Kidney International, Vol. 37 (1990), pp. 955–964

Chromogranin A in uremia: Progressive retention of immunoreactive fragments

RAY J. HSIAO, MATTHEW S. MEZGER, and DANIEL T. O'CONNOR

Department of Medicine, University of California and Veterans Administration Medical Center, San Diego, California, USA

Chromogranin A in uremia: Progressive retention of immunoreactive fragments. Chromogranin A is a soluble protein that is stored and released with catecholamines from their secretory vesicles. Its measurement is a probe of exocytotic sympathoadrenal activity, and in plasma it may also be a useful tool in the diagnosis of peptide producing endocrine neoplasms. Because we have found that chromogranin A is elevated in secondary (uremic) hyperparathyroidism, we systematically investigated the influence of renal dysfunction and its attendant hyperparathyroidism on chromogranin A in several subject groups: normal controls (serum creatinine ≤1.2 mg/dl), nonazotemic renal transplant recipients, nonazotemic subjects with glomerular disease (serum creatinine between 1.2 and 2 mg/dl), mid-range renal disease subjects (serum creatinine between 2 and 7.5 mg/dl), and end-stage renal disease subjects (serum creatinine >7.5 mg/dl). Plasma chromogranin A rose with deterioration of renal function, and the rise was independent of etiologic diagnosis, blood pressure, or indices of sympathoadrenal activity or hyperparathyroidism. Size fractionation of uremic plasma by gel filtration, and immunoextraction by region-specific anti-chromogranin A (anti-N-terminal, anti-C-terminal, and anti-mid-molecule) antibodies suggested that chromogranin A immunoreactivity circulates in uremia as lower molecular weight fragments of the parent chromogranin A molecule, with mid-molecule fragments the major constituent. This immunoreactivity is only minimally removed by peritoneal dialysis and is not at all hemodialyzable. The uremia-dose-dependent accumulation of chromogranin A immunoreactive fragments in renal failure suggests that the kidney is a major site of disposition or removal of the immunoreactivity. Furthermore, lack of detectable chromogranin A immunoreactivity in normal subjects' urine suggests that the immunoreactivity is destroyed as it is removed by the kidney. We conclude that plasma chromogranin A increases in proportion to degree of renal insufficiency and that renal function must therefore be controlled when using plasma chromogranin A in the investigation of amine or peptide hormone storage and release.

Chromogranin A [1, 2] is an acidic, monomeric protein which is stored and released with catecholamines by exocytosis from secretory vesicles in the adrenal medulla [3, 4]. We have found elevated plasma chromogranin A in subjects with peptide hormone producing neoplasia, such as pheochromocytoma, parathyroid adenoma or hyperplasia, thyroidal C cell disorders, carcinoid tumor, oat cell lung carcinoma, and pancreatic islet-cell tumor [5]. Indeed, measurement of circulating chromogranin A concentration may be a valuable diagnostic tool for these endocrine tumors [5]. In the evaluation of hyperparathyroidism, we also found elevated plasma chromogranin A concentration in patients with secondary (uremic) hyperparathyroidism [5]. Since uremia per se may alter peptide hormone metabolism [6], and since chromogranin A may be identical to parathyroid secretory protein/secretory protein I [7], we wondered whether the rise in chromogranin A in secondary hyperparathyroidism was the result of renal dysfunction alone, or hyperparathyroidism, or both.

In this study, we investigated the influence of renal dysfunction and the etiology of renal failure on plasma chromogranin A concentration, the removal of chromogranin A by either hemodialysis or peritoneal dialysis, and the correspondence of blood pressure or indices of sympathoadrenal or parathyroid function with plasma chromogranin A concentration. The results suggest that plasma chromogranin A increases progressively in renal failure, independently of etiologic diagnosis or parathyroid status.

Methods

Human samples

Serum or plasma samples were obtained for the measurement of chromogranin A, parathyroid hormone, creatinine and catecholamines from 37 healthy controls (26 male and 11 female) as well as subjects with various kinds and degrees of renal dysfunction (N = 105, 73 male and 32 female): nonazotemic renal transplant recipients (N = 5, 4 male and 1 female), nonazotemic glomerulonephritis (N = 8, 6 male and 2 female), mid-range renal disease (N = 30, 24 male and 6 female), and end-stage renal disease (N = 62, 39 male and 23 female). The criterion for renal function group was the serum creatinine: serum creatinine ≤1.2 mg/dl (range 0.5 to 1.2) for normal controls, serum creatinine <2 mg/dl (range 1.3 to 1.9) for nonazotemic renal transplant recipients, serum creatinine also <2 mg/dl (range 0.7 to 1.8) for the nonazotemic glomerulonephritis group, serum creatinine between 2 and 7.5 mg/dl (range 2 to 7.3) for the mid-range renal disease group, and serum creatinine >7.5 mg/dl (range 7.7 to 27) for the end-stage renal disease group.

To evaluate whether the rise in plasma chromogranin A in uremia is a result of augmented sympathoadrenal activity, samples were obtained for the analysis of plasma chromogranin A, creatinine, and catecholamines from three groups of patients: normal controls (N = 37), mid-range renal disease (N = 5), and hemodialyzed end-stage renal disease at the beginning of

Received for publication June 28, 1989
and in revised form October 16, 1989
Accepted for publication October 19, 1989

a hemodialysis treatment ($N = 9$). The end-stage renal disease patients were further stratified into two subgroups: a high chromogranin A group with plasma concentration >500 ng/ml, and a lower chromogranin A group with plasma concentration <500 ng/ml.

To determine if etiology of renal disease influences the concentration of plasma chromogranin A, the 62 patients from the end-stage renal disease group were subdivided into five groups according to their etiologic clinical diagnosis: glomerulonephritis ($N = 28$), diabetes mellitus ($N = 14$), polycystic kidney disease ($N = 7$), tubulointerstitial nephritis ($N = 6$), and hypertension ($N = 7$). Plasma chromogranin A concentration was then compared among these five groups using a one-way analysis of variance (ANOVA).

To determine whether plasma chromogranin A is removed by either hemodialysis or peritoneal dialysis, nine end-stage renal disease subjects were evaluated. In five subjects already on a thrice weekly hemodialysis regimen, arterial blood was obtained immediately before and after a four hour course of hollow-fiber dialyzer hemodialysis; plasma chromogranin A and total protein were measured. In addition, dialysate was also obtained for analysis after countercurrent exposure to blood across the hollow fiber membrane. Four subjects on continuous ambulatory peritoneal dialysis (CAPD) used Travenol 1.5% to 2.5% dextrose dialysate (Baxter-Travenol, Chicago, Illinois, USA), with an exchange volume of 2 liters. Dwell times were four hours ($N = 2$), five hours ($N = 1$), or 72 hours ($N = 1$). Venous blood and peritoneal dialysate were obtained at the end of each dwell for the measurement of chromogranin A and total protein concentration.

Plasma and urine samples were obtained from subjects with proteinuria ($N = 6$) and normal controls ($N = 6$) for the measurement of chromogranin A, creatinine and protein concentration. The criterion for proteinuria was ≥2+ urinary protein by color reaction on Chemstrip 6L (Boehringer Mannheim Diagnostics, Indianapolis, Indiana, USA).

Venous catheterization

Venous samples from a secondary (uremic) hyperparathyroid subject were obtained at different locations in the neck region for the analysis of parathyroid hormone and chromogranin A.

Gel filtration

To ascertain the size distribution of plasma chromogranin A immunoreactivity in renal failure, selected plasma samples (100 to 500 μl) from end-stage renal disease patients were gel-filtered at 2 ml per hour, collecting 0.5 ml fractions from a 55 by 0.9 cm column of Ultrogel ACA-22 (LKB Produkter, Bromma, Sweden) that was equilibrated and eluted with 0.15 M sodium chloride, 0.1% (wt/vol) ovalbumin, 0.1% (wt/vol) sodium azide, and 0.01 M sodium phosphate (pH 7.4). The column had previously been standardized for void volume (V_o) by elution of blue dextran, for total internal volume (V_t) by elution of potassium chloride and for the elution position of purified, ^{125}I-labeled human chromogranin A. Each column run included potassium chloride as an internal standard for V_t. The column was chosen to provide optimal separation of ^{125}I-labeled chromogranin A from both V_o and V_t [8]. The distribution coefficient (K_d) of the column for the elution volume (V_e) at any given peak was calculated as:

$$K_d = (V_e - V_o)/(V_t - V_o).$$

In vitro dialysis

Peritoneal dialysate from the continuous ambulatory peritoneal dialysis uremics ($N = 4$) and plasma from normals ($N = 6$) and uremics ($N = 6$) was obtained to study the in vitro dialyzability of chromogranin A immunoreactivity. One ml from each sample was dialyzed overnight at 4°C in vitro against 1 liter of buffer (10 mg/ml ovalbumin, 0.15 M sodium chloride, 0.01 M sodium phosphate, pH = 7.4) using standard cellulose dialysis tubing (approximate 5,000 dalton permeability cutoff; Spectrapor, Fisher).

Chromogranin A region-specific immunoextraction

Rabbit antisera were raised by intradermal immunization. The antibody directed against the human chromogranin A whole molecule recognized mid-molecule but not N- or C-terminal epitopes [9]. Antisera were also raised against a synthetic human/bovine synthetic N-terminal 17-mer (LPVNSPMNKGDTEVMKY) and a synthetic bovine chromogranin A C-terminal 16-mer (YELEKVAHQLEELRRG) by coupling them to the carrier keyhole limpet hemocyanin (Calbiochem, San Diego, California, USA) via bisdiazobenzidine [10] and injecting them intradermally [11]. These antisera all recognized human chromogranin A on immunoblots, at optimal titers (vol/vol) of 1:100 (anti-N-terminal, and anti-C-terminal) or 1:1000 (anti-whole/mid-molecule).

Anti chromogranin A antibodies were purified by ammonium sulfate precipitation [12], and then coupled to cyanogen bromide activated Sepharose (Pharmacia, Piscataway, New Jersey, USA) at 10 mg protein/ml beads [12]. Two hundred microliter plasma samples (obtained from one normal control and one end-stage renal disease patient) were then incubated with 10 mg (30 μl) of antibody-coupled Sepharose beads in a final volume of 600 μl in binding buffer (0.15 M sodium chloride, 10 mM sodium phosphate, 10 mM ethylenediamine tetraacetic acid, 0.01% Triton X-100, pH 7.4) at 4°C overnight with continuous shaking, after which the beads were removed by centrifugation. Plasma chromogranin A immunoreactivity was determined by radioimmunoassay both pre- and post-immunoextraction.

Chromogranin A radioimmunoassay

Human chromogranin A was measured by a soluble phase, double antibody radioimmunoassay, as previously described [5].

Other assays

Parathyroid hormone in serum was measured by three methods: a radioimmunoassay specific for the intact, whole molecule, or its N-terminal determinants [13] (INS-PTH; normal range, 11 to 24 pg/ml); a radioimmunoassay that detects parathyroid hormone as well as its C-terminal or mid-molecule fragments [13] (MM-PTH; normal range, 50 to 330 pg/ml); and a two-site immunoradiometric assay that detects only the intact, full length molecule [14] (IRMA; normal range, 10 to 65 pg/ml).

Protein concentration was determined by the Coomassie Brilliant Blue dye binding spectrophotometric method [15].

Creatinine was measured spectrophotometrically by a Beck-

Table 1. Chromogranin A values in subjects stratified by degree of renal insufficiency

Group	N	Sex m/f	Age years	SBP mm Hg	DBP mm Hg	S_{Cr} mg/dl	INS-PTH pg/ml	MM-PTH pg/ml	CgA ng/ml
Normal	37	26/11	41 ± 2	125 ± 3	75 ± 2	0.9 ± 0.1 (N = 20)[a]	12 ± 1 (N = 17)[a]	130 ± 8 (N = 17)[a]	50 ± 3
Renal transplant recipients	5	4/1	43 ± 7	122 ± 8	75 ± 6	1.6 ± 0.1	24 ± 5 (N = 4)[a]	1314 ± 430 (N = 4)[a]	143 ± 20
NAGD	8	6/2	49 ± 8	141 ± 3	84 ± 4	1.3 ± 0.2	18 ± 2 (N = 5)[a]	418 ± 92 (N = 5)[a]	195 ± 74
MRRD	30	24/6	55 ± 3	147 ± 5	91 ± 2	4.1 ± 0.3	44 ± 9 (N = 23)[a]	2023 ± 418 (N = 23)[a]	252 ± 39
ESRD	62	39/23	50 ± 2	143 ± 2	82 ± 2	13.3 ± 0.5	66 ± 9 (N = 59)[a]	7938 ± 1388 (N = 59)[a]	559 ± 90
Total	142	99/43	49 ± 1	138 ± 2	82 ± 1	7.9 ± 0.6 (N = 125)[a]	49 ± 5 (N = 108)[a]	4856 ± 830 (N = 108)[a]	326 ± 44
P value			< 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Abbreviations are: NAGD, nonazotemic glomerular disease (S_{Cr} < 2 mg/dl); MRRD, mid-range renal disease (S_{Cr} = 2–7.5 mg/dl); ESRD, end-stage renal disease (S_{Cr} > 7.5 mg/dl); SBP, systolic blood pressure; DBP, diastolic blood pressure; S_{Cr}, serum creatinine; INS-PTH, intact N-terminal specific parathyroid hormone (nl: 11–24 pg/ml); MM-PTH, C-terminal (mid-molecule) parathyroid hormone (nl: 50–330 pg/ml); CgA, chromogranin A (nl < 112 ng/ml); P values compare the 5 groups by one-way ANOVA test. Plus-minus values are means ± SEM.

[a] Only N samples were obtained.

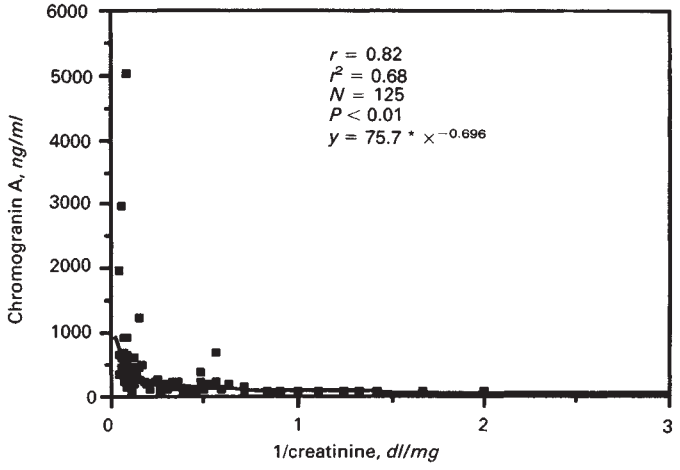

Fig. 1. *Plasma chromogranin A as a function of the inverse of serum creatinine in normal controls (N = 20), nonazotemic renal transplant recipients (N = 5), nonazotemic glomerular disease subjects (N = 8, serum creatinine <2 mg/dl), mid-range renal disease subjects (N = 30, serum creatinine = 2–7.5 mg/dl), and end-stage renal disease subjects (N = 62, serum creatinine >7.5 mg/dl).* Plasma chromogranin A rose with deterioration of renal function (r = −0.82, N = 125, P < 0.01).

man creatinine analyzer (Beckman Instruments, Palo Alto, California, USA).

Plasma norepinephrine and epinephrine were determined by the radioenzymatic method [16].

Statistical analysis

Results are reported as mean value ± the standard error of the mean (SEM), unless otherwise stated. Descriptive statistics (mean and standard error) and inferential statistics (one-way analysis of variance (ANOVA), paired and unpaired t-tests, simple regression, and multiple regression) were generated by statistics software packages (Cricket Graph and StatWorks, Cricket Software, Philadelphia, Pennsylvania) on a Macintosh microcomputer. Simple, logarithmic, and exponential correlations were calculated, and that with the highest r value is reported.

Results

In the 37 normal controls (Table 1) who were selected to represent a large range of ages in both sexes, chromogranin A concentrations did not vary with either age or sex, as previously reported [5] (P > 0.1 for both).

Table 1 displays plasma chromogranin A concentration along with serum creatinine level in five groups of subjects divided according to renal function. There was a stepwise increase in plasma chromogranin A concentration with deterioration of renal status, progressing from normal controls to end-stage renal failure subjects, and chromogranin A correlated with serum creatinine (r = 0.43, N = 125, P < 0.01). Since serum creatinine is inversely proportional to glomerular filtration rate, we also plotted chromogranin A against the inverse value of serum creatinine ($1/S_{Cr}$). An even more impressive inverse correlation linked $1/S_{Cr}$ and chromogranin A (Fig. 1, r = −0.82, P < 0.01).

Figure 2 presents the gel filtration elution profile for chromogranin A immunoreactivity in uremic plasma. The relatively small effective hydrodynamic size of the immunoreactivity suggests that the retained chromogranin A immunoreactivity in renal failure consists of fragments of the parent chromogranin A molecule. A similar gel filtration of normal human plasma did not yield a detectable peak, since the total chromogranin A immunoreactivity in normal plasma was beneath the lower limit of assay detection after the obligate dilution of gel filtration.

To further evaluate the size of these chromogranin A fragments, uremic plasma (N = 6), peritoneal dialysate from continuous ambulatory peritoneal dialysis patients (N = 4), and normal control plasma (N = 6) were dialyzed overnight against a physiologic solution (10 mg/ml ovalbumin, 0.15 M sodium chloride, 0.01 M sodium phosphate, pH = 7.4). There were no significant differences between pre-dialysis and post-dialysis values (Table 2), which suggested that these chromogranin A fragments were still too large to be dialyzed, that is, larger than

Table 2. In vitro dialyzability of chromogranin A immunoreactivity (ng/ml)[a]

Subjects	Fluid	*N*	Pre-dialysis	Post-dialysis	Paired-*t*	*P*
Normals	Plasma	6	41 ± 4	37 ± 4	1.39	0.22
Uremics	Plasma	6	655 ± 74	651 ± 107	0.10	0.93
Uremics	Peritoneal dialysate	4	27 ± 4	21 ± 2	2.13	0.12

Uremics: serum creatinine > 7.5 mg/dl.

[a] Plasma from normals ($N = 6$) and uremics ($N = 6$) and peritoneal dialysate from the continuous ambulatory peritoneal dialysis-treated uremics ($N = 4$) was dialyzed overnight at 4°C as described in the **Methods** section.

Table 3. Solid phase immunoextraction of human plasma chromogranin A immunoreactivity with anti chromogranin A region-specific antisera

	Normal control plasma CgA		ESRD subject plasma CgA		Buffer control CgA
	ng/ml	%	*ng/ml*	%	*ng/ml*
Pre-extraction	105	100	>1920	100	<5
Post-extraction with antibody coupled to beads:					
Anti mid-molecule antibody	18	17 (18/105)	900	<47 (900/>1920)	<5
Anti C-terminal antibody	99	94 (99/105)	1773	<92 (1773/>1920)	<5
Anti N-terminal antibody	93	89 (93/105)	>1920	100 (>1920/>1920)	<5
Preimmune rabbit serum	108	103 (108/105)	>1920	100 (>1920/>1920)	<5

Abbreviations are: CgA, chromogranin A; ESRD, end-stage renal disease.

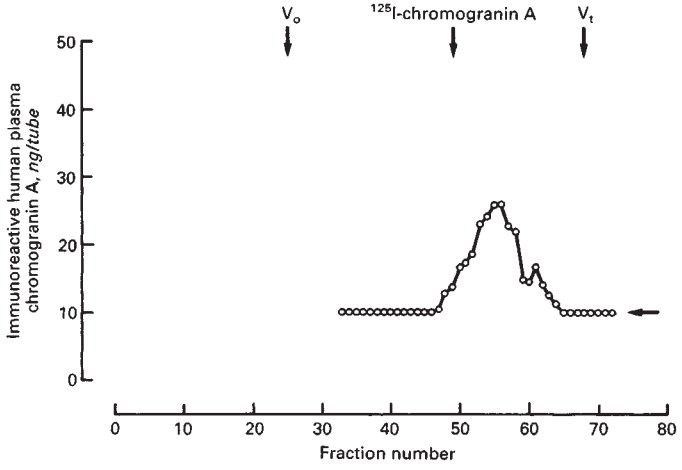

Fig. 2. *Characterization of the size of uremic plasma chromogranin A immunoreactivity by gel filtration.* The chromatography was performed with a 0.9 by 55 cm Ultrogel ACA-22 column, equilibrated and eluted with 0.15 M sodium chloride, 0.1% (wt/vol) sodium azide, 0.1% (wt/vol) ovalbumin, and 0.01 M sodium phosphate (pH 7.4). The column was standardized for void volume (V_o) by elution of blue dextran, total internal volume (V_t) by potassium chloride, and the elution position of purified, ^{125}I-labeled human chromogranin A. The horizontal arrow in the lower right-hand corner of the panel indicates the lower limit of detection in the radioimmunoassay in which these samples were assayed.

the approximate 5 kilodalton porosity cutoff of cellulose membranes.

To evaluate the region of the molecule to which these chromogranin A fragments correspond, plasma samples were immunoextracted with solid-phase region-specific anti-chromogranin A antibodies (Table 3). In both normal and uremic plasma, the anti-mid-molecule antibody efficiently extracted immunoreactivity, while the anti-N- and C-terminal antibodies were less effective, suggesting that the bulk of the circulating immunoreactivity consisted of mid-molecule chromogranin A fragments recognized by the radioimmunoassay.

Table 4 documents a lack of correlation between blood pressure and plasma chromogranin A. In untreated subjects in both the mid-range renal disease and end-stage renal disease groups, there was no relationship of chromogranin A to blood pressure ($P > 0.1$).

Table 5 compares plasma chromogranin A and corresponding plasma catecholamine concentrations in four groups: normal controls, mid-range renal insufficiency, end-stage renal disease, with plasma chromogranin A either <500 ng/ml or >500 ng/ml. Plasma chromogranin A concentration increased as a direct function of the rise in serum creatinine (Fig. 3), while plasma catecholamines rose in mid-range renal disease to a level as high as that seen in end-stage renal disease. These relationships suggest that the rise in plasma chromogranin A is the result of renal dysfunction alone, and is not tightly coupled to sympathoadrenal activation.

Figure 4 examines the relationship of parathyroid hormone (MM-PTH and INS-PTH) to renal function and plasma chromogranin A. Parathyroid hormone rose with increasing serum creatinine (for MM-PTH: $r = 0.327$, $N = 91$, $P < 0.01$; for INS-PTH: $r = 0.20$; $N = 91$, $P > 0.05$), and displayed an even more significant rise with decrease of glomerular filtration rate (1/creatinine, or $1/S_{Cr}$). There was a modest correlation between chromogranin A and parathyroid hormone (Fig. 4, $P < 0.05$).

To determine whether chromogranin A in end-stage renal disease is more tightly linked to renal function (creatinine) or parathyroid function (parathyroid hormone), we utilized multiple linear regression, with creatinine and parathyroid hormone (both MM-PTH and INS-PTH) as independent variables and

Table 4. Influence of blood pressure upon plasma chromogranin A in MRRD and ESRD in subjects not receiving antihypertensive drugs

Group	N	Age years	SBP mm Hg	DBP mm Hg	S_{Cr} mg/dl	INS-PTH pg/ml	CgA ng/ml
MRRD							
Normotensive	7	50 ± 6	137 ± 10	82 ± 2	3.1 ± 0.4	36 ± 11 (N = 6)[a]	174 ± 33
Hypertensive[b]	8	65 ± 2	172 ± 9	105 ± 4	4.5 ± 0.7	54 ± 18 (N = 2)[a]	184 ± 32
P value	—	<0.05	<0.01	<0.01	>0.1	>0.1	>0.1
ESRD							
Normotensive	16	52 ± 4	137 ± 5	74 ± 1	13.1 ± 0.9	75 ± 15 (N = 14)[a]	475 ± 51
Hypertensive[b]	4	37 ± 5	146 ± 6	95 ± 3	13.5 ± 4.5	139 ± 57	335 ± 114
P value	—	>0.1	<0.01	<0.01	>0.1	>0.1	>0.1

Abbreviations are: MRRD, mid-range renal disease (S_{Cr} = 2.0–7.5 mg/dl); ESRD, end-stage renal disease (S_{Cr} > 7.5 mg/dl); SBP, systolic blood pressure; DBP, diastolic blood pressure; S_{Cr}, serum creatinine; INS-PTH, intact N-terminal specific parathyroid hormone (nl: 11–24 pg/ml); CgA, chromogranin A (nl < 112 ng/ml). Plus-minus values are means ± SEM.

[a] Only N samples were obtained.

[b] Hypertensive = SBP > 160 mm Hg or DBP > 90 mm Hg or both.

Table 5. Chromogranin A versus catecholamines in renal failure

Group subgroup	N	Serum creatinine mg/dl	Plasma catecholamines NE pg/ml	Plasma catecholamines EPI pg/ml	Plasma CgA ng/ml
Normal controls	37	0.9 ± 0.1	219 ± 12 (N = 22)[a]	44 ± 6 (N = 22)[a]	50 ± 3
MRRD	5	3.0 ± 0.2	1013 ± 384	132 ± 24	211 ± 48
ERSD	9	16.9 ± 1.9	832 ± 127	75 ± 17	475 ± 50
with CgA					
<500 ng/ml	5	13.3 ± 2.0	830 ± 231	55 ± 11	351 ± 21
>500 ng/ml	4	21.4 ± 1.9	834 ± 88	99 ± 35	630 ± 7

Abbreviations are: NE, norepinephrine; EPI, epinephrine; CgA, plasma chromogranin A; MRRD, mid-range renal disease (S_{Cr} = 2–7.5 mg/dl); ESRD, end-stage renal disease (S_{Cr} > 7.5 mg/dl).

[a] Only N samples were obtained.

Table 6. Chromogranin A values in ESRD subjects: Relationship to creatinine and parathyroid hormone measurements analyzed by multiple linear regression

Dependent variable	Independent variables	Coefficient	t statistic	N	P[b]
(Chromogranin A) = A_1[a] × (creatinine) + B_1[a] × (MM-PTH) + a constant					
CgA	Creatinine	31	5.06	59	<0.01
	MM-PTH	0	0.09	59	0.93
	Constant	98	1.46		0.15
(Chromogranin A) = A_2[a] × (creatinine) + B_2[a] × (INS-PTH) + a constant					
CgA	Creatinine	30	5.13	59	<0.01
	INS-PTH	0.27	0.46	59	0.65
	Constant	88	1.24		0.22

Abbreviations are: ESRD, end-stage renal disease (S_{Cr} > 7.5 mg/dl); MM-PTH, C-terminal (mid-molecule) parathyroid hormone; INS-PTH, intact N-terminal specific parathyroid hormone; CgA, chromogranin A.

[a] A_1, A_2, B_1, B_2 are coefficients corresponding to creatinine, MM-PTH, and INS-PTH

[b] P values are obtained by multiple regression test

chromogranin A as the dependent variable. The results (Table 6) indicate that chromogranin A is most tightly linked to degree of renal insufficiency as assayed by serum creatinine ($P < 0.01$), while parathyroid hormone is a comparatively ineffective predictor of chromogranin A concentration ($P = 0.93$ for MM-PTH and $P = 0.65$ for INS-PTH).

Table 7 shows parathyroid hormone and chromogranin A concentrations during selective venous sampling in the neck region of a uremic secondary hyperparathyroid subject with elevated plasma chromogranin A. Parathyroid hormone concentration "steps up" at the right and left superior thyroidal veins, while chromogranin A remains constant, further suggesting that parathyroid corelease of chromogranin A/secretory protein I [7] is not a major determinant of plasma chromogranin A concentration in uremia.

To investigate whether the etiology of renal failure has any bearing on chromogranin A concentration, 62 end-stage renal disease patients (39 male and 23 female) were stratified into five groups according to underlying diseases which resulted in renal failure: glomerulonephritis, diabetes mellitus, polycystic kidney

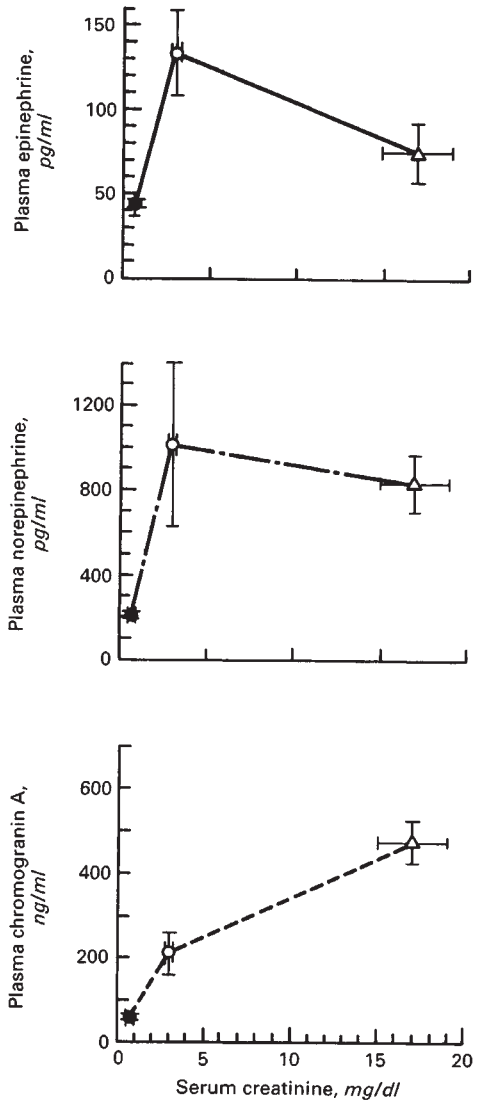

Fig. 3. *The comparative values of plasma epinephrine (pg/ml), norepinephrine (pg/ml) and chromogranin A (ng/ml) in normal controls (●), mid range renal disease (○) and end-stage renal disease (△).* Bars denote means ± SEM.

Table 7. Chromogranin A and parathyroid hormone distribution in selective venous samples from the neck region in a subject with secondary (uremic) hyperparathyroidism

Venous sampling site	Serum parathyroid hormone		Serum CgA
	INS *pg/ml*	IRMA *pg/ml*	*ng/ml*
R. vertebral	32	271	449
R. small vertebral	31	246	555
R. low internal jugular	35	319	491
R. superior jugular	37	282	461
Proximal L. innominate	36	315	445
L. vertebral	34	261	529
Low L. vertebral	36	264	529
Inferior thyroidal	33	353	542
Accessory inferior thyroidal	97	844	462
Thymic branch	35	279	452
R. superior thyroidal	116	5984	466
Deeper R. superior thyroidal	114	3328	503
Upper L. internal jugular	35	275	616
L. superior thyroidal	374	6128	471
Lower L. jugular	37	320	468
Superior vena cava	62	402	482

Abbreviations are: INS-PTH, intact N-terminal specific parathyroid hormone (nl: 11–24 pg/ml); IRMA-PTH, parathyroid hormone (intact, whole molecule) measured by immunoradiometric assay (nl: 10–65 pg/ml); CgA, chromogranin A (nl < 112 ng/ml).

disease, tubulointerstitial nephritis, and hypertension. A comparison by ANOVA showed no significant variation of chromogranin A by intergroup comparison (Table 8, $P = 0.56$).

To evaluate the effects of chronic therapeutic dialysis upon plasma chromogranin A, all 62 patients from the end-stage renal failure group were examined. All except three were maintained by either hemodialysis or peritoneal dialysis. Compared with dialyzed subjects, nondialyzed subjects had lower plasma chromogranin A concentration as well as lower serum creatinine concentration (Table 9). Thus, in the 59 dialyzed subjects, more severe uremia apparently both necessitated dialysis and resulted in greater accumulation of chromogranin A.

Mode of dialysis (hemodialysis versus peritoneal dialysis) did not influence plasma chromogranin A or serum creatinine (Table 10, $P > 0.1$).

When arterial plasma chromogranin A and total protein concentration were evaluated in samples obtained from paired pre-dialysis and post-dialysis periods in five hemodialysis subjects, there was no significant change in either chromogranin A (from 1307 ± 939 to 1325 ± 972 ng/ml, $P > 0.1$) or total protein (from 87 ± 5 to 93 ± 4 mg/ml, $P > 0.1$) upon hemodialysis (Fig. 5). Furthermore, chromogranin A immunoreactivity was not detectable in dialysate after countercurrent exposure to uremic blood ($N = 5$). These results indicate that uremic plasma chromogranin A immunoreactivity is not hemodialyzable.

Chromogranin A dialyzability was also evaluated during chronic ambulatory peritoneal dialysis (Table 11). Chromogranin A was detectable in peritoneal dialysate, and the amount of immunoreactivity in peritoneal dialysate was time dependent. In three patients who underwent four- to five-hour peritoneal dialysate dwell time, the mean chromogranin A immunoreactivity in the dialysate was 54 ± 6 ng/ml, with a plasma concentration of 792 ± 128 ng/ml. In one subject whose dwell time was 72 hours, the dialysate chromogranin A immunoreactivity approached that of plasma (640 vs. 660 ng/ml). The plasma chromogranin A clearance through peritoneal dialysis was 0.52 ± 0.08 ml/min (range 0.33 to 0.73), only a small fraction of the previously computed total body chromogranin A clearance in normal controls (147 ml/min) [17]. Chromogranin A specific activity (μg/mg protein) in peritoneal dialysate was substantially higher than that in plasma (Table 12).

Table 12 shows plasma and urinary chromogranin A immunoreactivity in normal controls ($N = 6$) and subjects with proteinuria ($N = 6$). No chromogranin A immunoreactivity was detectable in normal controls' urine, but chromogranin A was clearly present in proteinuric urine, albeit at a far lower concentration (ng/ml) than that found in matched plasma. Chromogranin A specific activity (μg/mg protein) was higher in

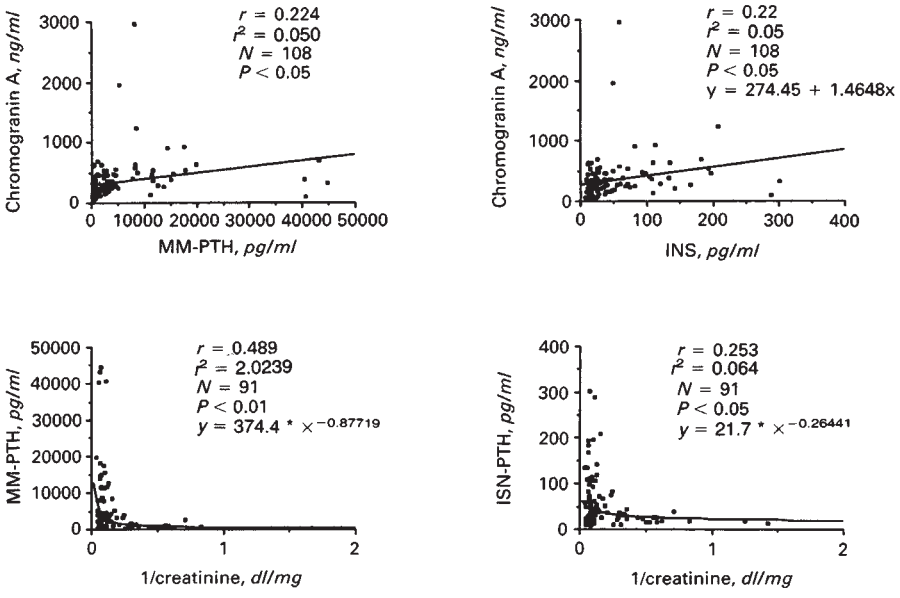

Fig. 4. *Relationships between chromogranin A, serum immunoreactive parathyroid hormone and renal function.* Parathyroid hormone activity was measured by either the MM-PTH assay (which detects PTH as well as its C-terminal or mid-molecule fragments [13]) or the INS-PTH assay (which detects the whole molecule or its N-terminal determinants [13]). There was a significant relationship between MM-PTH (left panels) and serum creatinine ($r = 0.327$, $N = 91$, $P < 0.01$) and an even more impressive correlation between MM-PTH and the reciprocal of serum creatinine ($r = 0.489$, $N = 91$, $P < 0.01$). The correlation between chromogranin A and MM-PTH, however, was less significant ($r = 0.224$, $N = 108$, $P < 0.05$). Compared with MM-PTH, INS-PTH (right panels) had a less significant relationship to creatinine ($r = 0.20$, $N = 0.91$, $P > 0.05$), reciprocal of creatinine ($r = 0.253$, $N = 91$, $P < 0.05$), or chromogranin A ($r = 0.22$, $N = 108$, $P < 0.05$).

Table 8. Chromogranin A values in ESRD subjects stratified by etiology of renal disease

Group	*N*	Age *years*	SBP *mm Hg*	DBP *mm Hg*	S_{Cr} *mg/dl*	INS-PTH *pg/ml*	CgA *ng/ml*
GN	28	47 ± 3	141 ± 3	83 ± 2	14.0 ± 0.9	71 ± 14 ($N = 25$)[a]	670 ± 175
DM	14	55 ± 3	149 ± 6	80 ± 3	12.7 ± 0.9	65 ± 22	392 ± 35
PCKD	7	60 ± 6	132 ± 7	77 ± 4	13.8 ± 1.4	75 ± 22	400 ± 33
TIN	6	36 ± 5	140 ± 8	83 ± 5	13.4 ± 2.2	67 ± 27	824 ± 432
HTN	7	56 ± 8	152 ± 9	82 ± 6	11.3 ± 1.1	41 ± 13	379 ± 59

Abbreviations are: GN, glomerulonephritis; DM, diabetes mellitus; PCKD, polycystic kidney disease; TIN, tubulointerstitial nephritis; HTN, hypertension; other abbreviations defined in Table 1. Plus-minus values are mean ± SEM.
[a] Only *N* samples were obtained.

Table 9. Influence of chronic therapeutic dialysis upon plasma chromogranin A in ESRD

Group	*N*	Age *years*	SBP *mm Hg*	DBP *mm Hg*	S_{Cr} *mg/dl*	INS-PTH *pg/ml*	CgA *ng/ml*
Undialyzed	3	56 ± 4	157 ± 16	89 ± 6	8.0 ± 0.3	78 ± 33	247 ± 12
Dialyzed[a]	59	50 ± 2	142 ± 2	81 ± 2	13.6 ± 0.5	65 ± 9 ($N = 56$)[b]	575 ± 94
P value	—	> 0.1	> 0.1	> 0.1	< 0.05	> 0.1	< 0.01

Abbreviations are: ESRD, end-stage renal disease ($S_{Cr} > 7.5$ mg/dl); SBP, systolic blood pressure; DBP, diastolic blood pressure; S_{Cr}, serum creatinine; INS-PTH, intact N-terminal specific parathyroid hormone (nl: 11–24 pg/ml); CgA, chromogranin A (nl < 112 ng/ml).
[a] Either hemodialysis ($N = 52$) or peritoneal dialysis ($N = 7$)
[b] Only *N* samples were obtained.

proteinuric urine than in matched plasma, reminiscent of the peritoneal dialysate result (Table 11).

Discussion

Chromogranin A was initially identified as a protein co-stored and co-released with catecholamines from secretory vesicles [1, 2]. Subsequently it was found in a variety of endocrine tissues [8, 18–22], and because its plasma concentration is elevated in patients with peptide-producing endocrine neoplasms [5], it may be a diagnostic tool for these endocrine tumors [5]. In a prior study [5], we found that uremic subjects with secondary hyperparathyroidism also had elevated plasma chromogranin A. Because uremia per se may perturb peptide hormone metabolism [6], and because of the known structural and immunologic similarities between chromogranin A and parathyroid secretory protein I [7, 22, 23], we investigated the effect of renal dysfunction and uremic (secondary) hyperparathyroidism on plasma chromogranin A.

We found that the kidney seems to play a role in chromogranin A disposition or catabolism. In uremic subjects, decreased glomerular filtration resulted in an increase in plasma chromogranin A concentration (Table 1, Fig. 1).

Uremic plasma gel filtration (Fig. 2) suggested that chromogranin A immunoreactivity consisted of fragments of the parent chromogranin A molecule. Results of region-specific, solid-phase immunoextraction of uremic plasma (Table 3) further

Table 10. Plasma chromogranin A values in dialyzed ESRD subjects stratified by type of dialysis

Group	N	Age years	SBP mm Hg	DBP mm Hg	S_{Cr} mg/dl	INS-PTH pg/ml	CgA ng/ml
Hemodialysis	52	52 ± 2	144 ± 3	82 ± 2	13.7 ± 0.6	64 ± 10 (N = 50)[a]	573 ± 106
Peritoneal dialysis	7	35 ± 3	127 ± 7	78 ± 4	12.8 ± 0.6	75 ± 18 (N = 6)[a]	587 ± 104
P value	—	< 0.01	< 0.05	> 0.1	> 0.1	> 0.1	>0.1

Abbreviations are: ESRD, end-stage renal disease (S_{Cr} > 7.5 mg/dl); SBP, systolic blood pressure; DBP, diastolic blood pressure; S_{Cr}, serum creatinine; INS-PTH, intact N-terminal specific parathyroid hormone (nl: 11–24 pg/ml); CgA, chromogranin A (nl < 112 ng/ml).
[a] Only N samples were obtained.

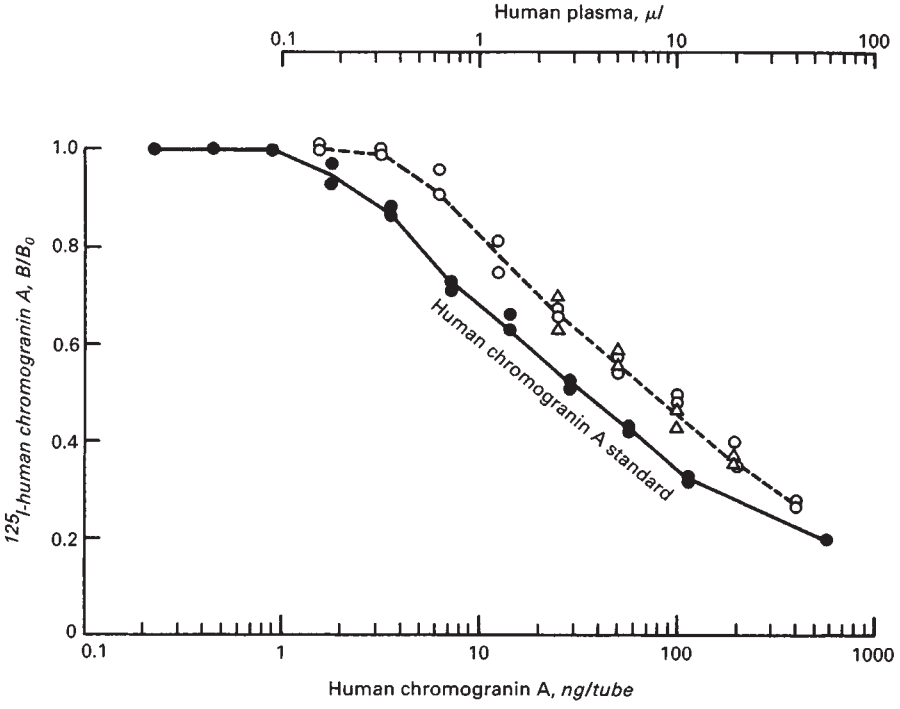

Fig. 5. *Standard curve of the human chromogranin A radioimmunoassay, and pre- and post-hemodialysis chromogranin A immunoreactivity in a uremic subject.* B/B_0 for ^{125}I-chromogranin A is plotted versus $\log_{10}$ of added unlabeled chromogranin A or uremic plasma. B = counts per minute (minus blank) for any given assay tube; B_0 = counts per minute (minus blank) for maximum-binding assay tubes, without added unlabeled standard or unknowns. Symbols are: (○----○) pre-hemodialysis chromogranin A immunoreactivity in a uremic subject; (△----△) post-hemodialysis chromogranin A immunoreactivity in the same uremic subject.

Table 11. Effect of continuous ambulatory peritoneal dialysis on chromogranin A in ESRD (N = 4)

Parameter	Mean ± SEM	Range
Chromogranin A *ng/ml*		
Plasma	759 ± 97	535 to 930
Dialysate	200 ± 147	43 to 640
Chromogranin A plasma clearance *ml/min*	0.52 ± 0.08	0.33 to 0.73
Total protein *mg/ml*		
Plasma	87 ± 7	62 to 99
Dialysate	7.2 ± 7.0	0.23 to 34
Chromogranin A specific activity *ng/mg protein*		
Plasma	8.0 ± 0.9	6 to 10
Dialysate	116 ± 36	19 to 190

Conditions of continuous ambulatory peritoneal dialysis are given in the **Methods** section.

Table 12. Plasma and urinary chromogranin A in subjects with proteinuria

	Normal controls	Proteinuria
N	6	6
Plasma or serum		
Chromogranin A *ng/ml*	41 ± 4	268 ± 85
Creatinine *mg/dl*	0.9 ± 0.1	3.6 ± 1.0
Total protein *mg/ml*	—	68 ± 10
Chromogranin A specific activity *ng/mg protein*	—	4.6 ± 1.5
Urine		
Chromogranin A *ng/ml*	Undetectable (< 5 ng/ml)	62 ± 16
Creatinine *mg/dl*	113 ± 44	80 ± 17
Total protein *mg/ml*	Undetectable	0.6 ± 0.2
Chromogranin A specific activity *ng/mg protein*	Undetectable	162 ± 48

support the idea of circulating chromogranin A fragments, most of which possess mid-molecule epitopes. Bidirectional (N-terminal and/or C-terminal) proteolytic processing of chromogranin A has been demonstrated in chromaffin granules [11, 24].

While we were unable to detect urinary chromogranin A immunoreactivity in normal controls, chromogranin A immunoreactivity was clearly present in the urine of subjects with proteinuria (Table 12), at a higher "specific activity" (μg chromogranin A/mg protein) than the corresponding plasma sample. Since chromogranin A, at a Stokes radius of 77 to 80 Å

[1] is unlikely to be filtered at the glomerulus as the intact molecule, the urinary chromogranin A immunoreactivity likely also represents chromogranin A fragments filtered by damaged glomeruli.

Both INS-PTH and MM-PTH rose as renal function deteriorated (Fig. 4), with an especially marked elevation of MM-PTH as a function of the fall in renal function ($1/S_{Cr}$); such changes in parathyroid hormone have previously been reported [24–26]. There was only a modest correlation ($r = 0.22$) between chromogranin A and the indices of parathyroid activity (MM-PTH and INS-PTH). Once a serum creatinine (renal function) effect on chromogranin A and parathyroid hormone (either MM or INS) was factored out, using multiple regression (Table 6), there was no further correlation between parathyroid function and chromogranin A concentration (Table 6). Furthermore, chromogranin A and parathyroid hormone values from neck venous catheterization illustrated a dissociation between parathyroid hormone and chromogranin A secretion in uremia (Table 7). Thus, we conclude that in uremic (secondary) hyperparathyroidism, parathyroid cosecretion of parathyroid hormone and chromogranin A/secretory protein I [7], is not a major determinant of the chromogranin A elevation.

Subjects who are dialyzed have higher plasma chromogranin A concentrations than those who are not dialyzed (Table 9), simply consistent with more advanced uremia, as suggested by higher serum creatinine. Chromogranin A is not affected by nor filtered by hemodialysis (Fig. 5). We detected chromogranin A immunoreactivity in peritoneal dialysate (Table 11). In both peritoneal dialysate (Table 11) and proteinuric urine (Table 12), chromogranin A specific activity (μg/mg protein) is higher than that of matched plasma. Perhaps the fragmented chromogranin A molecule in uremic plasma is small enough to allow filtration across both peritoneal and damaged glomerular membranes. The peritoneal dialysate chromogranin A immunoreactivity was not so small, however, as to be filterable across cellulose dialysis tubing (Table 2).

Finally, we found that neither etiologic diagnosis (Table 8), catecholamine release (Table 5, Fig. 3), nor blood pressure (Table 4) were closely linked to chromogranin A concentration in renal failure.

In conclusion, plasma chromogranin A rises with deterioration of renal function, and this elevation is apparently independent of augmented sympathoadrenal activity or hyperparathyroidism. Rather, the rise seems to reflect a retention of fragments of chromogranin A in uremia. Thus, when chromogranin A is used as a tool for evaluation of peptide or amine or peptide hormone storage and release, and especially in the diagnosis of neuroendocrine neoplasms, it is essential that subjects' renal status be taken into consideration.

Acknowledgments

This study was supported by the Veterans Administration, the National Institutes of Health, and the American Heart Association. We appreciate the technical assistance of Ms. Annie Chen and Ms. Justine Cervenka. Dr. David Endres (Nichols Institute, San Juan Capistrano, California, USA) assayed samples for parathyroid hormone. Drs. Thomas Ziegler, Roland Blantz, David Ward, and Robert Steiner allowed us to obtain samples from their patients with renal disease. Ms. Esther Carlton (Nichols Institute) supplied neck vein selective catheterization samples.

Reprint requests to Daniel T. O'Connor, M.D., Nephrology/Hypertension (V-111-H), V.A. Medical Center, San Diego, California 92161, USA.

References

1. Smith AD, Winkler H: Purification and properties of an acidic protein from chromaffin granules of bovine adrenal medulla. *Biochem J* 103:483–492, 1967
2. Smith WJ, Kirshner N: A specific soluble protein from the catecholamine storage vesicles of bovine adrenal medulla. I. Purification and chemical characterization. *Mol Pharmacol* 3:52–62, 1967
3. Blaschko H, Comline RS, Schneider FJ, Silver M, Smith AD: Secretion of a chromaffin granule protein, chromogranin, from the adrenal gland after splanchnic stimulation. *Nature* 215:58–59, 1967
4. Sage JH, Smith WJ, Kirshner N: Mechanism of secretion from the adrenal medulla. I. A microquantitative immunologic assay for bovine adrenal catecholamine storage vesicle protein and its application to studies of the secretory process. *Mol Pharmacol* 3:81–89, 1967
5. O'Connor DT, Deftos LJ: Secretion of chromagranin A by peptide-producing endocrine neoplasms. *N Engl J Med* 314:1145–1151, 1986
6. Rabkin R, Kitaji J: Renal metabolism of peptide hormones. *Miner Electrol Metab* 9(4–6):212–216, 1983
7. Cohn DV, Zangerle R, Fischer-Colbrie R, Chu LLH, Elting JJ, Hamilton JW, Winkler H: Similarity of secretory protein I from parathyroid gland to chromogranin A from the adrenal medulla. *Proc Natl Acad Sci USA* 79:6056–6059, 1982
8. O'Connor DT: Chromogranin: Widespread immunoreactivity in polypeptide hormone producing tissues and in serum. *Reg Peptide* 6:263–280, 1983
9. O'Connor DT, Pandian MR, Carlton E, Cervenka JH, Hsiao RJ: Rapid measurement of circulating human chromogranin A: In vitro stability, exploration of the neuroendocrine character of neoplasia, and assessment of the effects of organ failure. *Clin Chem* 35:1631–1637, 1989
10. Doolittle RF: *Of Urfs and Orfs: A Primer On How To Analyze Derived Aminoacid Sequences*. University Science Books, Mill Valley, California, 1986
11. O'Connor DT, Takiyyuddin M: Synthetic peptide epitopes: Clues to the structure, conformation, distribution and processing of chromogranin A. (abstract) *Clin Res* 36:187A, 1988
12. Harlow E, Lane D: *Antibodies: A Laboratory Manual*. Cold Spring Harbor Laboratory, New York, 1988
13. Endres D, Brickman A, Goodman W, Maloney N, Sherrard D: N- and C-terminal PTH radioimmunoassays in assessment of renal osteodystrophy. *Kidney Int* 21:132, 1982
14. Nussbaum SR, Zahradnik RJ, Lavigne JR, Brennan GL, Nozawa-Ung K, Kim LY, Keutmann HT, Wang C-A, Potts JT Jr, Segre GY: Highly sensitive two-site immunorediometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. *Clin Chem* 33:1364–1367, 1987
15. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. *Anal Biochem* 72:248–254, 1976
16. Peuler JD, Johnson GA: Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. *Life Sci* 21:625–636, 1977
17. O'Connor DT, Bernstein KN: Radioimmunoassay of chromogranin A in plasma as a measure of exocytotic sympathoadrenal activity in normal subjects and patients with pheochromocytoma. *N Engl J Med* 311:764–770, 1984
18. O'Connor DT, Burton DW, Deftos LJ: Chromogranin A: Immunohistology reveals its universal occurrence in normal polypeptide hormone producing endocrine glands. *Life Sci* 33:1657–1663, 1983
19. O'Connor DT, Burton DW, Deftos LJ: Immunoreactive human chromogranin A in diverse polypeptide hormone producing human tumors and normal endocrine tissues. *J Clin Endocrinol Metabol* 57:1084–1086, 1983

20. LLOYD RV, WILSON BS: Specific endocrine tissue marker defined by a monoclonal antibody. *Science* 222:628–630, 1983
21. WILSON BS, LLOYD RV: Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. *Am J Pathol* 115: 458–468, 1984
22. COHN DV, ELTING JJ, FRICK M, ELDE R: Selective localization of parathyroid secretory protein I/adrenal medulla chromogranin A in a wide variety of endocrine cells of the rat. *Endocrinology* 114: 1963–1974, 1984
23. KRUGGEL W, O'CONNOR DT, LEWIS RV: The amino terminal sequences of bovine and human chromogranin A and secretory protein I are identical. *Biochem Biophys Res Com* 127:380–383, 1985
24. WOHLFARTER T, FISCHER-COLBRIE R, HOGUE-ANGELETTI R, EIDEN LE, WINKLER H: Processing of chromogranin A within chromaffin granules starts at C- and N-terminal cleavage sites. *FEBS Lett* 231:67–70, 1988
25. ARNAUD CD: Hyperparathyroidism and renal failure. *Kidney Int* 4:89–95, 1973
26. FREITAG J, MARTIN KJ, HRUSKA KA, ANDERSON C, CONRADES M, LADENSON J, KLAHR S, SLATOPOLSKY E: Impaired parathyroid hormone metabolism in patients with chronic renal failure. *N Engl J Med* 298:29–32, 1978
27. HRUSKA KA, KORKOR A, MARTIN K: Peripheral metabolism of intact parathyroid hormone—role of liver and kidney and the effect of chronic renal failure. *J Clin Invest* 67:885–892, 1981