic agonism enhances the growth hormone (GH) response to GH-releasing hormone through an inhibition of hypothalamic somatostatin release in normal men. J Clin Endocrinol Metab 71:1581-1588, 1990

29. Pollock CA, Wyndham R, Collett PV, et al: Effects of erythropoietin therapy on the lipid profile in end-stage renal failure. Kidney Int 45:897-902, 1994

30. Jandeleit K, Ehmer B, Heintz B, et al: Hormonal and electrolyte changes under regular therapy with recombinant human erythropoietin in hemodialysis patients. Contrib Nephrol 91:57-60, 1991

31. Livnah O, Stura EA, Johnson DL, et al: Functional mimicry of a protein hormone by a peptide agonist: The EPO receptor complex at 2.8 Å. Science 273:464-471, 1996

32. Groopman JE, Molina JM, Scadden DT: Hematopoietic growth factors. Biology and clinical applications. N Engl J Med 321:1449-1459, 1989

33. Vihervuori E, Virtanen M, Koistinen H, et al: Hemoglobin level is linked to growth hormone–dependent proteins in short children. Blood 87:2075-2081, 1996

34. Jabs K: The effects of recombinant human erythropoietin on growth and nutritional status. Pediatr Nephrol 10:324-327, 1996