CYTOCHALASIN B INHIBITS THYROID SECRETION

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Summary:

The addition in vitro of cytochalasin B at concentrations of 0.5-3.0 μg/ml to prelabeled mouse thyroid glands interferes with release of iodine derived from thyroglobulin and blocks colloid endocytosis. It is suggested that cytoplasmic microfilaments are involved in the secretory process.

The widespread distribution of two prominent filamentous components present in cells has become apparent with the introduction of glutaraldehyde fixation for electron microscopy. These structures are the cytoplasmic microtubules and the smaller microfilaments. Investigations on the functional role of the microtubules have been considerably facilitated by the use of colchicine which leads to their disaggregation into smaller pieces or into dimers of the subunit (1-3). We have recently shown that agents known to disrupt microtubules blocked thyroid secretion suggesting a dependence of the secretory process on this organelle (4).

An approach to microfilament function has become available with the finding that cytochalasin B, a macrolide fungal metabolite obtained from Helminthosporium dematioideum (5-8), causes the disappearance of microfilaments and blocks a number of cellular processes including cytokinesis, single cell movement, glandular morphogenesis, axonal growth and cytoplasmic streaming. Microfilaments as well as microtubules are present in the thyroid (9). We now report that cytochalasin B blocks thyroid secretion in vitro as expressed either in terms of colloid droplet formation or as ^{131}I release into the medium.

Methods

The secretion of ^{131}I (as iodide and thyroxine) into the incubation medium from mouse thyroid glands prelabeled in vivo was measured as previously described (10). Cytochalasin B was dissolved in dimethylsulfoxide and added to the Earle's medium to a final dimethylsulfoxide concentration of 0.1%. Control flasks contained an equivalent concentration of dimethylsulfoxide. Tissues were incubated for 5 hr with or without the macrolide and/or TSH (0.6 units/mg) where indicated. Media and tissues were counted to < 2% counting error and the protein and non-protein iodide was determined chromatographically (10). Colloid droplets were counted in thyroids stained with the periodic acid-Schiff reaction as reported in a previous study (11).

Results and Discussion

Cytochalasin B, at a concentration of 3 µg/ml, blocked the formation of greater than 98% of colloid droplets in response to a 1 hr stimulation of the mouse thyroid by either TSH or cyclic AMP (Table 1). The number of colloid droplets present in the basal state was similarly reduced. At a concentration of 0.5 µg/ml approximately 80% inhibition was seen. The cells appeared histologically normal and no evidence of cell damage could be discerned. Dimethylsulfoxide alone, at a concentration of 0.1%, had no effect on this process.

Data for release of ^{131}I from prelabeled thyroids over a 5 hr incubation period are shown in Fig. 1. Cytochalasin B, at a concentration of 0.3 µg/ml, produced significant inhibition of TSH-stimulated release of ^{131}I without affecting basal release. At higher concentrations of the drug both basal and TSH-stimulated release of radioactivity rose but the ^{131}I was released primarily as iodoprotein. The release of non-protein ^{131}I in response to TSH was essentially abolished at 3 and 10 µg/ml cytochalasin B. The iodoprotein released by cytochalasin B was found by sucrose gradient centrifugation to be entirely in the form of 19S iodoprotein (i.e. thyro-

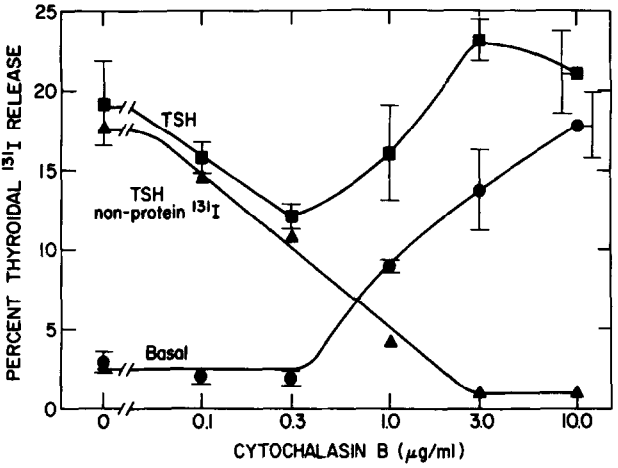

Fig. 1. The percent of thyroidal ^{131}I released into the medium in 5 hr with or without TSH (2.5 mU/ml) plotted as a function of the concentration of cytochalasin B. The percent of ^{131}I released as non-protein radioactivity in the presence of TSH was determined by multiplying the fraction of non-origin material determined chromatographically on pooled media by the mean total release. Points are mean ± S.E. of 3 (basal) or 4 (TSH stimulated) thyroids. Thyroids were labeled for 2 hr in vivo prior to incubation.

globulin). Release of iodoprotein has been shown previously to be a sensitive measure of cell damage (10). Concentrations of 10-30 μg/ml, but not lower levels, of cytochalasin B led to histologically obvious central necrosis.

Table 1

Effect of Cytochalasin B on TSH- and Cyclic AMP-Induced Colloid Droplet Formation by Mouse Thyroids In Vitro

	Basal	2mU/ml TSH	7mM cyclic AMP
	Colloid droplets per 50 follicles		
Control	40 ± 17	1281 ± 46	1450 ± 150
3 μg/ml Cytochalasin B	3 ± 2	22 ± 14	27 ± 6
0.5 μg/ml Cytochalasin B	5 ± 2	168 ± 16	273 ± 40

Thyroids were incubated 1 hr in Earle's solution with 0.1% dimethylsulfoxide and the specified concentration of cytochalasin B. All values are the mean ± S.E. for 3 thyroids. All follicles in a horizontal section through the center of a gland that met the criteria previously established (12) were counted and the number of droplets normalized to 50 follicles.

The present results show that thyroid secretion, as measured by colloid droplet formation and ^{131}I release, is inhibited by low concentrations of cytochalasin B (0.3-3.0 μg/ml). Since the action of cyclic AMP on secretion is also blocked, the inhibitory locus must lie between the generation of cyclic AMP and colloid resorption. The selective action of cytochalasin B on microfilaments therefore suggests a role for this organelle in thyroid secretion.

Colchicine, which also inhibits thyroid secretion (4), disaggregates microtubules without affecting microfilaments (7, 8). Conversely, cytochalasin B appears to have no effect on the microtubules (7, 8). Other differences between these two structural elements also exist. These include: 1) a difference in the diameters of the two elements - 40-70 Å for microfilaments vs. 200-250 Å for microtubules (7, 8, 12-14); 2) differences in the amino acid composition of the subunits (12, 13); and 3) differences in stability characteristics (12, 13). The present results thus suggest that both of these structural elements are required for thyroid secretion, although there is, as yet, no evidence for any interaction between them.

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