Prolactin Responses to Vasoactive Intestinal Polypeptide in Chronic Renal Failure Patients

Mohammed A. Arnaout[1], Yousef S. Hamzeh[1], Kamel M. Ajlouni[2]

Departments of Medicine, School of Medicine, University of Jordan[1], Amman, and Jordan University of Science and Technology[2], Irbid, JORDAN

Running Title: VIP, prolactin, chronic renal failure

Supported by University of Jordan and JUST.

Address correspondence to: M. A. Arnaout, M.D.
Endocrine Section
School of Medicine
University of Jordan
Amman, JORDAN

ABSTRACT

Prolactin response to intravenous bolus injection of 1 μg/kg vasoactive intestinal polypeptide was determined in 8 patients with chronic renal failure undergoing chronic hemodialysis and in 8 normal controls, age- and sex-matched. Plasma prolactin in chronic renal failure patients showed a blunted response following vasoactive intestinal polypeptide injection, whereas the controls showed significantly higher mean peak prolactin value over the baseline value ($p<0.002$).

On a separate day, each individual underwent a thyrotropin-releasing hormone (500 μg) challenge with the prolactin response determined. The chronic renal failure patients had significantly higher peak prolactin values ($p<0.002$), however, were not significantly different than those in control group.

In the group of chronic renal failure patients, the net prolactin increments (peak levels minus basal levels) were significantly higher with thyrotropin-releasing hormone than with vasoactive intestinal polypeptide ($p<0.02$). The net prolactin increments to thyrotropin-releasing hormone challenge were significantly higher in the control group than in chronic renal failure patients ($p<0.001$). Whereas, the peak values and the AUC were larger with the TRH challenge but were not significantly different.

The results demonstrate the lack of prolactin response to the stimulatory effect of vasoactive intestinal polypeptide in chronic renal failure. These data suggest that the responsiveness of plasma prolactin to vasoactive intestinal polypeptide is defective in these patients, the mechanism(s) of which are yet to be defined.

INTRODUCTION

Mild hyperprolactinemia is a common finding in patients with end stage renal disease (CRF) on chronic hemodialysis (1-6). A major mechanism for hyperprolactinemia in CRF appears to be reduced renal removal of prolactin (PRL) through tubular catabolism and/or urinary clearance (7). However, deficient PRL-inhibiting factor secretion had been suggested in CRF patients causing the PRL elevation (4). In addition, it has been shown that the PRL stimulation response to thyrotropin-releasing hormone (TRH) is blunted in CRF indicating a further disturbance at the pituitary level (1,3-4).

The physiological role of vasoactive intestinal polypeptide (VIP) in the regulation of prolactin secretion from the anterior pituitary has been proposed based on several lines of evidence. The localization of VIP within the paraventricular nucleus, median eminence, and anterior pituitary and in hypophyseal blood at levels greater than those in peripheral blood is supportive of VIP functioning as a hypophysiotrophic factor (8-10). VIP stimulates prolactin secretion *in vivo* and *in vitro* (11-13). Further lines of evidence include the VIP induced increase in prolactin mRNA (14) and the isolation of VIP specific receptors in anterior pituitary lactotrophes (15).

In the present report, in order to assess hypothalamic-pituitary regulation of PRL secretion, we have examined PRL secretion in response to the stimulatory effect of VIP and TRH in patients undergoing maintenance hemodialysis. In this study, the effect of intravenously administered VIP on plasma PRL concentration in a group of eight patients with CRF was studied.

MATERIALS AND METHODS

Volunteers

Eight patients, undergoing long-term hemodialysis for chronic renal failure with hyperprolactinemia, gave informed consent to participate in the studies with VIP after the study was approved by Human Research Committee of the University of Jordan School of Medicine. Women (n=4) were aged 40.0 ± 2.0 years (range 35 - 45 years), and men (n=4) aged 37.5 ± 5.5 (range 22 - 48 years). The patients had been receiving hemodialysis for 16.0 ± 2.1 months at the time of the study. Maintenance hemodialysis (two to three times per week) was instituted because of end stage renal insufficiency of various etiologies (Table I). All studies were done one day after having scheduled dialysis. Aluminum hydroxide (phosphate binder) and multivitamins were administered routinely to all patients, no anabolic drugs or hormones were given. The data were compared to a group of normal volunteers (age- and sex- matched) without the history of renal failure, hyperprolactinemia,or drug intake. The control group had normal kidney function, and the females had regular menses in the females and studied during the follicular phase.

VIP Administration

Synthetic porcine VIP (Bachem, Torrance, CA) was dissolved in normal saline, and sterilized by passage through a 0.22μm Millipore Millex-GV filter (Millipore Corp., Bedford, MA). After an overnight fast, all subjects were kept in a quiet room, and an indwelling venous catheter was placed in an antecubital vein. Following a 30 minute equilibrium period, VIP was given as a single IV bolus dose of 1μg/kg to all subjects (dose range 65-90 μg). Blood samples were obtained for hormone analyses

immediately before, and 5, 15, 30, 45, 60, 90, and 120 min after VIP injection.

On another day, the patients underwent a 500μg thyrotropin-releasing hormone (TRH, Abbott Laboratories, North Chicago, IL) challenge test with blood samples obtained for hormone analyses as in the VIP challenge test.

Ten microliters of Trasylol (Bayer; 10,000 KIU/ml) were added to each sample of blood, and samples were then centrifuged immediately at 4°C, and plasma was removed and frozen at -20°C until assay.

Radioimmunoassays:

Plasma VIP was measured in duplicate using an assay method previously reported (9). Prolactin (PRL) was measured in duplicate using material kindly provided by the National Hormone and Pituitary Program (NIDDK), and the University of Maryland School of Medicine (16). All VIP and PRL values were obtained from a single assay, with intraassay of variation of ≤5%. The PRL secretory responses were expressed as either absolute values (μg/l) or areas under the curve (AUC; μg/l.hr) calculated by trapezoid integration. Net increments in PRL levels were calculated as peak PRL levels minus basal PRL levels.

Statistics

Data were analyzed using repeated measure ANOVA and Student's t test for paired and unpaired data as appropriate. All data are depicted as mean ± SEM.

RESULTS

Clinical symptoms and signs and plasma VIP levels after VIP injection

Within a few minutes of VIP administration, both systolic and diastolic blood pressures decreased and pulse rate increased transiently and disappeared by 30 min after VIP injection. Obvious facial flushing was observed in all CRF patients and the control group. Intravenous VIP administration at a dose of 1 μg/kg resulted in a significant increase in plasma VIP, with a peak 5 min after the injection in all subjects. The VIP levels increased from undetectable baseline levels to 4.5 ± 1.2 μg/l ($p<0.005$ versus basal values) in CRF patients and to 1.7 ± 0.5 μg/l ($p<0.01$ versus basal values) in the normal controls (Figure 1). Plasma VIP levels fell to baseline by 45 min in the CRF patients and the normal controls.

Effect of VIP on plasma PRL levels in patients with chronic renal failure

In CRF patients, basal plasma PRL levels ranged from 24.4 - 45.8 μg/l, with a mean of 32.7 ± 2.9 μg/l (Table 2). Plasma PRL levels did not change significantly after VIP injection; the peak values, net increments in plasma PRL and AUC after VIP challenge were 36.5 ± 3.3 μg/l, 4.2 ± 1.7 μg/l and 1985.6 ± 215.2 μg/l.hr, respectively (Table 2). On the other hand, VIP caused significant increases in plasma PRL levels in the control group; the peak values were 41.6 ± 4.2 μg/l ($p<0.002$ versus basal values; $p<0.001$ versus peak values in CRF patients). In the control group, the net rise in plasma PRL and AUC after VIP injection were significantly greater than that in CRF patients ($p<0.0001$).

Figure 2 shows the pattern of plasma PRL responses to VIP. All patients failed to respond to VIP, except for patient #1 with > 50% increase in plasma PRL levels above baseline value.

Effect of TRH on plasma PRL levels in patients with chronic renal failure

Intravenous injection of TRH resulted in significant increase in plasma PRL levels in CRF patients (Table 2). The mean peak value (41.6 ± 4.2 μg/l) was significantly larger than the basal value ($p<0.002$). Net increments in plasma PRL and AUC after TRH injection were 10.6 ± 2.2 μg/l and 2203.5 ± 211.0 μg/l.hr; respectively. In control group, the peak PRL value was significantly larger than the basal values ($p<0.002$), (Table 2).

Furthermore, in CRF patients the net increase in PRL secretion with TRH challenge was significantly greater than with VIP challenge ($p<0.02$), whereas, the peak values and the AUC were larger with the TRH challenge but not significantly different.

DISCUSSION

This study demonstrates hyporesponsive prolactin stimulation to VIP in chronic renal failure with hyperprolactinemia. As compared to TRH challenge test, VIP injection resulted in no significant prolactin release. The pharmacokinetics of VIP was not significantly different in CRF patients as compared to controls.

The association of hyperprolactinemia and chronic renal failure that persisted despite dialysis treatment has been reported by various investigators (1-6). The incidence of hyperprolactinemia among patients

with CRF undergoing hemodialysis and receiving no PRL-modifying drugs ranges from 73 -91% in women, and 25 - 57% in men (4-5). Etiologically, none of our patients had symptoms suggestive of pituitary or central nervous system lesions, and none received medications known to stimulate PRL secretion.

Several factors may affect this increase in circulating PRL. Since the degradation of PRL is largely dependent on the kidney, increased PRL could possibly be a reflection of impaired renal clearance (17). In uremic patients the metabolic clearance rate of PRL was found to be depressed only by 25%, while its secretory rate was increased 4-folds (18). Modlinger et al. (19) showed that in man renal vein PRL concentration did not differ from those in the peripheral vein obtained simultaneously suggesting that the kidney does not play a significant role in PRL excretion or degradation. Furthermore, renal vein PRL from the diseased kidney was not different from that found in the contralateral normal kidney.

The hyperprolactinemia of uremic humans is characterized by a relative autonomy of the lactotrophes. Lactotrophes are resistant to suppression by dopamine (1,4), as well as hyporesponsive to stimulation by chlorpromazine or TRH (1,4), suggesting that a defect exists in the pituitary, involving either receptor binding or post-receptor event. Another finding supportive of a pituitary dysfunction is the markedly blunted PRL response to VIP.

In vitro immunoneutralization studies suggest positive regulation of prolactin secretion by pituitary VIP (20-22). The content of pituitary VIP mRNA is shown to be increased in hypothyroidism and replacement with l-thyroxine prevented this increase (23). Basal prolactin secretion from

cultured hypothyroid pituitary cells decreased significantly in the presence of anti-VIP antisera (22). In patients with CRF, hypothyroidism is common (high TSH levels in 6 of 8 patients in our group, Table 1) (17) and thus may contribute to hyperprolactinemia by increasing the VIP induced prolactin synthesis and release. However, the increased pituitary VIP mRNA in hypothyroidism may down-regulate the receptors to further VIP induced prolactin release. In addition, prolactin stimulation by TRH and by VIP involve different mechanisms (24), contributing to the difference in prolactin release by VIP and by TRH in our group of CRF patients.

In summary, the present study demonstrates hyporesponsive prolactin stimulation by VIP in hyperprolactinemic-chronic renal failure patients. The results suggest a defect in VIP induced prolactin release, the mechanism (s) of which are yet to be defined.

REFERENCES

1. Ramirez G, O'Neill WM, Jr. Bloomer HA, Jubiz W. Abnormalities in the regulation of prolactin in patients with chronic renal failure. J Clin Endocrinol Metab 1977;45:658-661.

2. Hagen C, Ølgaard K, McNeilly AS, Fisher R. Prolactin and the pituitary-gonadal axis in male uraemic patients on regular dialysis. Acta Endocrinologica 1976;82:29-38.

3. Gomez F, De La Cueva R, Wauters JP, Lemarchand-Beraud T. Endocrine abnormalities in patients under going long-term hemodialysis. The role of prolactin. Am J Med 1980;68:522-530.

4. Lim VS, Kathpalia SC, Frohman LA. Hyperprolactinemia and impaired pituitary response to suppression and stimulation in chronic renal failure: Reversal after transplantation. J Clin Endocrinol Metab 1979;48:101-107.

5. Cowden EA, Ratcliffe WA, Ratcliffe JG, Dobbie JW, Kennedy AC. Hyperprolactinemia in renal disease. Clin Endocr 1978;9:241-248.

6. Ølgaard K, Hagen C, McNeilly AS. Pituitary hormones in women with chronic renal failure: the effect of chronic intermittent hemo- and peritoneal dialysis. Acta Endocrinol 1975;80:237-246.

7. Strober W, Waldman TA. The role of the kidney in the metabolism of plasma proteins. Nephron 1974;13:35-66.

8. Rostene WH. Neurobiological and neuroendocrine functions of the vasoactive intestinal peptide (VIP). Prog Neurobiol 1984;22:103-129.

9. Arnaout MA, Garthwaite TL, Martinson DR, Hagen TC. Vasoactive intestinal polypeptide is synthesized in anterior pituitary tissue. Endocrinology 1986;119:2052-2057.

10. Shimatsu A, Kato Y, Matsushita N, Katakami H, Yanaihara N, Imura H. Stimulation by serotonine of vasoactive intestinal polypeptide release into rat hypophyseal portal blood. Endocrinology 1982;111:338-340.

11. Enjalbert A, Arancibia S, Ruberg M, Priam M, Bluet-Pajot MT, Rotsztejn WH, Kordon C. Stimulation of *in vitro* prolactin release by vasoactive intestinal peptide. Neuroendocrinology 1980;30:200-204.

12. Rotszteijn WH, Benoist L, Besson J, Beraud G, Bluet-Pajot MT, Kordon C, Rosselin G, Duval J. Effect of vasoactive intestinal peptide (VIP) on the release of adenohypophyseal hormones from purified cells obtained by unit gravity sedimentation. Neuroendocrinology 1980;31:282-286.

13. Reichlin S. Neuroendocrine significance of vasoactive intestinal polypeptide. Ann NY Acad Sci 1987;527:431-449.

14. Carrillo AJ, Pool TB, Sharp ZD. Vasoactive intestinal peptide increases prolactin messenger ribonucleic acid content in GH3 cells. Endocrinology 1985;116:202-206

15. Wanke IE, Rorstad OP. Receptors for vasoactive intestinal peptide in rat anterior pituitary glands: localization of binding to lactotrophes. Endocrinology 1990;126:1981-1990

16. Sinha YN, Selby FW, Lewis UJ, Vanderlaan WD. A homologous radioimmunoassay for human prolactin. J Clin Endocrinol Metab. 1973;36:509-16.

17. Emmanouel DS, Lindheimer M.D, Katz AI. Pathogenesis of endocrine abnormalities in uremia. Endocrine Reviews 1980;1:28-44.

18. Sievertsen B, Lim VS, Nakawatase C, Frohman LA. Hyperprolactinemia in chronic renal failure (CRF): Evidence for increased secretion rate (SR) and decreased metabolic clearance rate (MCR) of prolactin (PRL). Clin Res 1979;27:Abstract 260A.

19. Modlinger RS, Gutkin M. Plasma prolactin in essential and renovascular hypertension. J Lab Clin Med 1978;91:693-697.

20. Hagen TC, Arnaout MA, Scherzer WJ, Martinson DR, Garthwaite TL. Antisera to vasoactive intestinal peptide inhibit basal prolactin release from dispersed anterior pituitary cells. Neuroendocrinology 1986;43:641-645

21. Nagy G, Mulcahey JJ, Neill JD. Autocrine control of prolactin secretion by vasoactive intestinal peptide. Endocrinology 1988;122:364-366

22. Lam KSL, Reichlin S. Pituitary vasoactive intestinal peptide regulates prolactin secretion in the hypothyroid rat. Neuroendocrinology 1989;50:524-528

23. Segerson TP, Lam KSL, Cacicedo L, et al. Thyroid hormone regulates vasoactive intestinal peptide (VIP) mRNA levels in the rat anterior pituitary gland. Endocrinology 1989;2221-2223

24. Bjoro T, Sand O, Ostberg BC, Cordeladze JO, Torjesen P, Gautvik KM, Haug E. The mechanisms by which vasoactive intestinal peptide (VIP) and thyrotropin releasing hormone (TRH) stimulate prolactin release from pituitary cells. Biosci Rep 1990;10:189-199

Table I. Clininal features and endocrine data on 8 patients with chronic renal failure

Case No	Age (yr)	Sex	Diagnosis	Duration of dialysis (month)	PRL (μg/l)	GH (μg/l)	TSH (mU/l)	LH (IU/l)	FSH (IU/l)
1	40	F	Chronic Glomerulonephritis	22	44	23	8	6	12
2	48	M	Chronic Glomerulonephritis	24	30	36	2	105	60
3	22	M	Chronic Glomerulonephritis	13	25	19	5	31	7
4	40	M	Chronic Glomerulonephritis	18	46	13	9	149	15
5	35	F	Undetermined	14	35	14	8	9	9
6	45	F	Mixed Connective Tissue Disease	6	31	8	5	70	113
7	40	M	Chronic Interstitial Nephritis	12	24	12	3	40	21
8	40	F	Chronic Interstitial Nephritis	20	27	7	5	7	8

Table 2. Plasma prolactin levels (µg/l) and area under curve (AUC, µg/l/hr) in response to VIP (1µg/kg BW, iv), and TRH (500µg, iv) in chronic renal failure patients.

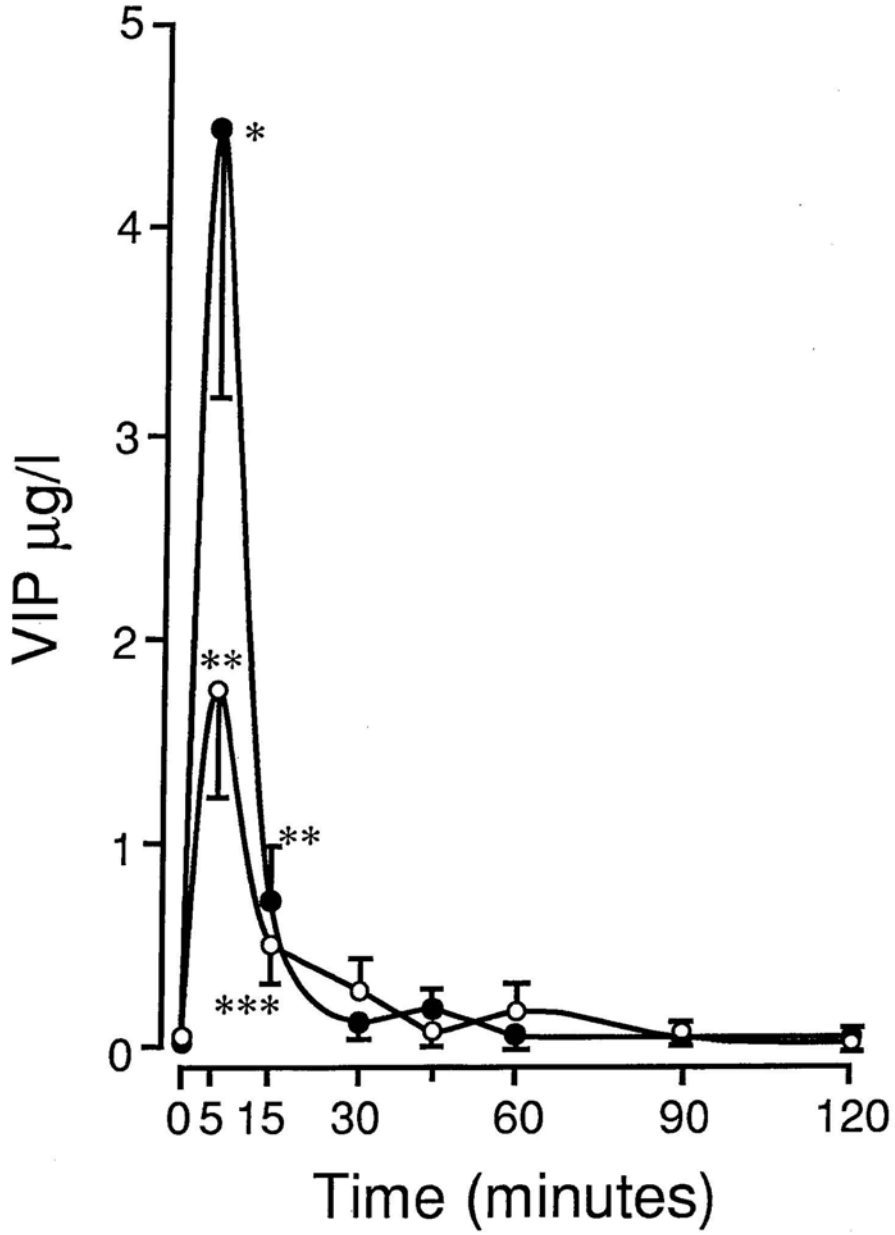
Case No.	VIP Test				TRH Test			
	Basal	Peak	Net increase	AUC	Basal	Peak	Net increase	AUC
1	30.9	45.8	14.9	2329.2	44.8	58.4	13.6	2842.4
2	44.0	46.0	2.0	2349.6	35.3	45.4	10.1	2318.7
3	30.3	31.8	1.5	1625.5	27.0	34.0	7.0	1655.9
4	35.2	36.7	1.5	2146.0	28.5	46.9	18.4	2332.7
5	24.7	29.2	4.5	1176.2	18.3	34.0	15.7	1566.0
6	45.8	49.3	6.5	2729.8	40.8	55.9	15.1	2970.5
7	24.4	26.2	1.8	1508.8	28.1	29.2	1.1	1692.5
8	26.5	27.2	0.7	1635.1	25.0	28.7	3.7	1669.9
Mean ± SEM	32.7 ± 2.9	36.5 ± 3.3	4.2 ± 1.7	1946.5 ± 183.7	31.0 ± 3.1	41.6 ± 4.2[a]	10.6 ± 2.2[b]	2131.1 ± 199.8
CONTROLS								
Mean ± SEM	12.4 ± 2.9	60.3 ± 9.1[a]	47.9 ± 6.6	1205.0 ± 78.2	12.4 ± 2.9	45.3 ± 5.0[a]	32.9 ± 5.8	1552.0 ± 156.3
p value vs Controls	<0.0001	<0.001	<0.0001	<0.0001	<0.0001	NS	<0.001	<0.005

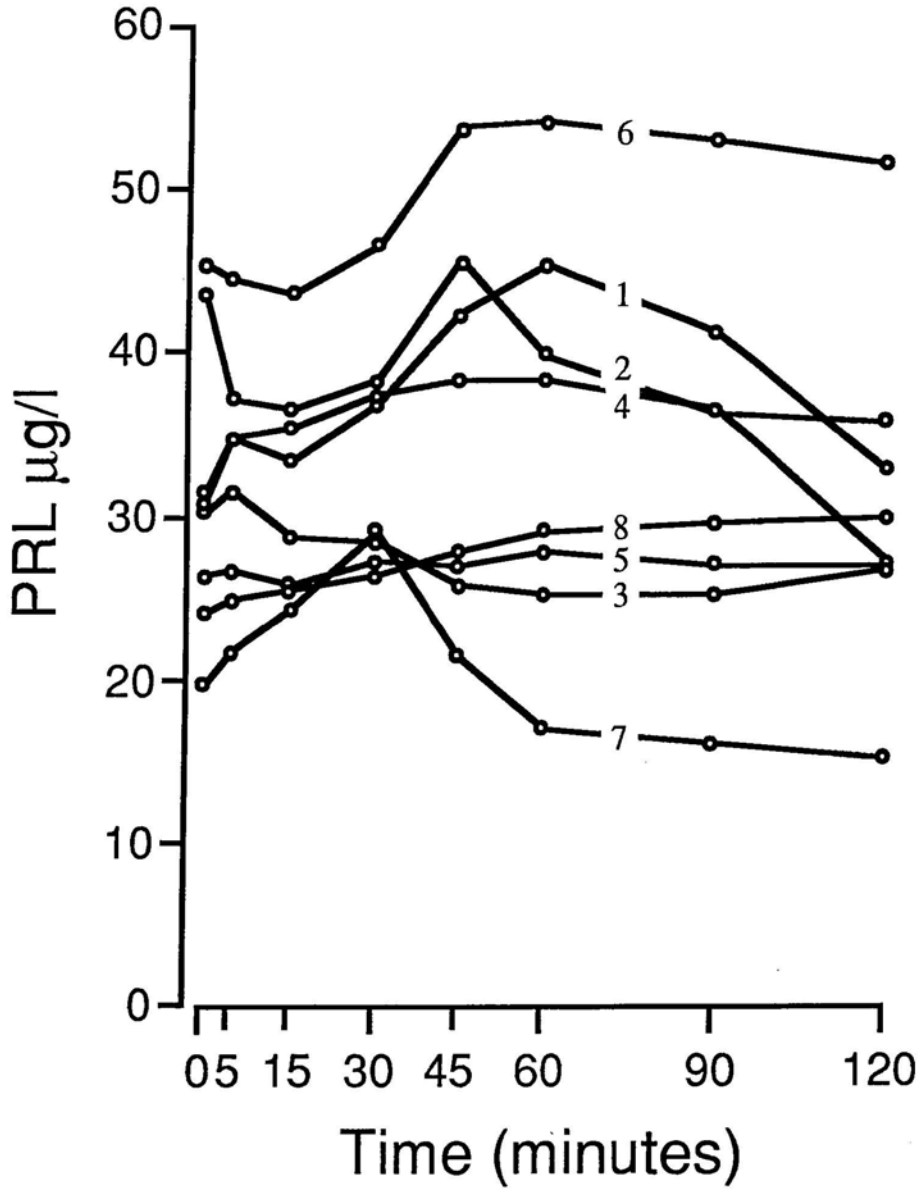
[a] $p<0.002$ versus basal; [b] $p<0.02$ versus net increase after VIP, NS = not significant

LEGENDS TO FIGURES

Figure 1. Radioimmunoassayable plasma vasoactive intestinal polypeptide (VIP) concentrations after intravenous bolus injection of VIP 1μg/kg in chronic renal failure patients (●—●) and normal subjects (O—O). The mean ± SEM are shown. *, $p<0.005$, **, $p<0.01$, ***, $p<0.025$, versus baseline levels.

Figure 2. Plasma PRL responses to VIP (1μg/kg BW, iv) in 8 chronic renal failure patients. Numbers indicate the patient # in table 2.


5
4
3
2
1
0
VIP µg/l
*
**
**
***
0 5 15 30 60 90 120
Time (minutes)


60
50
40
30
20
10
0
PRL µg/l
6
1
2
4
8
5
3
7
05 15 30 45 60 90 120
Time (minutes)