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Evidence of Calcitonin-Induced Inhibition of Calcitonin Secretion in Porcine Thyroid Slices (39154)

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(Introduced by P. Kumaresan)

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The influence of the calcium ion on calcitonin (CT) secretion has been examined in a variety of mammalian and submammalian species using various *in situ*, *in vivo*, and *in vitro* experimental techniques (1–7). Although calcium is an established CT secretagogue, it appears that the calcium ion is a much more potent stimulus to CT secretion *in vivo* and *in situ* than *in vitro* (5–7).

It has been proposed that tissue manipulation or deterioration may be responsible for the unresponsiveness of CT secretion observed *in vitro* (7). On the other hand, Bell (4) suggested that CT accumulation in the incubation medium may retard subsequent release of the hormone. Similarly, hormonal self-inhibition has been observed in other endocrine tissues such as the pancreas (8) and anterior pituitary (9, 10). It has been suggested that "internal" feedback mechanisms may be involved in the secretory regulation of several endocrine systems (10).

In the present study, increasing the volume of incubation and replacing the incubation medium at 5-min intervals were observed to enhance calcium-stimulated CT secretion in a closed *in vitro* system.

Materials and methods. Fresh porcine thyroid glands from mature hogs of mixed breeding were obtained for each experiment from a local abattoir. The glands were removed immediately after slaughter and placed in chilled aerated saline. Thyroid slices (approximately 0.5 mm thick) were taken from the lateral aspects of the thyroid lobes where we have previously observed the greatest calcitonin concentrations (personal communication). The thyroid slices were pooled and washed several times over a period of 30–45 min in chilled saline. Thyroid slices used in each experiment were taken from a single thyroid. Forty to 50 mg of the tissue slices (four to five pieces) were weighed into 10- or 25-ml Erlenmeyer flasks, and incubated on a gyrorotary incubator at 37°C for 30 min under an atmosphere of 95% O_2 and 5% CO_2. Incubations were conducted in 2 ml of an oxygenated Krebs–Ringer bicarbonate buffer containing 5.6 m*M* glucose except as otherwise indicated. After incubation the medium was separated from the tissue and stored at −20°C for subsequent CT assay. The CT concentration in the incubation media was determined by a specific porcine CT radioimmunoassay which has previously been described (11). Pure porcine CT (Lot #4688C-140A, Lederle Laboratories) was used for labeling with I^{125} and as the assay standard. Equilibrium incubations were conducted at 4°C for 6 days in a 0.05 *M* veronal buffer containing 0.02 *M* disodium EDTA and 0.5% bovine albumin. Bound and free labeled fractions were separated by talc adsorption. Each CT determination was performed in duplicate on several dilutions of incubation medium. The Students *t* test for nonpaired data was used to compare individual group means.

Results. In the initial phase of this study the influence of replacing the incubation medium with fresh medium at 5-min intervals was examined. In this experiment, 2.5 m*M* calcium significantly increased total CT secretion in both the control group ($P < 0.005$) and the experimental group which received medium replacement at 5-min intervals ($P < 0.025$). However, the total calcium-stimulated CT secretion during the 30-min incubation period was much greater

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in the medium replacement group ($P < 0.05$). Basal CT secretion was not altered by medium replacement (see Fig. 1).

The cumulative secretion of CT at each time interval in groups which received fresh medium replacement during the 30-min incubation is presented in Fig. 2. The calcium-stimulated CT secretion rose most rapidly during the first 10 min of incubation but continued to rise during the entire 30-min incubation. The cumulative calcium-stimulated secretion was significantly greater than control at each interval ($P < 0.005$). Tissue in the calcium-deficient media released most of its total secretion during the first 5 min of incubation.

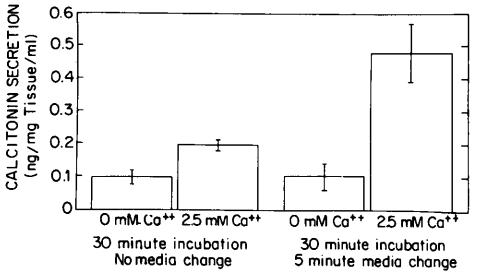

FIG. 1. Influence of incubation medium change on calcium-stimulated calcitonin secretion. The control groups (left) were incubated in a volume of 2 ml for 30 min. The 2-ml incubation volume in the experimental groups (right) was replaced with fresh medium at 5-min intervals during a 30-min incubation. Each vertical bar represents the mean total CT secretion from six determinations ± SE during the 30-min incubation.

In separate experiments, the influence of increasing the volume of incubation medium on calcium-stimulated CT secretion was examined (see Fig. 3). In this study, 50 mg of thyroid slices were incubated in 2, 5, 10, or 15 ml of incubation medium containing 0 or 2.5 m*M* calcium. Increasing the volume of incubation from 2 to 15 ml increased calcium-stimulated CT secretion from 135 to 675%. The calcium-stimulated increase in CT secretion in the 10- and 15-ml volumes of incubation was significantly greater than the calcium-stimulated increase observed in the 2 ml volume ($P < 0.05$; $P < 0.01$, respectively).

Discussion. The ability of the calcium ion to stimulate CT secretion *in vivo* has been established by a number of investigators (1–3). We have recently observed that calcium is the most potent CT secretagogue among the alkaline earth cations (12), and it appears to be physiologically important in the regulation of CT secretion (13). Therefore, the unresponsiveness of CT secretion to the calcium ion *in vitro* is inconsistent with the secretory response observed *in vivo* (5–7). In a study with cultured pieces of human medullary carcinoma, Gautvik and Tashjian (6) reported that a calcium concentration of 20 m*M* was required to produce a significant 2-fold increase in CT

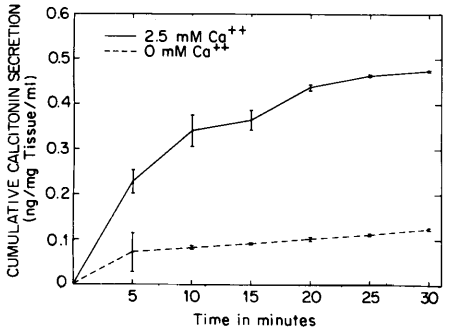

FIG. 2. Influence of calcium stimulation on cumulative CT secretion. The incubation medium in each flask was changed at 5-min intervals. Each point represents the mean cumulative CT secretion from six determinations at the time indicated ± SE. The broken and solid lines represent cumulative CT secretion in incubation media containing 0 and 2.5 m*M* calcium, respectively.

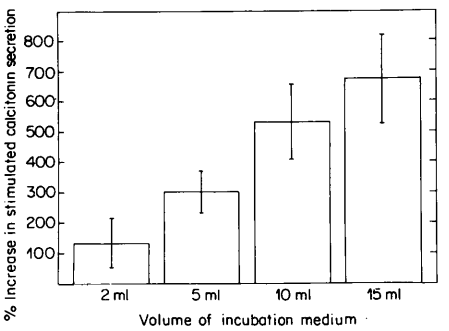

FIG. 3. Influence of increasing the volume of incubation on calcium-stimulated CT secretion. Each vertical bar represents the mean % increase in CT secretion ± SE from six estimates of CT secretion in media containing 2.5 m*M* calcium compared to six estimates of CT secretion in the same volume of 0 calcium media. The data are pooled from two experiments using two different thyroids.

release; whereas, a calcium infusion in the same patient before removal of the medullary carcinoma, which elevated plasma calcium by 3 mg/100 ml, produced a greater than 8-fold increase in circulating CT levels. Similarly, Feinblatt and coworkers (5) have observed an inconsistent response to calcium stimulation in cultured avian ultimobranchial glands. In a study of CT secretion from porcine thyroid slices, Bell (4) reported that increasing incubation medium calcium above 1.25 m*M* did not enhance secretion in a volume of 2 ml but did significantly increase CT secretion in a volume of 32 ml.

The calcium-stimulated CT secretion observed in volumes of 2 to 15 ml in the present study (Fig. 3) is consistent with the findings of Bell (4). In addition, the results of the incubation medium replacement experiments (see Fig. 1 and 2) argue in favor of a volume expansion influence on stimulated CT secretion *in vitro*. Taken together, these data suggest that CT accumulation in the medium during incubation may reduce the secretory responsiveness to calcium. The existence of direct feedback inhibition in the pancreas (8) and anterior pituitary (9) has also been proposed. Presently, the influence of CT accumulation on CT secretagogues other than calcium is being examined.

The sparse and uneven distribution of C-cells in the mammalian thyroid (14) may account for part of the meager secretory response to calcium *in vitro*. On the other hand, this factor would not explain the observed unresponsiveness of cultured ultimobranchial tissue (5, 7). However, tissue deterioration and oxygen effects on the cell membrane have been proposed (5, 7). Whatever the precise reasons for the limited secretory responsiveness, it is apparent from the results of the present study that the incorporation of volume expansion methods into the *in vitro* model will produce a calcium-stimulated secretory response which more closely mimics the secretory response observed *in vivo*.

Summary. In the present study, the possibility of calcitonin-induced self-inhibition in porcine thyroid slices was examined. Replacing the incubation medium at 5-min intervals during incubation and increasing the volume of incubation from 2 to 15 ml were observed to enhance calcium-stimulated calcitonin secretion *in vitro*.

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