Immunological, Biochemical, and Enzymic Validation of Radioimmunoassays Specific to the Amino and Carboxy Terminal of Human Calcitonin

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We characterized rabbit antisera to synthetic human calcitonin that specifically recognize the amino- and carboxy-terminal regions of calcitonin (antisera I-506 and HMS-385, respectively), by both classical (parallelism of dose–response curves) and (owing to the unavailability of a variety of analog fragments of human calcitonin) biochemical plus enzymic alteration of synthetic human calcitonin. We used nonequilibrium double-antibody assay conditions and incubation times of $48 + 72 + 24$ h. Final concentrations in the assay tube (1 mL) for I-506 and HMS-385 were 1:100 000 and 1:200 000, respectively. Midpoint of the calcitonin dose–response curve was 95 and 425 pg in the I-506 and HMS-385 assays, respectively. The intra- and inter-assay CV was <8 and 17%, respectively, for each assay. The HMS-385 antiserum was shown to recognize the products of chymotryptic digestion plus oxidation and reduction of the amino-terminal disulfide bond of calcitonin in addition to complete immunological recognition of a synthetic carboxy-terminal hexapeptide fragment. The I-506 antiserum failed to recognize the calcitonin fragments after chymotrypsin digestion and the carboxy-terminal hexapeptide fragment. Immunoreactivity in the I-506 assay was also reduced by about 90% after oxidation and reduction of the amino-terminal disulfide bond. HMS-385 cross reacted completely with rat and human calcitonin; I-506 was completely specific only to human calcitonin. Neither antiserum bound salmon, porcine, or bovine calcitons. The immunological determinants for the HMS-385 and I-506 antisera evidently reside in the amino acid sequence of the extreme carboxy-terminal region and the conformation plus amino acid sequence of the amino terminal, respectively. In normal human sera, immunoreactive calcitonin was undetectable (<10–20 ng/L) in 87 and 53% of samples with the carboxy- and amino-terminal antisera, respectively. In 97% of all normal sera analyzed with both antisera, immunoreactive calcitonin was <200 ng/L. In contrast, higher concentrations of carboxy- than amino-terminal immunoreactivity were noted in extracts of normal thyroids and sera from cancer patients.

Additional Keyphrases: reference intervals • thyroid cancer • concentration in normal human thyroid tissue • various $M_r$ forms of calcitonin in serum • site of immunological determinants • peptide hormones • specificity of antiserum

Human calcitonin is a 32-residue peptide that is found in low concentration in serum from normal human subjects (1). The normal concentration of calcitonin is debated among laboratories (2–8), in part because of the variation among antisera in their ability to recognize the various multiple molecular mass forms of serum immunoreactive calcitonin (9–11). A detailed investigation into the identity of these multiple forms of calcitonin would aid in understanding their role, both in physiological and pathological conditions. However, radioimmunoassays with well-defined specificities for particular amino acid sequences of human calcitonin must be developed to aid in identifying the multiple forms of calcitonin in serum.

Here we detail the characterization of antiseras that are specific to the amino- and carboxy-terminal regions of calcitonin. Because no large number of synthetic analogs to human calcitonin is available, we determined antisera specificity by examining the immunological cross reaction with: (a) a single synthetic carboxy-terminal hexapeptide analog of human calcitonin, (b) the fragments of calcitonin produced by incubation with trypsin (EC 3.4.4.4) and α-chymotrypsin (EC 3.4.4.3), (c) the products formed by oxidation and reduction of the amino-terminal disulfide bond, (d) sera from patients with medullary carcinoma of the thyroid, (e) crude extracts of normal human and rat thyroids, (f) purified preparations of porcine and bovine calcitonin, and (g) a synthetic preparation of salmon calcitonin.

We also report data on the concentration of amino- and carboxy-terminal immunoreactive calcitonin in extracts of normal human thyroids and in sera from both normal adult volunteers and patients with medullary carcinoma of the thyroid.

Materials and Methods

Radioiodination

Synthetic human calcitonin (Ciba Geigy Ltd., Basel, Switzerland; lot no. E-10235) was radioiodinated by previously published procedures (12). In brief, into a "V-vial" (Kontes, Vineland, NJ 08360; cat. no. K-749001) was placed: 4 μg of calcitonin (10 μL, in 17.3 mmol/L acetic acid); 10 μL of 0.5 mol/L sodium acetate, pH 5.0; 3 μg of lactoperoxidase (EC 1.11.1.7, lot no. 200141; Calbiochem-Behring Corp., La Jolla, CA 92112) in 10 μL of the sodium acetate buffer; and 2 mCi of Na$^{125}$I in 4 μL of 0.1 mol/L sodium hydroxide (Amersham, Arlington Heights, IL 60005; cat. no. IMS.300). The reaction was begun by adding hydrogen peroxide (10 μL, 6 mg/L; J. T. Baker Chemical Co., Phillipsburg, NJ 08865). A similar aliquot of hydrogen peroxide was added at 5 min, and the reaction was stopped at 10 min by adding 200 μL of a mixture of 50 mmol/L sodium acetate, 25 mmol/L sodium azide, pH 5.0. The reaction mixture was added to 10 mL of 0.1 mol/L tris(hydroxymethyl)aminomethane buffer, pH 9.5, and layered on a 1 × 4 cm QAE-Sephadex column (Pharmacia Fine Chemicals, Inc., Piscataway, NJ 08854). The radioiodinated calcitonin was eluted from the resin with a gradient of 0.0–0.3 mol/L sodium chloride in the tris(hydroxymethyl)-aminomethane buffer. The $^{125}$I-labeled calcitonin generally exhibited a specific activity of 4350 kCi/mol. The tracer was stored in assay buffer at 4 °C and was used for three weeks in the assay.

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Production of Antiserum

Antiserum I-506 was raised in rabbits immunized with synthetic unconjugated human calcitonin (Ciba-Geigy) and was generously provided by Dr. B. G. England (University of Michigan).

In our laboratory, female Dutch-Belted rabbits were immunized (13) with 200 μg of the same synthetic human calcitonin preparation without conjugation to a carrier protein. Rabbits were bled from the marginal ear vein, and the sera were stored at −70 °C. The bleeding obtained 16 weeks after the initial immunization of rabbit HMS-385 was used for characterization.

Radioimmunoassay Procedure

The standard calcitonin buffer (pH 7.5) contained, per liter, 50 mmol of phosphate, 150 mmol of sodium chloride, 1 g of bovine serum albumin, plus 0.1 g of sodium azide, and ethylenediaminetetraacetic acid, disodium salt. Using nonequilibrium assay conditions, we first incubated (sample or standard plus antiserum) for 48 h at 4 °C. Then approximately 25 000 cpm of 125I-labeled calcitonin tracer was added, along with 4 μg of rabbit IgG (Miles Laboratories, Inc., Elkhart, IN 46515), and the incubation was continued for 72 h at 4 °C. Goat antirabbit gamma-globulin was added, and the tubes were incubated for an additional 24 h at 4 °C. Hereafter, incubation intervals are referred to as “48 + 72 + 24 h.” Incubation volume was 1 mL, and the final concentrations of I-506, HMS-385, and goat antirabbit gamma-globulin were 1:100 000, 1:200 000, and 1:240, respectively. Human serum did not exceed 20% of the final volume in all test tubes of the assay. Synthetic calcitonin was added in increasing amounts to charcoal-extracted normal human serum at 4 °C, and the samples were analyzed to determine the percentage analytical recovery of immunoreactive calcitonin.

Studies of Antisera Specificity

We examined the degree of parallelism between the standard calcitonin dose–response curve and curves obtained with serial dilutions of: (a) sera from patients with medullary carcinoma of the thyroid, (b) extracts of normal human and rat thyroids, (c) purified porcine (lot no. K533-114; Armour Pharmaceutical Co., Kankakee, IL 60901) and bovine (lot no. 400170; Calbiochem-Behring Corp.) calcitonin, and (d) synthetic salmon (lot no. K713-081; Armour Pharmaceutical Co.) calcitonin. Thyroids were obtained from humans at autopsy and from rats killed by exposure to a carbon dioxide atmosphere.

With a scalpel, the thyroids were minced into 2- to 3-mm cubes at 4 °C and homogenized (Polytron, Model PCU-2-110; Brinkmann Instruments, Westbury, NY 11590) in the presence of a serine protease inhibitor (14), phenylmethyl sulfonylfluoride (2 mmol/L). The clear supernatant fluid after centrifugation (6000 × g) was lyophilized and stored at −70 °C.

We synthesized an analog of human calcitonin that included the carboxy-terminal hexapeptide region [Orn(Ala)IleGly-ValGlyAlaPro-NH2], by the solid-phase procedure (15), purified it by gel permeation chromatography, and characterized it as to amino acid composition. We examined the effect of oxidation and reduction of the amino-terminal disulfide bond on immunoreactivity by oxidizing synthetic human calcitonin with performic acid (16) and by reducing with dithiothreitol (17). The respective products were examined by isoelectric focusing, as described previously (12).

In addition, synthetic human calcitonin was incubated at 37 °C for increasing intervals with either trypsin (lot no. TRL3-36N681), in a substrate-to-enzyme weight ratio of 100:1, or α-chymotrypsin (lot no. 1450CDI 37E871) in a ratio of 100:1 and 10 000:1. Before incubation, the trypsin was treated with L-1-tosylamide-2-phenylethyl-chloromethyl ketone (lot no. 97C-0164) to inhibit the contaminant α-chymotryptic activity (18). Similarly, α-chymotrypsin was treated with p-tosyl-L-lysine chloromethyl ketone (lot no. 67C-0447) to inhibit the contaminant trypsin activity (19). The inhibitors were purchased from Sigma Chemical Co., St. Louis, MO 63176, and the enzymes from Worthington Biochemical Corp., Freehold, NJ 07728. Synthetic calcitonin was incubated with trypsin in a pH 8.1 buffer consisting of, per liter, 0.46 mol of tris(hydroxymethyl)aminomethane and 11.5 mmol of calcium chloride, and with α-chymotrypsin in a pH 7.8 buffer containing, per liter, 80 mmol of tris(hydroxymethyl)aminomethane and 0.1 mol of calcium chloride. Heat-inactivated (65 °C for 30 min) normal human plasma was added at the end of each incubation period. The incubation mixtures were frozen rapidly in solid CO2/acetone and stored at −70 °C until analyzed.

Sera

Blood was drawn by venipuncture from normal adult volunteers and from patients with medullary carcinoma of the thyroid. The sera obtained on centrifugation were frozen at −70 °C until analyzed.

Results

Assay Characteristics

The incubation intervals we selected for adding reagents to the nonequilibrium assay systems were derived from preliminary experiments (data not shown). Utilizing the 48 + 72 + 24 h incubation periods, we could quantitatively detect calcitonin (Figure 1) over the range of 4-1000 and 50-600 pg by the I-506 and HMS-385 assays, respectively. Slopes of the regression lines were each equal to 0.99. With respect to the human calcitonin dose–response curve, the midpoint was 90–100 pg and 400–450 pg in the I-506 (Figure 2, A) and HMS-385 (Figure 2, B) assays, respectively. The minimum detectable dose varied among assays, depending upon the specific activity of the tracer and the additions to the standard curve required to make it homologous with the tubes con-
taining the unknowns. In buffer alone, the minimal detectable dose was 2 and 8 pg per assay tube in the I-506 and the HMS-385 assay, respectively. The intra-assay and inter-assay coefficients of variation for either assay did not exceed 8 and 17%, respectively.

Specificity of Antisera

In both assays, data for dilutions of sera from patients with medullary carcinoma of the thyroid and extracts of normal human thyroids paralleled the synthetic human calcitonin standard dose–response curve (Figure 2, panels A and B). Dilutions of rat thyroid extract yielded an inhibition curve that paralleled the human calcitonin curve in the HMS-385 assay (Figure 2, panel B), but not in the I-506 assay (Figure 2, panel A). Salmon, porcine, and bovine calcitonins were not immunoreactive in either assay.

We examined antisera recognition of the synthetic carboxy-terminal hexapeptide analog. With the I-506 antiserum, the analog exhibited about 0.2% cross reaction (Figure 2, insert of panel A), while it was essentially equivalent to calcitonin in the HMS-385 assay (Figure 2, insert of panel B). Therefore, the immunological determinant for the HMS-385 assay resides in at least the carboxy-terminal hexapeptide region of calcitonin.

We investigated the immunological determinant for the I-506 assay by analysis for immunoreactivity after exposing calcitonin both to serine proteases or to chemicals that would oxidize or reduce the amino-terminal disulfide bond. Figure 3 depicts the immunological activity of calcitonin after increasing intervals of incubation with either trypsin (panel A) or high (panel B) and low (panel C) concentrations of α-chymotrypsin. Analysis of the incubation mixtures containing trypsin indicated that no loss of immunoreactive calcitonin was detectable by the HMS-385 assay during the 6 h of incubation, but a 35% loss was noted by the I-506 assay (panel A). Analysis of the incubation mixtures containing α-chymotrypsin indicated that with the I-506 assay there was an almost complete loss of immunoreactive calcitonin after incubation for 10 min with the higher enzyme concentration (panel B). For the same mixtures the HMS-385 assay showed a 30% decrement in immunoreactive calcitonin by 45 min of incubation. With the lower concentration of α-chymotrypsin (panel C), the HMS-385 assay detected no loss of immunoreactive calcitonin after even 2 h of incubation, while the I-506 assay again revealed a progressive decrease during the first hour of incubation.

Incubation mixtures were submitted to isoelectric focusing, to aid us in identifying the points of enzymic cleavage of human calcitonin and therefore the immunological determinant for antiserum I-506. The isoelectric focusing patterns (electrophoretograms) of the mixtures after 1- and 6-h incubations with trypsin are depicted in Figure 4, panels A and B.
Fig. 3. Immunological activity of calcitonin incubated with serine proteases

Synthetic calcitonin (100 μg) was incubated with trypsin at a substrate-to-enzyme weight ratio of 100:1 (panel A) and α-chymotrypsin at 100:1 (panel B) and 100,000:1 (panel C). Normal human serum was added at the end of each incubation, and the samples were frozen in a solid CO2/acetone bath and stored at −70°C until analyzed with antisera I-506 (○-○) and HMS-385 (▲-▲)

respectively. The electrophoretogram of the 1-h trypsin incubation mixture, in which no loss of immunoreactivity was noted by either assay, indicated that the major peak of immunoreactive calcitonin had an isoelectric point at pH 8.0. A similar isoelectric point for human calcitonin was reported previously from this laboratory; in addition, a minor peak at 6.6 to 6.7 was also shown to be present in the synthetic hormone preparation (12). Peptides with isoelectric points of 8.8 and 9.8 to ≥10.0 and retaining the carboxy-terminal immunological determinant for HMS-385 were noted in the 1-h trypsin incubation mixture. Incubation for 6 h with trypsin produced an increase in immunoreactive forms of pI ≥8.8. In addition, analysis of the calcitonin incubated with α-chymotrypsin revealed the presence of peptides identical to those observed in the electrophoretogram of the 6-h trypsin incubation mixture (data not shown). Therefore, the loss of immunoreactivity detected by the I-506 assay in both the α-chymotrypsin and the longer incubations with trypsin was associated with increased concentrations of carboxy-terminal-immunoreactive peptides with isoelectric points of 8.8 and 9.8 to >10.0. These data suggest that the immunological determinant for the I-506 antiserum encompassed a portion of the peptide that was extremely susceptible to peptide bond cleavage by chymotrypsin-like enzymes.

We undertook additional characterization of the immunological determinant for the I-506 antiserum by electrofocusing of the reaction products formed either by reduction or oxidation of the amino-terminal disulfide bond of human calcitonin. Analysis indicated that both reduction (Figure 5, panel A) and oxidation (Figure 5, panel B) of the disulfide bond not only caused the expected shift in the peptide's isoelectric point but also decreased the peptide's immunoreactivity by about 90% as measured by the I-506 assay, when compared to the level of immunoreactivity detected by the HMS-385 assay.

Thus, the data on the enzymic and chemical modification of calcitonin suggest that the immunological determinant for the I-506 antiserum includes both the amino acid sequence

![Graph](image-url)

Fig. 4. Electrophoretogram of calcitonin incubated for 1 h (panel A) and 6 h (panel B) with trypsin

The electrofocusing columns were run for 24 h at 4°C, reaching 1000 V and 1.5 mA. Column eluents were analyzed by use of antisera I-506 (○-○) and HMS-385 (▲-▲)
Fig. 5. Electrophoretogram of calcitonin after dithiothreitol reduction (panel A) or performic acid oxidation (panel B) of the amino-terminal disulfide bond.

Column eluents were analyzed by use of antisera I-506 (0-0) and HMS-385 (A-A).

and the conformation of the amino-terminal region of human calcitonin.

Amino- and Carboxy-terminal Immunoreactive Calcitonin in Sera and Tissue Extracts

Analysis of sera from human volunteers indicated that 89% of the samples had concentrations of calcitonin that were below the detection limits of the HMS-385 carboxy-terminal assay, while 53% were undetectable with the I-506 amino-terminal assay (Figure 6). If we consider the differences in sensitivity between the assays and examine those samples with concentrations of ≤50 ng/L (pg/mL), then 95% of the HMS-385 and 70% of the I-506 observations fall below 50 ng/L. With the I-506 assay, 3% of the observations were in the range of 200–300 ng/L and the remaining 27% were in the range of 50–200 ng/L. Thus, in a normal adult population, a high percentage of individuals have more amino- than carboxy-terminal immunoreactive calcitonin. In contrast, a higher level of carboxy- than of amino-terminal immunoreactive calcitonin was noted in extracts of normal human thyroids (Table 1) and serum from patients with medullary carcinoma of the thyroid (Table 2).

Discussion

A brief comment on the chemistry of the calcitonins will

![Graph](image-url)

Fig. 6. Distribution of immunoreactive calcitonin concentrations in sera from normal adult volunteers as detected by the I-506 (hatched bars) and HMS-385 (open bars) assays. Numbers within the bars denote the number of samples comprising the observations. Charcoal-extracted normal human serum (200 μL) was added to obtain data for each point on the standard curve.

![Table](image-url)

Table 1. Immunoreactive Calcitonin in Extracts of 20 Normal Human Thyroids

<table>
<thead>
<tr>
<th>Identn. no.</th>
<th>Calcitonin, μg/mL</th>
<th>Identn. no.</th>
<th>Calcitonin, μg/mL</th>
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<tbody>
<tr>
<td></td>
<td>I-506</td>
<td></td>
<td>HMS-385</td>
</tr>
<tr>
<td>1</td>
<td>0.3</td>
<td>11</td>
<td>10.0</td>
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<tr>
<td>2</td>
<td>5.6</td>
<td>13</td>
<td>3.8</td>
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<tr>
<td>3</td>
<td>0.2</td>
<td>15</td>
<td>1.1</td>
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<tr>
<td>4</td>
<td>0.4</td>
<td>16</td>
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<tr>
<td>5</td>
<td>0.1</td>
<td>17</td>
<td>1.7</td>
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<tr>
<td>6</td>
<td>1.5</td>
<td>18</td>
<td>0.8</td>
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<tr>
<td>7</td>
<td>1.9</td>
<td>19</td>
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<tr>
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<tr>
<td>9</td>
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<td>10</td>
<td>0.5</td>
<td>22</td>
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*Expressed as micrograms of immunoreactive calcitonin per gram of lyophilized thyroid extract.
provide a background for the observations on our rabbit antiserum to human calcitonin.

Calcitonins are unique among peptide hormones because of the lack of amino acid homology among species (Figure 7). In general, the calcitonins can be divided into three classes according to amino acid homology (20, 21): (a) human and rat (93.8% homology); (b) ovine, bovine, and porcine (87.5%); and (c) salmon and eel (90.6%). The only features shared among all the peptides are: (a) an amino-terminal disulfide bonded ring structure that has six of the seven amino acids in common; (b) leucine, glycine, and prolineamide at positions 9, 28, and 32, respectively; and (c) a total of 32 amino acids.

The high degree of amino acid heterogeneity among the calcitonins has generally necessitated the use of homologous antigens for development of species-specific radioimmunoassay. Thus, our rabbit antiserum to human calcitonin behaved as expected and did not bind calcitonins of bovine, porcine, or salmon origin. The high degree of amino acid homology between rat and human calcitonin (94%) explains why our carboxy-terminal-specific HMS-385 antiserum exhibited complete immunological identity between the human and rat calcitonins. The carboxy-terminal specificity of antisera to human calcitonin probably accounts for their usefulness in assays for rat calcitonin (22-24). However, some antisera to human calcitonin, like our I-506 antiserum, reportedly are not usable for measuring rat calcitonin (25). Although the 94% homology between rat and human calcitonin suggests that antiserum to them could be used interchangeably, apparently the amino acid differences between the peptides (at residues 16 and 28) are responsible for altering the conformation and thus the immunological identity of the two peptides. This is important, because in the majority of cases the immunological determinant for an antiserum directed against a peptide is identified by using short-chain synthetic analogs of the peptide. Such an approach presumes that the conformation of the analog is identical to that of the parent peptide. Obviously, from our observations with rat and human calcitonins in the I-506 assay, there is a difference in conformation, and thus the use of analogs would not be appropriate for identifying the determinant of this antiserum.

We identified the immunological determinant for antiserum I-506 by examining the retention of immunoreactivity following both chemical and enzymic alteration of human calcitonin. The early work on porcine calcitonin (26) showed that it is extremely resistant to the action of trypsin, even though it has two trypsin-sensitive arginine residues, unless the peptide is first either reduced and alkylated or oxidized with performic acid. They also reported on the lability of the leucine bond at position 9 (common among species) to scission by \( \alpha \)-chymotrypsin. Our data on the retention of the immunoreactivity of human calcitonin incubated with trypsin demonstrate that calcitonin resists degradation by trypsin. The decrease in immunoreactivity observed with prolonged exposure to trypsin was associated with residual contaminant
α-chymotrypsin activity in the particular lot of trypsin. The extremely rapid loss of immunoreactivity noted by the I-506 assay in the α-chymotrypsin incubates indicates that a portion of the immunological determinant for this antisera resides in the region of leucine at position 9. The initial scission at leucine 9 by α-chymotrypsin would generate two peptides, i.e., calcitonin 1–9 and 10–32. The 10–32 sequence would be slightly more basic than calcitonin, because the amino group of glycine (position 10) has a higher dissociation constant (pKd = 9.8) than does the α-amino group of cystine (pKα = 8.9). The 10–32 sequence probably corresponds to the pI 8.8 peptide noted in Figure 4. The small amino-terminal 1–9 fragment lost immunoreactivity in both assays. Although calcitonin has several potential α-chymotrypsin cleavage points closer to the carboxy-terminal that would probably preserve the determinant for antisera I-506, these peptides were not observed in our electrophoretograms. This would suggest that the initial action of α-chymotrypsin is to cleave human calcitonin at the leucine 9 position and then to degrade the 10–32 sequence into smaller and more basic peptides similar to those at pH 9.8 to 10.0 in Figure 4.

The enzymic data, in conjunction with the observed loss of immunoreactivity in the I-506 assay of calcitonin submitted to oxidation and reduction, clearly indicate that the immunological determinant for the I-506 antisera resides in the amino-terminal portion of the peptide and is probably associated with both the linear amino acid sequence and the conformation of the peptide in this region.

With respect to physiological fluids, the specific immunoreactive calcitonin components would, in addition to calcitonin, include potential precursor(s) of calcitonin and metabolic fragments. In the present study, 97% of the observations obtained with the amino-terminal I-506 assay in a relatively large population of adult volunteers were less than 200 ng/L, while all the observations with the carboxy-terminal HMS-385 assay were less than 150 ng/L. In fact, immunoreactive calcitonin was undetectable (<10 to 20 ng/L) in 87 and 53% of the samples in the HMS-385 and I-506 assays, respectively.

Our data on the basal concentrations of calcitonin contrast, in some respects, with earlier reports (1, 27). Heath and Sizemore (27) noted a significant difference in mean concentrations of calcitonin between men and women—49 and 31 ng/L, respectively. However, Parthomere and Deftos (1) noted no sex-related differences, and a mean concentration of 24 ng/L. In both reports, most patients had detectable calcitonin, but this was not true in our study. The apparent discordance is probably ascribable to differences in reagents and methodology among the laboratories.

With most radioimmunoassays, it should be remembered that the estimate of antigen concentration is calculated from a negative observation, i.e., the number of counts not bound to antibody when compared to appropriately prepared controls. Therefore, any factors (specific or nonspecific) that prevent the primary antibody from binding the labeled ligand (when applicable, the secondary antibody from binding the primary antibody) would be interpreted as a positive ligand concentration. Thus, it is essential that in each assay the test tubes denoting 100% binding, nonspecific background, and each point on the standard curve should be handled like the tubes containing the unknowns that are being assayed. In this regard, we characteristically include charcoal-extracted normal human serum in both the control tubes and those in the standard curve, to compensate for the nonspecific suppressive effect of serum on the antigen–antibody reactions. Our data indicate that there was no loss in either nonlabeled (Figure 1) or labeled calcitonin (unpublished observations) during our seven-day assay. Plasma, or some semblance thereof, was not added to the standard curve in the report of Heath and Sizemore, ostensibly because it caused a loss of immunoreactivity in calcitonin stored for 12 months at −18 °C. However, the more appropriate experiment would have been to determine the effect of plasma on labeled and nonlabeled calcitonin during the assay. The absence of plasma from the standard curve would help to explain the differences between the observations in our laboratory and Sizemore’s. From the report of Parthomere and Deftos (1), it is difficult to determine if plasma was added to the standard curve but, regardless, their carboxy-terminal antisera is more sensitive and exhibits higher affinity for calcitonin than our carboxy-terminal HMS-385. They reported a mean of 24 ng/L for the basal concentration of calcitonin in normal subjects, while we cannot detect less than 20 ng/L with the HMS-385 assay. The difference between 20 and 24 ng/L is not statistically significant when one considers the error associated with measurements at the upper end of the dose–response curve. Therefore, if we consider the relative sensitivities of the respective carboxy-terminal specific antisera, the data are compatible between our laboratories.

The higher carboxy-terminal immunoreactive calcitonin observed in extracts of normal thyroids may be due to either the presence of calcitonin precursors that are not recognized by the I-506 assay or the metabolism of calcitonin associated with any autolysis that may have occurred between death and autopsy. The relatively high concentrations of carboxy-terminal immunoreactive calcitonin in the serum of patients with medullary carcinoma of the thyroid suggest that, at least in the diseased state, the thyroid may be releasing more precursors to calcitonin than the 32-amino-acid calcitonin peptide. Our observations on the differences in amino- and carboxy-terminal immunoreactive calcitonin in sera and tissue extracts will be elaborated in a subsequent communication from this laboratory.

In summary, we have characterized antisera specific to the amino- and carboxy-terminal of human calcitonin. We have shown that the serum concentrations of immunoreactive calcitonin in normal adults were largely undetectable by both assays, e.g., <10–20 ng/L. In addition, we observed higher concentrations of carboxy- than amino-terminal immunoreactive calcitonin, both in extracts of normal human thyroids and in serum from patients with medullary carcinoma of the thyroid.

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