PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 165, 215–217 (1980)

Immunoreactive Calcitonin in Pheochromocytomas (40960)[1]

ROBERT S. WEINSTEIN AND LORRAINE F. IDE

Department of Medicine, Section of Metabolic and Endocrine Disease, Medical College of Georgia, Augusta, Georgia 30912

Abstract. Immunoreactive calcitonin (iCT) was evaluated in serum and tumor extracts from four successive, normocalemic patients with sporadic pheochromocytoma to determine how commonly these tumors contain CT-like immunoreactivity. Levels of iCT were measured by a nonequilibrium radioimmunoassay sensitive to both the intact molecule and the 11–32 amino acid region of human CT. With this assay, iCT is detectable in 56% of normal fasting adults ($n = 52$). The normal mean was 42 pg/ml with a normal range (mean ± 3 SD) of undetectable to 80 pg/ml. Serial dilutions of serum from a patient with medullary carcinoma of the thyroid and of acetic acid extracts of the pheochromocytomas gave displacement curves indistinguishable from the human standard. Patient "A" had an elevated serum iCT level of 477 pg/ml. After surgery for the pheochromocytoma, his basal serum iCT was normal and there was no abnormal elevation in iCT after pentagastrin stimulation. CT-like immunoreactivity was present in each of the four tumor extracts (2500–8600 pg iCT/g wet wt) and absent from an omental lymph node removed from patient A. Hypercalcitoninemia in patients with sporadic pheochromocytoma may not represent a concurrent medullary carcinoma of the thyroid since the presence and secretion of CT-like immunoreactivity from pheochromocytomas may be more common than previously realized.

If a patient has an elevated serum immunoreactive calcitonin (iCT) and a pheochromocytoma, the presence of multiple endocrine neoplasia, type 2, is usually assumed (1). Elevated iCT levels are, however, found in a substantial proportion of patients with nonthyroidal tumors (2). Furthermore, there have been reports of CT-like immunoreactivity in an occasional pheochromocytoma (3–6, 8–11). The purpose of the present study was to examine whether pheochromocytomas commonly contained iCT-like immunoreactivity and the relationship of this finding to hypercalcitoninemia.

Materials and methods: Extraction procedure. Four patients with histologically proven adrenal pheochromocytoma were studied. Their ages ranged from 9 to 54 years. All were normocalcemic (serum calcium, 9.0–10.3 mg/dl). No other affected family members were discovered. Portions of the tumors and of an omental lymph node from patient "A" were promptly frozen at −70°C and later extracted by hand mincing into 0.5-mm fragments in 0.1–0.5 *N* acetic acid (0.5 ml/100 mg wet wt tumor). Serial dilutions of the supernatant were assayed for iCT. Paired control tubes with equivalent aliquots of acetic acid in assay buffer were examined to determine whether the extraction procedure would modify the assay detection of iCT. Values for the extraction were discarded if the control tube showed >1 SD change from the initial binding control. Heat inactivation and neutralization of the extracts did not significantly alter the detection of iCT. An aliquot of the extract from patient A (1.1 ml) was neutralized and chromatographed on a column of Sephadex G-50, 1.5 × 40 cm in assay buffer and the eluant examined for iCT.

Calcitonin radioimmunoassay. Serum iCT was determined by a nonequilibrium radioimmunoassay using a goat antiserum (G1) to synthetic human CT and human CT (Beckman, Lot No. B0903, 0.56 mg/vial) for standard and ^{125}I tracer. This antiserum detects both the intact molecule and the 11–32 amino acid region of human CT (12). The assay buffer was 0.05 *M* phosphate buffer, pH 7.4, containing 0.05 *M* EDTA and 0.1% sodium azide. Final volume was 0.5 ml after the addition of Sephadex G-50

[1] Supported in part by the Biomedical Research Support Grant of the Medical College of Georgia.

0037-9727/80/100215-03$01.00/0

purified tracer on Days 3–4. Protein concentrations in all tubes were kept constant by the use of pooled blood bank sera, previously checked for undetectable iCT and found to have no deleterious effects on the labeled hormone. Aliquots of the pheochromocytoma extracts (5–20 μl) were brought up to 100 μl with this pooled sera. The standard curve also contained 100 μl of pooled blood bank sera per tube. Phase separation was achieved with dextran-coated charcoal. Both bound and free-labeled hormones were counted for 10 min or 10,000 cpm/tube. All samples were analyzed in triplicate in multiple dilutions. Figure 1 illustrates a standard curve prepared with G1 antiserum at a final dilution of 1:62,500. Increasing volumes (5–15 μl) of diluted serum from a patient with medullary carcinoma of the thyroid and of a pheochromocytoma extract (5–20 μl) caused displacement curves indistinguishable from the standard. The assay detection limit was 10 pg/tube, so that the minimum tissue concentration detectable was 100 to 200 pg/g wet wt. Recovery of synthetic CT (50–500 pg) added to pooled blood bank sera was 84 to 108%. The recovery rate was not used to alter the assayed results. With this assay, iCT is detectable in 56% of normal fasting adults (n = 52) and absent from thyroidectomized patients (n = 4). The normal mean was 42 pg/ml with a range (mean ± 3 SD) of undetectable to 80 pg/ml. The mean coefficients of variation for the intra- and interassay controls were 10.9 and 14.7%, respectively, over a 10-month period. There was no cross reactivity with human ACTH, glucagon, growth hormone, pentagastrin, procine insulin, α-methyl-*para*-tyrosine, phenoxybenzamine, or salmon CT.

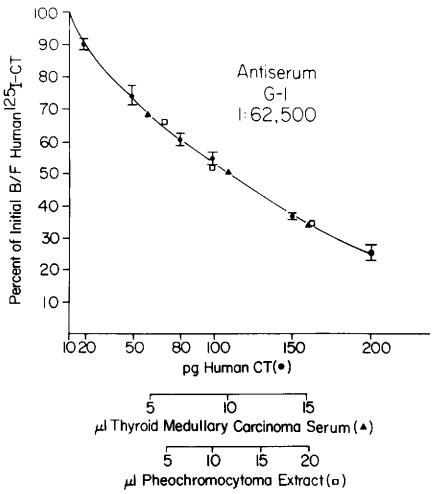

FIG. 1. Comparison of tracer displacement curves of synthetic human CT standard, serum from a patient with medullary carcinoma of the thyroid, and acetic acid extract of a pheochromocytoma from Patient A. The brackets indicate the range of three to four replicates in a single assay. Concentration scales along the abscissa are adjusted as shown to allow superimposition of each curve.

Serum calcium was determined by AutoAnalyzer. The normal range is 8.8–10.8 mg/dl.

Results. Table I illustrates the values of iCT in the serum and in extracts of the pheochromocytomas of our patients. Although serum iCT was variable, all four extracts contained CT-like immunoreactivity. An omental lymph node removed from patient A during surgery did not contain CT-like immunoreactivity.

Gel filtration of the extract from patient A revealed a broad peak of iCT coeluting with the ^{125}I synthetic human CT tracer.

Patient A demonstrated hypercalcitoninemia (477 pg/ml) that decreased (200, 43, and 71 pg/ml) during 1 month of therapy with α-methyl-*para*-tyrosine and phenoxybenzamine (Table I). Reduction of the hypertensive episodes and of the hypercalcitoninemia appeared to occur at the same time. After removal of a solitary left

TABLE I. SERUM LEVELS AND EXTRACTED CT-LIKE IMMUNOREACTIVITY IN FOUR PATIENTS WITH PHEOCHROMOCYTOMA

Patient	Serum iCT[a] (pg/ml)	Pheochromocytoma extract iCT[b] (pg/g wet wt)
A	477,200,43,71	2588
B	72,74,28	8608
C	N.D.[c]	3405
D	N.D.	4860

[a] Normal mean is 42 pg/ml with range undetectable to 80 pg/ml (mean ± 3 SD)

[b] 0.5 ml 0.1–0.5 N acetic acid/100 mg tumor.

[c] Not detectable.

adrenal pheochromocytoma, the blood pressure and basal iCT were normal. Postoperative stimulation with pentagastrin (Peptavlon, Ayerst, 0.5 μg/kg iv) did not result in an abnormal elevation of serum iCT. His basal serum iCT has remained normal over a 10-month period.

Discussion. Heath and Edis (4) and others (5, 6) have demonstrated still higher levels of serum calcitonin in patients with pheochromocytoma and express caution about potential confusion with multiple endocrine neoplasia, type 2. Our findings suggest that the presence of CT-like immunoreactivity in sporadic pheochromocytoma may be more common than previously appreciated and that serum iCT in patients with sporadic pheochromocytoma may be unpredictable. Hypercalcitoninemia does not necessarily indicate medullary carcinoma of the thyroid and, if misinterpreted, could result in unnecessary surgery. On the other hand, failure to recognize multiple endocrine neoplasia, type 2, could lead to missing disease in the contralateral adrenal due to the high incidence of bilateral adrenal tumors in this familial disorder (4, 7). When hypercalcitoninemia is found in a patient with pheochromocytoma, the possibility of multiple endocrine neoplasia, type 2, or the ectopic secretion of iCT must be considered. In sporadic pheochromocytoma, hypercalcitoninemia should be confirmed after adrenal surgery.

There is great variability in the literature regarding the concentration of CT-like immunoreactivity found in extracts of pheochromocytomas. Some workers have reported nanogram-per-gram levels (4, 8–11) while others have noted values a 1000-fold greater (3, 5, 6). Differences may be related to the immunochemical heterogeneity of iCT (13), different extraction procedures or CT antisera, calculation of iCT relative to fat-free weight rather than wet weight, and the variability of the tumors themselves. Furthermore, marked differences in the iCT content of different areas of a medullary carcinoma of the thyroid have been reported (14).

Matched circulating iCT levels have not correlated with tumor concentrations in any study including ours (2, 6, 9, 11). The factors controlling the ectopic secretion of calcitonin are unknown.

The authors gratefully acknowledge B. S. Roof, M.D., Medical University of South Carolina, for the generous donation of the calcitonin antiserum and Glen W. Sizemore, M.D., Mayo Clinic, for advice on the extraction procedure.

1. Steiner, A. L., Goodman, A. D., and Powers, S. R., Medicine **47**, 371 (1968).
2. Schwartz, K. E., Wolfsen, A. R., Forster, B., and Odell, W. D., J. Clin. Endocrinol. Metabol. **49**, 438 (1979).
3. Voelkl, E. F., Tashjian, A. H., Jr., Davidoff, F. F., Cohen, R. B., Perlia, C. P., and Wurtman, R. J., J. Clin. Endocrinol. Metabol. **37**, 297 (1973).
4. Heath, H., and Edis, A. J., Ann. Intern. Med. **91**, 208 (1979).
5. Milhaud, G., Calmettes, C., Jullienne, A., Ribeiro, F., and Moukhtar, M. S., *in* "Proceedings of the Fifth International Congress of Endocrinology" (V. H. T. James, Ed.), Vol. 2, p. 430. Excerpta Medica, Amsterdam (1977).
6. Raue, F., Bayer, J. M., Rahn, K. H., Herfarth, Ch., Minne, H., and Ziegler, R., Klin. Wochenschr. **56**, 697 (1978).
7. Graze, K. G., Spiler, I. J., Tashjian, A. H., Jr., Melvin, K. E. W., Cervi-Skinner, S., Gagel, R. F., Miller, H. H., Wolfe, H. J., DeLellis, R. A., Leape, L., Feldman, Z. T., Reichlin, S., N. Engl. J. Med. **299**, 980 (1978).
8. Abe, K., Adachi, I., Miyakawa, S., Tanaka, M., Yamaguchi, K., Tanaka, N., Kameya, T., and Shimosato, Y., Cancer Res. **37**, 4190 (1979).
9. Kaplan, E. L., Sizemore, G., Hill, B. J., and Peskin, G. W., Clin. Res. **20**, 724 (1972).
10. Kalager, T., Glück, E., Heimann, P., and Myking, O., Brit. Med. J. **2**, 21 (1977).
11. Trump, D. L., Livingston, J. N., and Baylin, S. B., Cancer **40**, 1526 (1977).
12. Roof, B. S., Weinstein, R. S., Vujic, I., and Burdash, N. M., Biomedicine **30**, 82 (1979).
13. Sizemore, G. W., and Heath, H., J. Clin. Invest. **55**, 1111 (1975).
14. Trump, D. L., Mendelsohn, G., and Baylin, S. B., N. Engl. J. Med. **301**, 253 (1979).

Received January 14, 1980. P.S.E.B.M. 1980, Vol. 165.