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A Radioimmunoassay for Serum Calcitonin in the Rat (39430)

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Despite extensive utilization of the rat to characterize the physiologic and pathophysiologic roles of calcitonin and factors which influence the secretion of the hormone, no radioimmunoassay has been developed with sufficient sensitivity to measure serum calcitonin in this species. Recently, Burford and associates (1) reported the important observation that human and rat calcitonin are closely related immunologically and that antisera to human calcitonin might be used to measure calcitonin in the rat. The hormone, however, could not be measured in the peripheral circulation with their assay. We now describe studies with a radioimmunoassay originally developed for human calcitonin (2, 3) which has sufficient sensitivity to permit characterization of calcitonin secretion in sera from normal rats.

Methods and materials. Male Sprague-Dawley rats weighing between 125 and 200 g were utilized. They were fasted overnight and blood samples were obtained by orbital puncture with the animals under light ether anesthesia for determination of calcium (4) and calcitonin. Thyroparathyroidectomized male Sprague-Dawley rats (Hormone Assay Laboratories, Inc.) weighing between 150 and 165 g were similarly studied. Sera from these animals were used for the radioimmunoassay. Calcium, 4 or 4.8 mg/100 g body wt, as calcium gluconate was given as a single intraperitoneal injection.

Thyroid glands were removed with the animals under ether anesthesia. The glands were freeze-dried and extracted three times with 1 ml of butanol:acetic acid:water (75:7.5:21) as previously described (5). The three extracts were combined, freeze-dried and dissolved in 1 ml of 0.6% acetic acid with 1% bovine serum albumin. The extracts were then serially diluted in the buffer for the radioimmunoassay.

Radioimmunoassay. The procedure described by Sizemore *et al.* (2) for radioimmunoassay of human calcitonin was employed with antiserum from chicken 513 (3). This was kindly supplied by Dr. Glen Sizemore. Serum from thyroparathyroidectomized rats was added to the buffer instead of human athyreotic plasma at a concentration of 5%. The buffer was 0.1 *M* tris(hydroxymethyl)aminomethane, pH 7.8, with kallikrein-trypsin inhibitor (Trasylol), 500 kIU/ml (FBA Pharmaceuticals, Inc.). The incubation volume was 0.5 ml and the final concentration of antiserum was 1:30,000 dilution. For each serum sample, incubations were carried out without antiserum to determine "damage" to the labeled hormone. Fifty microliters of serum samples were used for the assay which was carried out in triplicate. Human synthetic calcitonin (Ciba-Geigy Corp.) was labeled with ^{125}I by the method of Tashjian (6). About 15,000 cpm of ^{125}I-labeled calcitonin per tube was incubated. Bound and free [^{125}I]calcitonin were separated by dextran-charcoal (7). The sensitivity of the assay is 3 pg and corresponds to a lower detection limit of 60 pg/ml in serum. The coefficient of variation within assays was 22.6% and between assays was 32.6%. The same assay was used when the effects of calcium were examined for determination of basal and stimulated values.

Correlation coefficient was calculated with a 9810A Calculator (Hewlett-Packard).

Results. The radioimmunoassay curves with human calcitonin standard and extracts from thyroid glands of two rats and serum from a normal rat are shown in Figs. 1A and 1B, respectively. Based on these results the two glands were estimated to contain 100 and 250 ng of calcitonin, respectively.

Serum calcitonin was not detectable in each of five thyroparathyroidectomized rats even after calcium administration had brought the serum calcium to within the

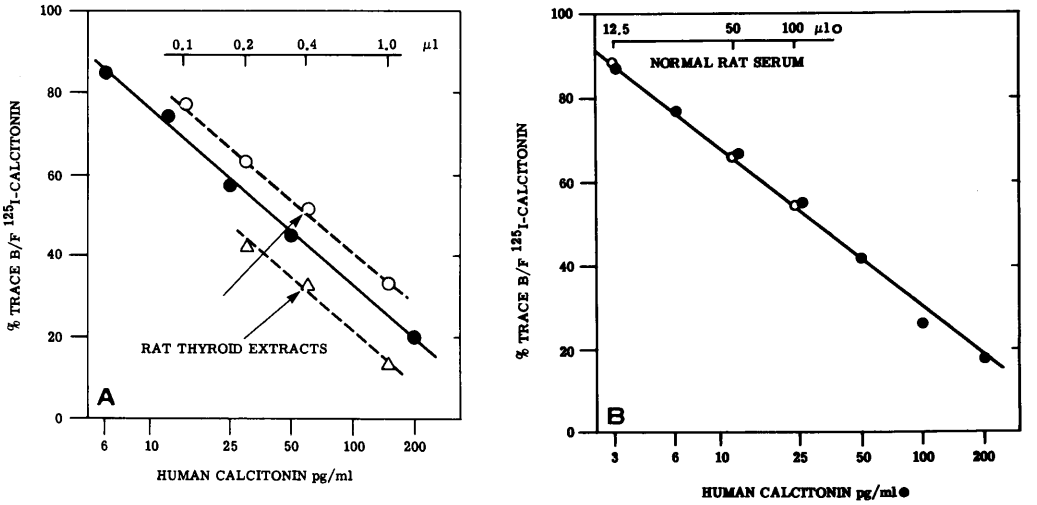

FIG 1. Immunoassay curve for ^{125}I-labeled human calcitonin with antiserum CK-513 against synthetic human calcitonin. Displacement by unlabeled synthetic human calcitonin and by two thyroid extracts from normal rats (A) and by serum from a normal rat (B) are shown.

normal range or above (Fig. 2). In contrast, basal calcitonin ranged from 71 to 175 pg/ml in seven animals and serum calcitonin had increased to a range of from 180 to 322 pg/ml by 30 min after calcium administration. In this study serum calcitonin was not detected in one normal rat either before or after calcium administration.

Basal serum calcitonin ranged from below the limits of detection (<60 pg/ml) to 650 pg/ml. Mean serum calcitonin was comparable in male and female rats both in the basal state and after calcium, 4.8 mg/100 g body wt (Table I, Fig. 3).

The relationship between serum calcium and serum calcitonin in the basal state and 30 min after the administration of calcium is shown in Fig. 3. The correlation coefficient was not significant in the fasting basal state ($r = 0.030$, $p > 0.1$) but was statistically significant when values 30 min after calcium administration were included ($r = 0.868$, $p < 0.001$).

Discussion. The results of the present studies show that serum calcitonin changed in a physiologically predictable manner. Calcitonin was not detectable in sera from thyroparathyroidectomized rats regardless of whether the rats were hypocalcemic or whether they had been given calcium and showed normal or high serum calcium values. In contrast, increases in serum calcium produced by intraperitoneal administration of the ion produced increases in serum calcitonin in each of the normal animals in which basal levels of calcitonin could be measured. Finally, extracts of each of two thyroid glands from rats diluted out in a manner identical to that of the human calcitonin standard. These results provide strong evidence for the validity of the assay.

Previous studies in the rat have indicated that the parafollicular cells are apparently well adapted to secrete calcitonin in response to acute stimulation (9). Whereas chronic hypercalcemia leads to intrathyroidal depletion of the hormone, sustained hypocalcemia produces striking increases in the thyrocalcitonin content in the thyroid. In subjects with hypocalcemia, marked increases in calcitonin secretion are produced by calcium and pentagastrin (10). Thus, with hypocalcemia there is increased storage of the hormone. These studies provide evidence that calcitonin synthesis and secretion are poorly coordinated (9). We believe this lack of coordination accounts for the variation in serum calcitonin in the basal state and after calcium administration found in the present study and for the variation in release of calcitonin by pork thyroid slices in the basal state and in response to a variety of stimuli described in previous studies from our laboratory (11, 12). It also may be the

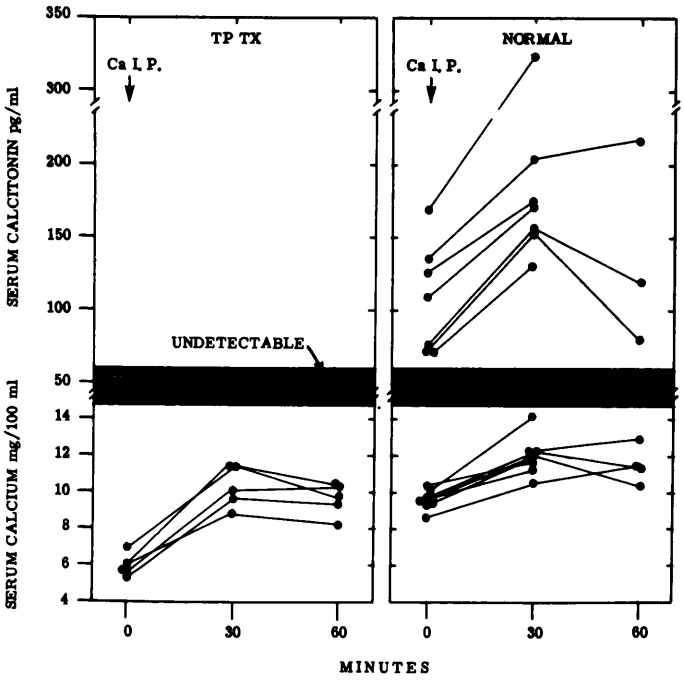

FIG. 2. The effects of intraperiotneal (ip) injection of calcium (Ca), 4 mg/100 g body wt, on serum calcitonin and serum calcium in five thyroparathyroidectomized (TPTX) and in eight normal male rats.

TABLE I. THE EFFECTS OF CALCIUM[a] ON SERUM CALCIUM AND SERUM CALCITONIN IN NORMAL MALE AND FEMALE RATS[b]

		Serum Calcium (mg/100 ml)			Serum Calcitonin (pg/ml)		
Sex	*N*	Control	30 min	*P* value	Control	30 min	*P* value
Male	7	9.5 ± 0.1	13.3 ± 0.7	<0.001	517 ± 30	2,271 ± 311	<0.001
Female	8	9.5 ± 0.1	14.1 ± 0.8	<0.001	417 ± 30	2,858 ± 175	<0.001
		NS	NS		NS	NS	

[a] 4.8 mg/100 g body wt ip.
[b] Results are given as means ± SE.

cause for the difference in quantity of calcitonin in extracts of rat thyroids observed in the present report as compared to previous studies (1) and for the inability of Talmadge and associates (13) to detect calcitonin in peripheral blood of normal Holtzman rats with a radioimmunoassay for rat calcitonin which has a lower limit of detection of 240 pg/ml. In this study, values as high as 600 pg/ml were noted during feeding when highly purified rat calcitonin was used as a standard. In subsequent studies, Cooper *et al.* (14) found that mean serum calcitonin was increased acutely to above 2,000 pg/ml after calcium was administered intravenously and to about 500 pg/ml after calcium was administered by mouth in Holtzman rats. Basal levels were not detected (<120–240 pg/ml). This difference in basal values as compared to our own may reflect strain differences, differences in the radioimmunoassay itself, or differences in physiological status of the animals at the time of study.

Serum calcitonin has previously been measured by bioassay in a system which utilized tissue culture of long bones of fetal rats labeled *in vivo* with ^{45}Ca (8). It was found that the concentration in serum from normal rats after parathyroidectomy was less than 0.05 MRC milliunit per milliliter (180 pg/ml). Thirty minutes after calcium administration, values as high as 1 to 2

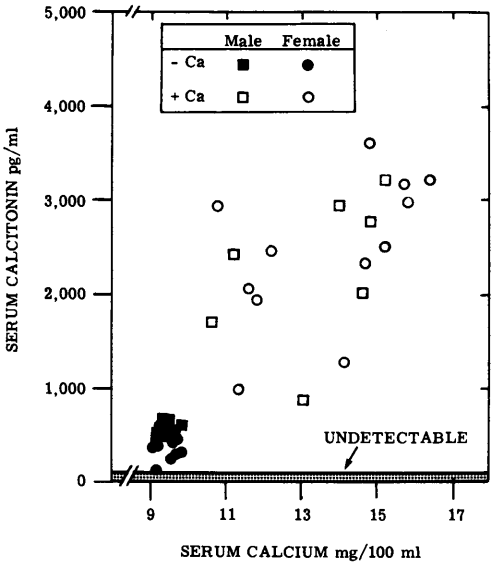

FIG. 3. Relationship between serum calcitonin and serum calcium in normal male rats given either no calcium (−Ca) or calcium (+Ca), 4.8 mg/100 g body wt 30 min previously. The correlation coefficient is significant if values after calcium are included ($r = 0.868, p < 0.001$) and is not significant for the unstimulated values ($r = 0.030, p > 0.1$).

MRC milliunits per milliliter (3600–7200 pg/ml) were found. These latter values are higher than those herein reported by radioimmunoassay. As noted already, there is evidence that calcitonin is stored in hypocalcemic states (9) and an exaggerated increase in serum calcitonin in response to calcium challenge has been reported (10). Therefore, the high values obtained by bioassay in the study just cited may reflect this phenomenon to some extent.

Summary. A radioimmunoassay for serum calcitonin in the rat is described. Materials for assay of human calcitonin are employed. The sensitivity of the assay is 3 pg and corresponds to a lower limit of detection of 60 pg/ml for peripheral serum. The normal range is up to 650 pg/ml in rats weighing between 125 and 200 g. Serum calcitonin is not detectable in thyroparathyroidectomized animals. It increases and varies directly with the serum calcium after intraperitoneal administration of the ion. Values for serum calcitonin in male and female rats are comparable under basal conditions and after calcium. The radioimmunoassay should allow the further characterization of calcitonin secretion in response to physiologic and pathophysiologic stimuli in this species.

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