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Lack of Direct Effects of Calcitonin and Parathyroid Hormone on *in Vitro* Secretion of One Another from Rat Thyroparathyroid Glands[1,2] (40769)

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In most mammals the thyroid and parathyroid glands exist in a close anatomical relationship. Whether or not this proximity of the two different endocrine glands is of any physiological significance has never been established. However, past reports have suggested that the thyroid and parathyroid glands might directly influence the secretion of parathyroid hormone (PTH) and calcitonin (CT), respectively (1–3). Fisher *et al.* (1) and Abe and Sherwood (2) observed increased release of PTH *in vitro* from porcine parathyroid slices exposed to medium containing added CT. Gittes and Irvin (3) originally reported results in rats which suggested that removal of either the parathyroid glands or the thyroid gland (CT) impaired the ability of the animal to combat induced hypercalcemia, they concluded that the parathyroid glands contained a calcitonin "releasing factor."

Recently, we developed a system for studying concurrent *in vitro* secretion of PTH and CT from baby rat thyroparathyroid glands (4, 5). In the present study we have used this system to reexamine whether the parathyroid gland (or PTH) affects the secretion of CT (3) and whether the thyroid gland (or CT) affects the release of PTH (1, 2). The effects of both endogenous and exogenous CT and PTH were studied by incubating thyroid and parathyroid glands separately and together and by adding known amounts of CT and PTH to medium bathing them.

Materials and methods. Incubation procedure. Details of the incubation procedure have been reported previously (4, 5). In brief, thyroid and parathyroid glands were removed from 8-day-old rats (Zivic-Miller, Allison, Penn.) and incubated for 8 hr in a serum-free, chemically defined medium (modified Eagle's minimum essential medium). Each flask, containing glands from 1 pup in 2 ml of medium, was gassed with 95% 0_2–5% CO_2 and incubated with constant shaking at 37°C. An aliquot of medium from each flask (0.3 ml) was removed at 4 hr, and the remaining medium was removed at 8 hr. Samples of medium were analyzed by atomic absorption spectrophotometry to verify calculated calcium concentrations, and the remainder of the medium was stored at −20°C until used for the radioimmunoassays. Typically, each experiment consistd of six groups each containing six flasks. Two calcium concentrations in medium, 1 and 2.5 m*M*, were studied in each experiment.

Endogenous hormone effects. Figure 1A schematically depicts the experimental design. To examine possible effects of endogenous CT and PTH on one another the following conditions were employed: (i) In-

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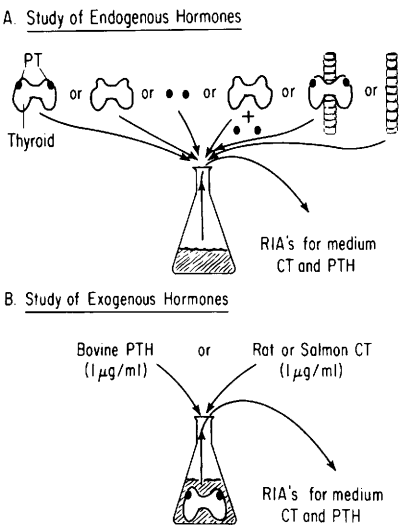

FIG. 1. Schematic representation of experimental design for testing possible effects of endogenous (A) or exogenous (B) PTH and CT on secretion of one another *in vitro*.

cubation of entire thyroparathyroid gland complexes separated from the trachea (usual procedure, Refs. 4, 5); (ii) incubation of entire thyroparathyroid gland complexes still attached to the trachea; (iii) incubation of thyroid and parathyroid glands in separate flasks; and (iv) separation of thyroid and parathyroid glands and then incubation in the same flask. All surgery was performed under ether anesthesia using a dissection microscope. Glands were removed still attched to the trachea by transecting the trachea 2–3 mm both cephalad and caudad with respect to the thyroid gland.

Exogenous hormone effects. Figure 1B schematically depicts the design of these experiments. To study possible effects of CT and PTH on secretion of one another, the two hormones were added directly to the incubation medium at the beginning of the experiment. For this aspect of the study, entire thyroparathyroid complexes separated from the trachea were incubated. To examine possible effects of PTH on CT release, bovine 1-84 PTH was added to medium at a concentration of 1 μg/ml. To study possible effects of CT on PTH release, either native rat CT or synthetic salmon CT was added to medium at a concentration of 1 μg/ml.

Hormones. Bovine 1-84 PTH was purchased from Inolex Pharmaceutical Company (Glenwood, Ill.) and had a specific biological activity of 250 units/mg. Synthetic salmon CT was kindly donated by Dr. J. P. Aldred, Armour Pharmaceutical Company (Kankakee, Ill.) and had a specific biological activity of 5000 units/mg. Purified native rat CT was prepared in our laboratory as described previously (6) and had a specific biological activity of 260 units/mg.

Radioimmunoassays. Details of the radioimmunoassays for CT and PTH have been reported earlier (4, 5, 7). For CT, the assay was conducted using a chicken antiserum to rat CT at 1:10,000 (final dilution), and pure native rat CT was used as unlabeled reference standard and for iodination with ^{125}I. For PTH, assays were conducted using a guinea pig antiserum to bovine 1-84 PTH (final dilution 1:80,000) which crossreacts with rat PTH (4). Pure bovine 1-84 PTH, donated by Dr. Henry Keutmann (Massachusetts General Hospital), was employed as unlabeled reference standard and for iodination with ^{125}I.

Statistical analyses. Results of hormone assays were calculated using nonlinear regression analysis on an IBM 1130 computer (5, 8). If all values exceeded the lower limit of detectability of the immunoassay, then mean values (± SEM) were calculated for each group, and the significance of differences between mean treatment groups was determined using either an F test or a two-tailed Student's t test (9). If any of the values in a treatment group were below the lower limit of detectability of the assays for CT or PTH, then mean values could not be calculated, and significance of differences between treatment groups was evaluated using the nonparametric rank sum test of Wilcoxon (9). A P value ≤ 0.05 was considered significant.

Results. Effects of medium Ca. As shown in Fig. 2 and 4–6, the results of all experiments conducted confirmed our previous finding (4, 5) that, using the system described, appropriate changes in secretion of CT and PTH were observed in response to

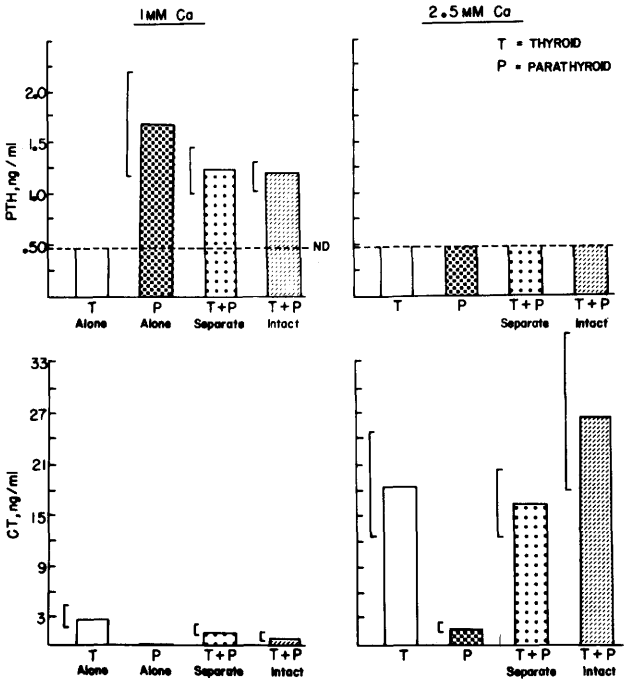

FIG. 2. Concurrent changes in immunoreactive CT and PTH released into medium after incubation of rat thyroids and parathyroids for 8 hr in 1 or 2.5 m*M* Ca. Glands were incubated either separately (T,P) or together, either intact (T + P intact) or after having been separated surgically (T + P separate), according to the procedure diagrammed in Fig. 1A. Each bar represents the mean value for six flasks, and the bracket shows the SEM. Horizontal dashed line shows the lower limit of assay detectability (ND = nondetectable).

an alteration in the concentration of calcium in the medium. Figures 2 and 4 illustrate that glands incubated in 2.5 m*M* Ca produced CT levels in medium at 8 hr which were 5- to 10-fold higher than levels produced by glands incubated in 1 m*M* Ca. Furthermore, secretion of CT was linear over the 8-hr period (Fig. 4). Figures 2, 5, and 6 illustrate that parathyroids incubated in 1 m*M* Ca secreted 5 to 10 times as much PTH at 1 m*M* Ca than at 2.5 m*M* Ca and secretion was linear over the 8-hr period studied (Figs. 5 and 6). Effects of medium calcium on both hormones were statistically significant ($P < 0.01 - < 0.001$) in all experiments.

Effects of endogenous hormones. Figure 2 illustrates that incubation of thyroid and parathyroid glands separately or together (intact thyroparathyroid complex or separated glands present in the same flask) had no significant effect on the amount of CT or PTH released into the medium. Secretion of PTH was demonstrable at 1 m*M* Ca and suppressed at 2.5 m*M* Ca. No PTH was found in medium in which only thyroid glands were incubated, even at 1 m*M* Ca. Levels of CT in the medium were high in 2.5 m*M* Ca and low in 1 m*M* Ca. At 1 m*M* Ca, CT was not measurable in medium in which only parathyroid glands had been incubated. At 2.5 m*M* Ca a small amount of CT secretion was observed with this group, most likely due to a small amount of thyroid tissue inadvertantly removed during dissection of the parathyroid gland.

Results shown in Fig. 3 indicate that dissection of thyroparathyroid glands away from the trachea does not damage the gland complexes and lead to nonspecific hormone release. Levels of PTH at 1 m*M* Ca (top graph) and of CT at 2.5 m*M* Ca (bottom graph) were not significantly different whether the gland complex had been dis-

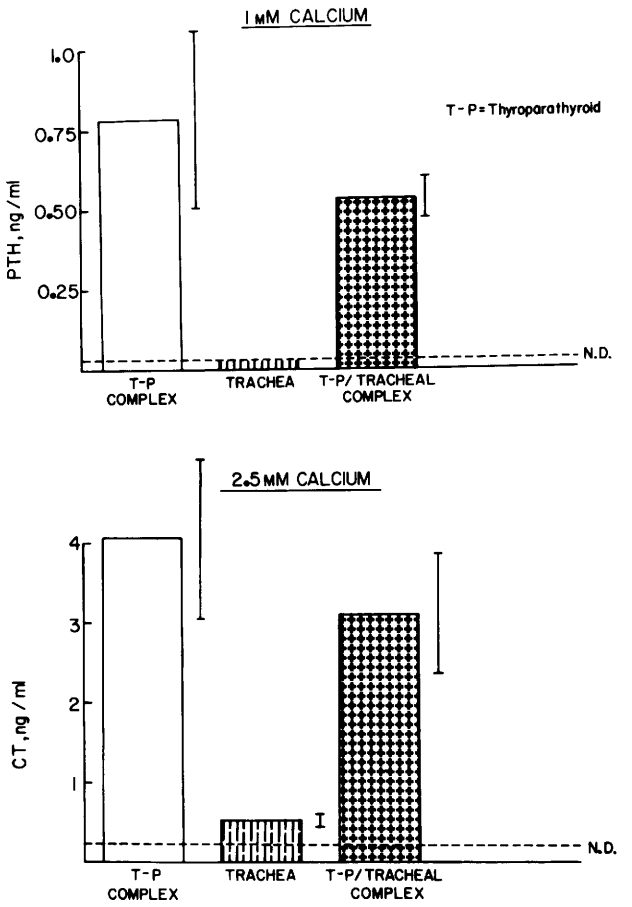

FIG. 3. Concurrent changes in immunoreactive CT and PTH released into medium after incubation of rat thyroparathyroid complexes for 8 hr in 2.5 or 1 m*M* Ca, respectively. Gland complexes were incubated either attached to the trachea or not according to the procedure diagrammed in Fig. 1A. See Fig. 2 legend for additional details.

sected out from the trachea or whether it was left untouched and incubated still attached to the trachea. With only trachea present, no measurable PTH was found in the medium (top graph) and only a small amount of CT was found (bottom graph), probably due to remnants of thyroid tissue not removed from the tracheal segment.

Effects of exogenous hormones. Figure 4 shows that addition of a large amount of bovine PTH to the medium had no significant effect on the CT released from thyroparathyroid complexes. Appropriate increases in CT release occurred in response to an increase in the medium calcium concentration and measurement of PTH in the medium verified the fact that the thyroid gland was exposed to a high level of extracellular PTH.

Figures 5 and 6 illustrate that addition of a large quantity of either homologous (rat) CT (Fig. 5) or heterologous (salmon) CT (Fig. 6) to the medium had no significant effect on the secretion of PTH from the parathyroid glands in the thyroparathyroid complexes. Appropriate decreases in PTH release occurred in response to an increase in the medium calcium concentration and measurement of CT in the medium in the experiment shown in Fig. 5 verified that the glands were exposed to a high concentration of CT.

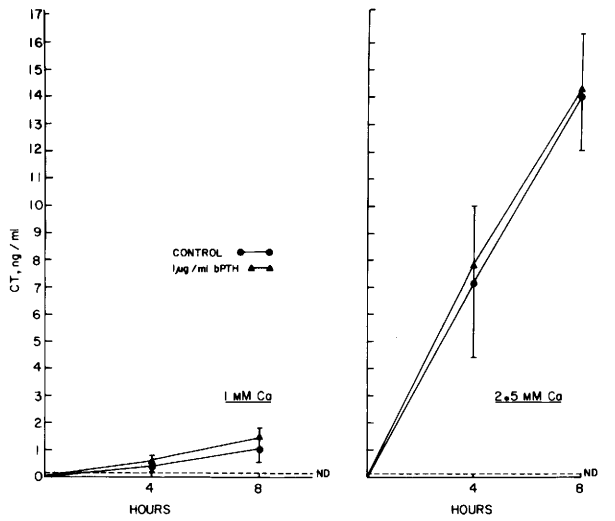

FIG. 4. Changes in immunoreactive CT released into medium from rat thyroparathyroids incubated in 1 or 2.5 m*M* Ca in the presence or absence of added bovine PTH as depicted in Fig. 1B. Each point represents the mean value for six flasks and the vertical line shows the SEM. In the medium containing added PTH, the level of PTH, measured by radioimmunoassay, exceeded the upper limit of detectability for the assay (i.e., >200 ng/ml).

Discussion. It is well established that secretion of both PTH and CT are regulated by changes in the concentration of calcium in extracellular fluids. Hypercalcemia stimulates CT secretion and suppresses PTH release while hypocalcemia increases PTH secretion and suppresses CT release. In addition to calcium, a variety of other agents have been found to alter secretion of PTH and CT both *in vivo* and *in vitro*. However,

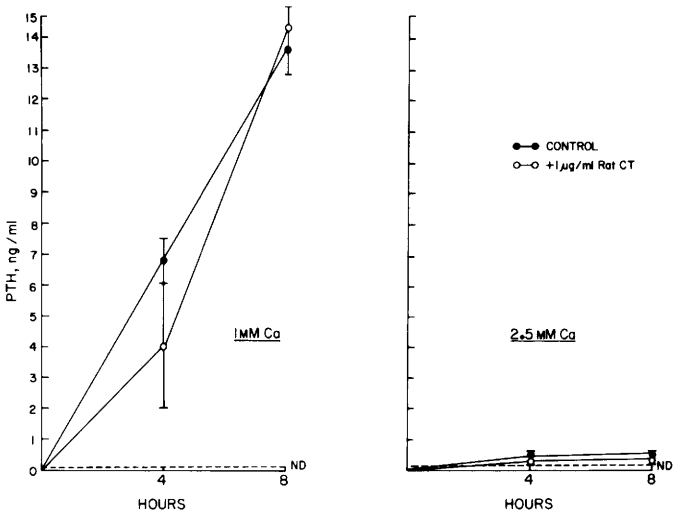

FIG. 5. Changes in immunoreactive PTH released into medium from rat thyroparathyroids incubated in 1 or 2.5 m*M* Ca in the presence or absence of added rat CT as depicted in Fig. 1B. See Fig. 4 legend for additional details. In the medium containing added CT, the level of CT, measured by radioimmunoassay, exceeded the upper limit of detectability for the assay (i.e. >60 ng/ml).

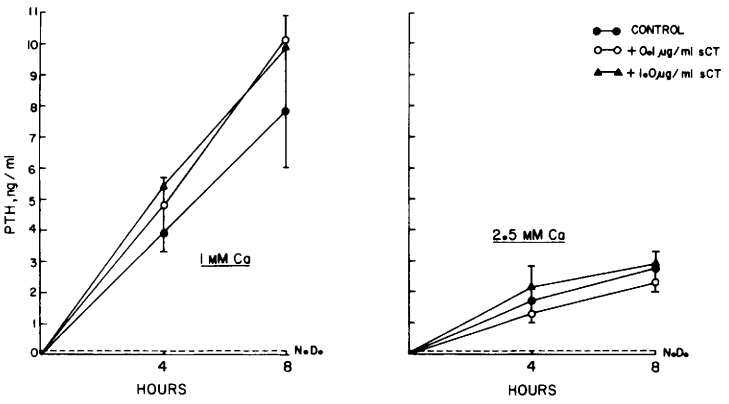

FIG. 6. Changes in immunoreactive PTH released into medium from rat thyroparathyroids incubated in 1 or 2.5 m*M* Ca in the presence or absence of added salmon CT as depicted in Fig. 1B. See Fig. 4 legend for additional details.

the physiological role of agents other than calcium in regulating release of PTH and CT has not been established.

An intriguing, potentially important question is whether or not the thyroid and parathyroid glands, by secreting CT and PTH, respectively, or by releasing some unidentified factor, directly influence the secretory activity of one another, i.e., by some means other than by their ability to alter the level of plasma calcium. The question is prompted in part by the fact that the thyroid and parathyroid glands are closely associated anatomically in most mammals, including the rat. Furthermore, a few reports have suggested that the parathyroid gland (or PTH) might influence CT release. For example, Gittes and co-workers originally provided evidence for a direct CT releasing factor of parathyroid origin (3, 10, 11) but subsequently this finding could not be confirmed (12). More recently studies by Fischer *et al.* (1) and Abe and Sherwood (2) suggested that addition of high doses of porcine or salmon CT to medium bathing slices of porcine parathyroid glands elevated PTH secretion even when the concentration of calcium in the medium was high (2.0 or 2.75 m*M*). However, these *in vitro* findings have not been confirmed, and the limited studies which have addressed this question *in vivo* (13) indicated that secretion of PTH was not responsive to the quantity of CT administered but rather only to the extent and duration of the ensuing CT-induced hypocalcemia.

Our present results clearly show that CT and PTH did not directly affect the secretion of one another. This was the case whether the hormones were added to the medium (Figs. 4–6) or whether they were secreted from the glands themselves (Fig. 2). The use of glands incubated together or separately (Fig. 2) also makes it unlikely that factors other than CT or PTH released from the thyroid and parathyroid, respectively, play any significant regulatory role with respect to secretion of PTH and CT. Likewise, it appears unlikely that physical contact between the two glands has any influence on secretion of the two hormones. Clearly, since appropriate responses in release of PTH and CT were observed in response to changes in medium Ca (Figs. 2, 4–6), failure of the hormones to alter secretion of one another cannot be attributed to a lack of responsiveness of the thyroid C-cell or parathyroid chief cell in the system employed.

Our findings support the conclusion by Toverud *et al.* (12) that the parathyroid gland does not affect CT release. Our observation that neither salmon nor rat CT influences secretion of rat PTH *in vitro* is in conflict with the reports of Fischer *et al.* (1) and Abe and Sherwood (2). The reasons for the discrepancy in their findings and ours are not readily apparent. One possibility is

that species differences are involved since they used porcine or bovine parathyroid tissue. Another difference in the procedures is that the medium used by Fischer *et al.* (1) contained 10% serum while ours was serum free. A third possible explanation is that in our experiments where CT was added to medium the peptide did not readily penetrate the intact, encapsulated parathyroid gland whereas their thinly sliced porcine or bovine glands were more permeable to the CT present in the incubation medium. Further studies will be required to resolve this question. At present, however, we conclude from our present studies that, in the rat, CT and PTH likely do not directly affect the secretion of one another.

Summary. Thyroid and parathyroid glands from 8-day-old rat pups were incubated both separately and together for 8 hr in a serum-free medium. In addition, thyroparathyroid complexes were incubated in the presence or absence of added bovine PTH or rat or salmon CT. Secretion of PTH and CT was measured using radioimmunoassays that detect rat PTH and rat CT. In all experiments, secretion of CT was 5- to 10-fold greater in 2.5 m*M* Ca than in 1 m*M* Ca. Conversely, PTH release was 5- to 10-fold higher in 1 m*M* Ca than in 2.5 m*M* Ca. PTH secretion was no different whether the thyroid gland was present with the parathyroids or not, and CT release from thyroids was not affected by the presence or absence of the parathyroid glands. The results support the conclusion that the thyroid gland (CT) exerts no direct influence on the ability of the parathyroids to release PTH and that the parathyroid (PTH) exerts no direct effect on the release of CT from the thyroid gland.

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