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TSH-STIMULATED INCREASES IN CALCIUM UPTAKE AND CALMODULIN LEVELS IN THYROID CELLS

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This is the first report to show that polypeptide hormone increases cellular calmodulin contents. In cultured porcine thyroid cells, 6 days' exposure to TSH (above 0.02 mU/ml) increased cellular calmodulin contents. Six days' exposure to TSH also increased calcium uptake in thyroid cells. This TSH-stimulated increase in calcium uptake was partly due to the increase in cellular calmodulin contents.

Thyrotropin (TSH) regulates many of the functions of thyroid follicular cells through the action of cAMP(1), the well known second messenger of hormone action. During recent years calcium has been shown to function as another regulatory signal for numerous cell events(2). There is now increasing evidence that calmodulin, the calcium-dependent regulatory protein, may be the major intracellular mediator of the effects of calcium on cellular metabolism(3). Calmodulin is implicated in both hormone action and secretion.

We will show that TSH augments calcium uptake and also increases thyroid cell calmodulin levels, indicating that TSH-induced increases in calcium uptake are partly due to the increases in calmodulin.

MATERIALS AND METHODS

Thyroid cell culture: Thyroid cells were obtained from porcine thyroid glands as described previously(4). Freshly isolated cells were suspended (3×10^6 cells/ml) in Eagle's minimum essential medium (MEM) supplemented with 10% new born-calf serum and antibiotics (penicillin, 200 units/ml; streptomycin, 50 µg/ml). Cells were cultured as a suspension at 37°C in a 95% air: 5% CO_2 water-saturated atmosphere.

Calcium uptake: After 6 days' culture in the presence of graded doses of TSH, thyroid cells were washed twice at room temperature with phosphate-buffered saline glucose (PBSG) containing 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.45 mM $CaCl_2$, 0.5 mM $MgCl_2$ and 5.6 mM glucose (pH 7.4). Calcium uptake was measured as described by Wollheim et al.(5). The washed cells were

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suspended in PBSG and then distributed into polystyrene tubes (0.4 ml volume) containing 200 µl of a mixture of dibutyl- and dinonylphthalate (10:3 vol/vol) layered on top of 20 µl of 6 M urea. The 50 µl of PBSG, containing the thyroid-cell suspension, was carefully placed against the walls of the tubes to leave an air-layer between the buffer and the oil mixture. The incubation was started by adding another 50 µl of warm (37°C) PBSG containing 0.8 µCi of $^{45}CaCl_2$ and 1.4 µCi [6,6'(n)^{3}H] sucrose (4 µM), as a marker of extracellular space. The tubes were incubated in a water bath at 37°C. The incubation was stopped and the cells were separated from the incubation buffer (usually after 5 min of incubation) by centrifugation for 15 sec at 8,000 g in a Greiner microfuge (type 2F1). By this procedure the thyroid cells were effectively separated from the buffer by passage through the phthalate mixture and into the urea layer. The bottom of the tubes were cut above the urea layer and placed in 5 ml Instagel (Packard Instrument International S.A., Zurich, Switzerland) for liquid scintidlation spectrometry. Calcium uptake was calculated from the calcium space in excess of the [^{3}H] sucrose space. The sucrose space reached a maximum within 1 min of incubation and remained constant over 30 min.

Calmodulin levels in thyroid cells: Calmodulin was measured as described by Inaba et al.(6). The thyroid cells, cultured for 6 days in the presence of graded doses of TSH, were collected, washed twice with a medium consisting of 125 mM borate buffer, pH 8.4, 1 mM ethyleneglycol-bis-(β-aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA) and 75 mM NaCl, suspended and then homogenized with all-glass homogenizers. Aliquots were then removed for protein determination. The homogenates were heat-treated at 90°C for 5 min and rapidly cooled to 4°C by immersion into a methanol: dry ice bath. The heat-treated samples were then centrifuged at 10,000 g for 30 min and the supernatants were used for the assay. Calmodulin levels were measured by RIA using CAABCO calmodulin kits (CAABCO, Houston, Texas). All samples were determined in the same assay run. Calmodulin levels were expressed as µg of calmodulin per mg of protein.

Materials etc.: TSH was obtained from Armour Pharmaceutical Company (Phoenix, Arizona, U.S.A.); new-born calf serum and Eagle's MEM from Flow Laboratories (Irvine, Scotland, U.K.) and $^{45}CaCl_2$ and [6,6'(n)^{3}H] sucrose from the Radiochemical Centre (Amersham, England). All other chemicals were of the highest purity available commercially. Experiments were conducted at least 4 times. Typical data and the final concentrations of TSH are shown in the text and the figures. Student's t-test was used for statistical evaluations. Protein concentrations were determined according to Lowry(7).

RESULTS

TSH-stimulated increases in calcium uptake (Fig. 1A) Porcine thyroid cells were cultured in the presence of graded doses of TSH for 6 days and the calcium uptake of the thyroid cells was then studied. Graded doses of TSH augmented calcium uptake of the cells: 0.02 mU/ml TSH augmented calcium uptake significantly and 0.05-0.10 mU/ml TSH augmented it maximally.

TSH-stimulated increases in calmodulin levels (Fig. 1B) Thyroid cells were cultured in the presence of graded doses of TSH for 6 days and then calmodulin contents of the cells were measured. Graded doses of TSH augmented cellular calmodulin contents: 0.02 mU/ml TSH augmented calmodulin levels significantly and 0.05-0.10 mU/ml TSH augmented them maximally.

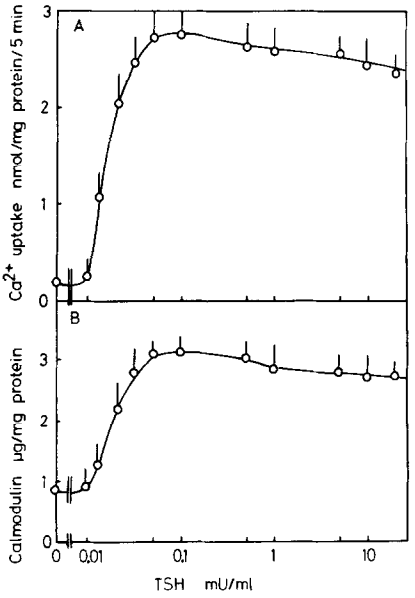

Fig. 1. Thyroid cells were cultured in the presence of graded doses of TSH for 6 days and calcium uptake (A) and calmodulin levels (B) of the cells were studied. A: After 6 days' culture, the washed cells were suspended in PBSG and calcium uptake was measured as described in the text. B: After 6 days' culture, calmodulin contents were measured as described in the text. Each value is the mean+SE of 3-5 determinations.

DISCUSSION

This is the first report to show that polypeptide hormone increases cellular calmodulin contents. TSH increases cellular calmodulin contents, and also increases calcium uptake of thyroid cells. This TSH-stimulated increase in calcium uptake is partly due to the increase in cellular calmodulin contents.

Calmodulin has been considered to play an important role in regulating endocrine cells. Chafouleas et al.(8) investigated a variety of hormonally regulated systems to determine whether calmodulin is selectively elevated and concluded that in no instance did it appear to be so. Since then there have been no other reports that showed modulation of calmodulin levels by polypeptide hormones.

The physiological significances of the TSH-stimulated increases in calcium uptake and calmodulin are not known at present. It is established that calcium has an informational role in many tissues(2) and also in the thyroid. Recent evidence has indicated that calmodulin acts as an intracellular calcium receptor and mediates the calcium regulation of an extensive range of

fundamental cellular activities, including cyclic nucleotide and glycogen metabolism, protein phosphorylation, microtubule assembly and disassembly, calcium flux and activities of tryptophan-5'-monooxidase and phospholipase A_2(9,10). The fact that calmodulin regulates so many physiological processes indicates the possibility that alteration of the intracellular level of this protein could also be an important mechanism in the control of responses mediated through calcium. Thus increases in calmodulin levels caused by polypeptides hormones in endocrine cells have been expected, though there have been no such reports yet.

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