Possible Role of Microtubules in Thyroid Secretion

John A. Williams and J. Wolff

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND 20014

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Abstract. Colchicine and other microtubule-active agents have been found to block the release, stimulated by either thyroid-stimulating hormone or by dibutyryl cyclic adenosine 3′:5′-monophosphate, of 131I from previously 131I-labeled mouse thyroid glands in vitro. The time and concentration characteristics of these inhibitors are consistent with their actions on microtubules in other systems. [3H]colchicine was also shown to be bound to a soluble 68 protein of bovine thyroid slices similar to the protein identified in other systems as a microtubular subunit. The demonstrated inhibition of colloid droplet formation and absence of an effect on thyroidal adenyl cyclase or cyclic 3′:5′-phosphodiesterase suggests a colchicine-sensitive role for microtubules in colloid endocytosis in the thyroid gland.

The effects of colchicine on spindle fibers and a number of other intracellular systems concerned with cell structure and movement have been ascribed to dissolution of microtubules.1–6 These actions occur in the micromolar concentration range and are associated with the binding of colchicine to a specific protein believed to be a subunit of the microtubule.7–12 Recently, colchicine has been shown to block insulin secretion by isolated islets of Langerhans.13 We have therefore studied the effects of colchicine (and other compounds that act on microtubules) on thyroid secretion and found them to block secretion in a manner suggesting an effect on the microtubules. A thyroidal colchicine-binding protein similar to those previously identified as a microtubular subunit has also been demonstrated.

Methods. Effects of agents on thyroid secretion: The effects of microtubule-active agents on thyroid secretion were studied by use of a mouse thyroid system.14,15 Thyroid glands were labeled with 131I for 2 hr in vivo, removed on the trachea, and incubated in vitro for 6 hr. Agents were tested for their ability to inhibit release of 131I in response to thyroid-stimulating hormone (TSH) or N6-2-O-dibutyryl 3′:5′-cyclic AMP (Bu2cAMP). After preincubation with the agent alone, the gland was transferred to another flask containing the agent and TSH (2 mU/ml) or Bu2cAMP (10−4 M) as specified. Controls were preincubated in Earle's solution. During preincubation only 1–2% of thyroidal 131I was released by all groups. All agents were dissolved directly in Earle's solution except for podophyllotoxin and griseofulvin which were dissolved in ethanol and brought to a final concentration of 0.25% ethanol. Controls received the same amount of ethanol, which by itself had no effect on either basal or stimulated release.

Adenyl cyclase and diesterase assays: Adenyl cyclase assays were performed with bovine thyroid membranes.16 Cyclic 3′:5′-nucleotide phosphodiesterase was measured by method I of Butcher and Sutherland17 using the 200 × g supernatant solution of homogenates of 8–10 pooled mouse thyroids.
Uptake and distribution of $[^3H]$colchicine: We studied the uptake of $[^3H]$colchicine (New England Nuclear, specific activity 2.5 Ci/mmol) by bovine thyroid glands. Slices weighing 50–60 mg were incubated at 37°C in 3 ml of Earle’s solution containing bovine serum albumin (2 mg/ml) and 0.2 µCi/ml of $[^3H]$colchicine at the concentrations indicated in the text. After 0.5–6 hr, the slices were lightly blotted, rinsed three times for 3 min each in Earle’s solution and dissolved in scintillation vials in 0.5 ml of Soluene (Packard). 15 ml of Bray’s solution was then added and, after the decay of chemiluminescence, radioactivity was measured. Quenching was corrected for by the use of an internal standard.

To evaluate the state of intracellular $[^3H]$colchicine, we incubated 500 mg of bovine thyroid gland slices at 37°C in Earle’s solution containing $[^3H]$colchicine (1 µCi/ml, 2 × 10⁻⁴ M) for 2 hr, and homogenized them in 3 volumes of 250 mM sucrose–10 mM MgCl₂–10 mM phosphate buffer (pH 7.0)–0.1 mM GTP. Homogenization and all subsequent operations took place at 2°C. The homogenates were centrifuged for 1 hr at 100,000 × g and aliquots of the original homogenate and supernatant solution were taken for determination of radioactivity as above. Colchicine binding to macromolecules in the 100,000 × g supernatant solution was tested by gel filtration on a 1.5 × 15.0 cm Sephadex G-100 column eluted with 10 mM phosphate buffer (pH 7.0)–10 mM MgCl₂–0.1 mM GTP. Thirty 1.5-ml fractions were collected and 0.2 ml of each was added to 10 ml of Aquasol (New England Nuclear) for radioactivity determination. Protein was estimated as $A_{280}$ read against an eluent-buffer blank.

Linear 5–28% sucrose gradients, 4.8 ml per tube, were prepared containing 100 mM KCl, 10 mM Na phosphate buffer (pH 7.0), and 10 mM MgCl₂. A 0.2-ml aliquot from the bound colchicine peak was layered over the buffered sucrose gradient and centrifuged at 2°C for 3.5 hr at 65,000 rpm in the Spinco Model L2-65B centrifuge. Crystalline bovine serum albumin and crystalline rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) were added as markers. Protein was determined by fluorescence read at 340 nm and radioactivity by counting in the Aquasol system.

Thin-layer chromatography on silica-gel chromatogram sheet was carried out using methanol or n-butanol saturated with ammonia and water according to the method of Wilson and Friedkin.¹²

Results. Inhibition of thyroid secretion in vitro by colchicine: Preincubation with colchicine was required to give inhibition of the TSH effect. Fig. 1, top, shows a dose–response curve of thyroid $^{131}$I release during a 4-hr incubation after a 2-hr preincubation. Note the steep decrease of the TSH effect between 10⁻⁷ and 10⁻⁴ M and the lack of effect of colchicine on basal release at concentrations up to 10⁻³ M. In other experiments (not shown) colchicine, at concentrations above 10⁻³ M, released iodoprotein; this has been shown to indicate thyroid damage.¹³

Fig. 2 shows the per cent inhibition of the TSH response as a function of colchicine concentration and different preincubation times. Increasing the duration of preincubation increases the degree of maximal inhibition and decreases the concentration required for 50% inhibition; the latter are as low as 1.5 × 10⁻⁷ M after a 4-hr preincubation.

Colchicine also inhibited the release of thyroidal $^{131}$I in response to Bu₂cAMP (Table 1). As noted previously,¹⁶ 1 × 10⁻⁴ M Bu₂cAMP stimulated approximately the same release of $^{131}$I from the mouse thyroid as did 2 mU/ml TSH. This stimulated release was inhibited to about the same extent at two different concentrations of colchicine.

To help localize the site of inhibition, we fixed thyroids after preincubation and incubation with or without colchicine and TSH, and examined them histo-
Fig. 1. Effects of colchicine *(top)* and vinblastine *(bottom)* on thyroidal $^{131}$I release from mouse thyroid glands labeled *in vivo* for 2 hr, preincubated 2 hr *in vitro* with the specified concentration of colchicine or vinblastine, and then incubated 4 hr with or without TSH (2 mU/ml). Each point is the mean ±SE of 6-10 thyroids.

logically. Whereas colchicine alone had no effect on glandular morphology, it virtually abolished TSH-induced colloid droplet formation (Fig. 3).

Since the action of TSH on thyroid secretion is believed to be mediated by cyclic AMP, possible sites of inhibition include inhibition of adenyl cyclase and stimulation of cyclic 3':5'-nucleotide phosphodiesterase. However, no effect of colchicine on basal, TSH-, or fluoride-stimulated adenyl cyclase activity of purified bovine thyroid membrane could be detected (Table 2). Moreover, no effect on phosphodiesterase was seen (Table 2).

On the assumption that colchicine acts on microtubules, other agents known to affect this organelle were tested. Vinblastine was found to inhibit TSH-induced secretion (Fig. 1, *bottom*). Of interest is the flattened dose–response curve characteristic of the actions of this agent on other systems.\[^{18}\] Table 3 gives the 50% inhibitory concentration for TSH-induced $^{131}$I release as well as the relative concentration at which mitosis is blocked for a number of spindle inhibitors. Of the well-studied agents, only griseofulvin failed to inhibit thyroid secretion.
Demonstration of a thyroidal colchicine-binding protein: The action of colchicine on microtubule-associated functions has recently been related to colchicine binding of a protein thought to be a microtubule subunit. The uptake and binding of \[^{3}H\] colchicine by bovine thyroid gland slices was therefore studied. Fig. 4 shows the uptake of colchicine by thyroid slices at three different concentrations. Both the rate of equilibration and the final amount taken up are dependent on the colchicine concentration in the medium. It is probable that the time and concentration dependence for inhibition of TSH-induced \[^{131}I\] release (Fig. 2) can be explained as due to the need to attain effective tissue concentrations. Homogenization in the sucrose-GTP buffer and centrifugation showed that over 95% of the radioactivity was in the 100,000 × g supernatant fraction. When an aliquot of this material was passed through a Sephadex G-100 column a bound fraction was separated from free colchicine. A typical elution profile is shown in Fig. 5A. The bound-colchicine peak emerged immediately behind the protein peak, which elutes in the void volume and is primarily thyroglobulin. Colchicine-binding activity was essentially abolished by pronase digestion. Thin-layer chromatography of a concentrated solution of the bound fraction gave a single spot with \(R_f\) similar to that of the original \[^{3}H\] colchicine and carrier.

**Table 1.** Colchicine inhibition of TSH- and B\(u\)cAMP-stimulated \(^{131}I\) release from mouse thyroids in vitro.

<table>
<thead>
<tr>
<th>Colchicine (M)</th>
<th>Control</th>
<th>2 mU/ml TSH</th>
<th>10(^{-4}) M B(u)cAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.3 ± 0.7</td>
<td>16.8 ± 1.0</td>
<td>13.1 ± 0.5</td>
</tr>
<tr>
<td>3 × 10(^{-7})</td>
<td>3.2 ± 0.2</td>
<td>9.1 ± 0.9</td>
<td>8.5 ± 1.6</td>
</tr>
<tr>
<td>3 × 10(^{-4})</td>
<td>3.5 ± 0.6</td>
<td>6.2 ± 0.6</td>
<td>3.9 ± 0.6</td>
</tr>
</tbody>
</table>

All values are mean ± SE for 3–6 thyroids preincubated for 2 hr in Earle's medium to which colchicine had been added and then incubated 4 hr with TSH or B\(u\)cAMP also present as specified. Release during the 4-hr incubation is expressed as a percentage of the original thyroid content.
FIG. 3. The effect of colchicine \((3 \times 10^{-4} \text{ M})\) on the TSH-induced colloid droplet response of mouse thyroid gland \textit{in vitro} preincubated for 2 hr and incubated for 4 hr. Both \(A\) and \(B\) contained 1 mU/ml TSH in the incubation medium while \(B\) contained colchicine in both the preincubation and incubation media. Note abundant colloid droplets in \(A\) and their almost complete absence in \(B\). Thyroids were fixed in Bouin’s solution, sectioned at 6 \(\mu\)m, and stained by the periodic acid–Schiff reaction followed by hematoxylin.

When an aliquot from the bound-colchicine peak was subjected to sucrose gradient centrifugation the labeled material sedimented with a sedimentation coefficient of 6 S between the serum albumin and glyceraldehyde-phosphate-dehydrogenase markers (Fig. 5B). No radioactivity was present in the 19S (thyroglobulin) region. A similar sedimentation coefficient has been obtained for the colchicine-binding protein of rat thyroid gland. Thus [\(^3\)H]colchicine is taken up by the thyroid gland and bound to a 6S protein in the 100,000 \(\times\) g supernatant solution.

**Discussion.** The results demonstrate inhibition of thyroid secretion \textit{in vitro} by colchicine and other microtubule-active agents by both blockage of colloid droplet formation and release of thyroidal \(^{131}\)I. If the inhibitory effect is mediated via

<table>
<thead>
<tr>
<th>Adenyl cyclase activity*</th>
<th>Cyclic 3':5'-nucleotide phosphodiesterase activity</th>
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<tbody>
<tr>
<td>(nmol cAMP/mg protein/10 min)</td>
<td>(nmol P(_i)/mg protein/hr)</td>
</tr>
<tr>
<td>Basal</td>
<td>NaF</td>
</tr>
<tr>
<td>Control</td>
<td>0.68</td>
</tr>
<tr>
<td>10(^{-4}) M colchicine</td>
<td>0.68</td>
</tr>
</tbody>
</table>

* Representative experiment, one of four on different bovine thyroid membrane fractions all showing similar results.
† Values are means ± SE for three experiments done in duplicate on pooled mouse thyroid.

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**TABLE 2. Effects of colchicine on thyroidal adenyl cyclase and cyclic 3'-5'-nucleotide phosphodiesterase.**
Table 3. Effects of spindle poisons on thyroid secretion.

<table>
<thead>
<tr>
<th>Agent</th>
<th>50% inhibitory concentration on 131I release</th>
<th>Relative spindle potency compared to colchicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colchicine</td>
<td>2 × 10⁻⁵ M</td>
<td>1.0</td>
</tr>
<tr>
<td>Colcemide</td>
<td>6 × 10⁻⁵ M</td>
<td>0.1†</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>6 × 10⁻⁷ M</td>
<td>0.4†</td>
</tr>
<tr>
<td>Podophyllotoxin†</td>
<td>3 × 10⁻⁷ M</td>
<td>0.5§</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>No effect at 10⁻⁶ M</td>
<td>2.0†</td>
</tr>
</tbody>
</table>

* Obtained from inhibitory curves similar to those of Fig. 1. Preincubation time of 2 hr in all experiments.
† Deysson, 1968.
‡ Kindly supplied by Dr. W. J. Gensler, Boston University.

the microtubule this implies a previously unrecognized dependence of thyroid secretion on this organelle. It is thus worthwhile to examine the evidence that the effect is so mediated.

Colchicine and related agents were found to be inhibitory at the same low concentrations in which they interfere with microtubule-mediated functions in other systems.¹⁻⁶ Thyroid secretion also shares with these other systems the time and concentration dependence for inhibitory activity (Fig. 2)³,¹⁹ and the more gradual dose–response curve characteristic of vinblastine¹⁸ (Fig. 1). Furthermore, the relative potency of podophyllotoxin and colcemide are consistent with their potencies on the dissolution of spindle fibers⁹,¹⁸ (Table 3). The chemical requirements for inhibition of thyroid hormone secretion are thus seen to be quite analogous to those that characterize other microtubular systems.

Another property of microtubule-containing systems is the presence of 6S colchicine-binding protein in the soluble fraction of homogenates.⁷⁻¹² The thyroid gland appears to contain a similar protein (Fig. 5). Since the thyroid gland has also been shown to contain microtubules, both in the occasional ciliated cells²⁰⁻²² as well as in the normal epithelial cell²³⁻²⁵ it appears likely that the site

Fig. 4. Uptake of [¹H]-colchicine by beef thyroid slices as a function of time and concentration of colchicine. Uptake is expressed as picomoles of colchicine per mg thyroid (wet weight) calculated from the specific activity of colchicine. Each point is the mean ± SE of three values, each determined in duplicate.
FIG. 5. (A) Gel filtration on Sephadex G-100 of the 100,000 × g supernate from the homogenate of bovine thyroid slices incubated for 2 hr in 2 × 10⁻⁴ M [³H]colchicine medium. (B) Sucrose gradient sedimentation of protein-bound [³H]colchicine peak separated by gel filtration on Sephadex G-100. Fractions are numbered from the bottom of the tube. Crystalline bovine serum albumin (sedimentation coefficient 4.2 S) and glyceraldehyde-3-phosphate dehydrogenase (7.4 S) were added as markers.

of action of colchicine and related compounds is, in fact, on the microtubule of the thyroid.

The current concept of the mechanism of secretion of the thyroid hormone is that TSH stimulates adenyl cyclase present in the basal membrane and thereby increases cellular levels of cyclic AMP, which in turn stimulates endocytosis at the apical membrane. The resorbed colloid droplets fuse with lysosomes, the thyroglobulin is digested there, and hormone is eventually released to the circulation. ³⁴ The site of action of colchicine is clearly beyond the stage of the accumulation or maintenance of cyclic AMP since colchicine also inhibited the Bu₂cAMP stimulated ¹³¹I release and since neither adenyl cyclase nor the 3'·5' cyclic nucleotide phosphodiesterase activities are altered substantially by high concentrations of colchicine (Table 2). Our results therefore suggest that microtubules may play a role in thyroid secretion through an effect on endocytosis.

Abbreviations: TSH, thyroid-stimulating hormone; Bu₂cAMP, N⁴·2-O-dibutyryl 3·5'-cyclic AMP.

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7 Wilson, L., and M. Friedkin, Biochemistry, 6, 3126 (1967).