Human thyroid cells in monolayer retain the ability to secrete tri-iodothyronine in response to thyrotrophin

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ABSTRACT

Confluent monolayer cultures of human thyroid cells secreted low levels of immunoassayable tri-iodothyronine (T₃) and this process could be stimulated by TSH in a concentration-dependent manner. The characteristics of the response to TSH were related to the age of the thyroid cell culture both in terms of the relative sensitivity to TSH and the quantity of T₃ released. Cells which had been in culture for 2–3 days (primary cultures) secreted high levels of T₃ under unstimulated and TSH-stimulated conditions with a median effective dose (ED₅₀) for TSH of 0·030 mu. TSH/ml. However, cells which had been subcultured and consequently had been in culture for a longer period of 6–7 days secreted lower levels of T₃ under basal and stimulated conditions. This was approximately 30% of that released from primary cultures with an ED₅₀ for TSH of 0·1 mu. TSH/ml. Reorganization of human thyroid cells into follicular structures was seen during growth with TSH but these cultures showed little response to subsequent acute stimulation by TSH; the return of a diminished, less sensitive response to TSH was seen after a recovery period of 8 h. The time-course of T₃ release was dependent on the TSH concentration with low TSH concentrations stimulating T₃ secretion after increased incubation periods. Human thyroid cells had lost the ability to concentrate and organify free iodide after several days in culture but were still secreting T₃. This indicates the presence of intracellular stores of T₃ which are released on stimulation with TSH, rather than new synthesis of T₃.


INTRODUCTION

The culture of human thyroid cells is a well-established technique. Two forms of isolated thyroid cell preparations are used experimentally: monolayer cultures, or cells cultured in the presence of agents that increase intracellular cyclic AMP levels (thyrotrophin (TSH), dibutylryl cyclic AMP or thyroid-stimulating immunoglobulins) which initiate their rearrangement into two-dimensional structures resembling thyroid follicles.

The morphological and biochemical behaviour of these thyroid cell culture systems has been widely studied with particular reference to the importance of cellular organization in thyroid hormone synthesis and secretion. Monolayer cultures of porcine thyroid cells have lost the capacity to concentrate and organify iodide whereas TSH-treated cells actively concentrate iodide, iodinate thyroglobulin and synthesize thyroid hormones. Cells in monolayer retain the ability to synthesize thyroglobulin but secrete non-iodinated thyroglobulin whereas reorganized cells secrete iodinated thyroglobulin (Lissitzky, Fayet, Giraud et al. 1971; Winand, Wadeleux, Etienne-Deceerf & Kohn, 1976; Wadeleux, Etienne-Deceerf, Winand & Kohn, 1978). These studies indicate the necessity of a follicular organization for iodide transport, organification and synthesis of thyroid hormones. Nevertheless freshly dispersed hog thyroid cells have been shown to synthesize and secrete thyroid hormones during short-term incubations (Rousset, Poncet & Mornex, 1976; Rousset & Mornex, 1981) and thyroid hormones have also been demonstrated in the culture medium of 4-day cultures of non-associated sheep thyroid cells (Dickson, 1966).

There has only been one report of thyroid hormone release by cultured human thyroid cells (Bidey, Marsden, Anderson et al. 1977). In this study of TSH-stimulated cellular differentiation of human thyroid cells it was found that immunoassayable thyroxine
(T₄) and tri-iodothyronine (T₃) were released from thyroid cells in monolayer. Levels of T₃ and T₄ were increased in the medium from associated cells cultured in the presence of TSH but significant levels were also released from unsupplemented control cells in monolayer culture during a period up to 6 days. The results of these experiments demonstrated the continued ability of human thyroid cells in monolayer to secrete thyroid hormones under chronic stimulation.

Our study investigates for the first time the ability of confluent monolayer cultures of human thyroid cells to secrete T₃ in response to acute stimulation with TSH.

**MATERIALS AND METHODS**

**Materials**

Medium 199, phosphate-buffered saline (PBS), fetal calf serum, glutamine, penicillin, streptomycin and trypsin were all obtained from Gibco-Europe Ltd, Paisley, Strathclyde. Dispase was obtained from Boehringer Corporation, Lewes, E. Sussex, bovine serum albumin (fraction V powder) from Armour Pharmaceutical Co., Eastbourne, E. Sussex and TSH (bovine pituitary, 1 unit/mg) from Sigma, London. [¹²⁵I]Tri-iodothyronine (>200 Ci/mmol) was obtained from Amersham International plc, Bucks. All other chemicals were of analytical grade. All tissue-culture plastics were obtained from Sterilin, Teddington, Middx.

**Cell isolation and culture**

Isolated human thyroid cells were obtained from samples of thyrotoxic tissue and non-toxic goitres from surgery as previously described (Ollis, Munro & Tomlinson, 1982). The method basically involved dispersion of tissue by both mechanical and enzymatic means. Isolated cells were plated out in 9 cm Petri dishes at a concentration of 2–5 x 10⁵ cells/ml medium (Medium 199 + 10% fetal calf serum supplemented with 2 mm-glutamine, 0.22% bicarbonate, 100 μg penicillin/ml and 100 μg streptomycin/ml). After 24 h the cells were rinsed with PBS to remove non-adherent blood cells, fresh medium was added and the cells were grown to confluence (approximately 2–3 days) at 37°C in an atmosphere of 5% CO₂/95% air in a water-saturated incubator. At confluence the cells were passaged using 0.125% (w/v) trypsin in 5-3 mM-KCl, 137 mM-NaCl, 4.2 mM-NaHCO₃, 5 mM-glucose, 0.5 mM-disodium EDTA (Puck’s saline) for 3–5 min at 37°C. The disaggregated cells were pelleted by centrifugation at 500 g for 5 min and resuspended in supplemented Medium 199. The cells were plated out into six-well culture dishes (3.5 cm diameter) and grown to confluence.

**Thyroid hormone incubation**

The investigation was to determine the characteristics of the response to TSH of human thyroid cells in monolayer in relation to cell age, time of incubation and the presence of TSH during the cell growth phase. Cells in the primary culture and after subculturing were initially used to investigate cell age. Subsequently all following data were obtained from subcultured cells in order to increase cell number and to rule out the possibility of the presence of intact follicles in the primary culture which could be isolated during the dispersion process. Studies into the effects of TSH present during the cell growth period on subsequent secretion of T₃ used cells which had been grown with TSH since subculturing up to the day of incubation (2–3 days). The effect of a recovery period from chronic stimulation by TSH over this period was investigated by maintaining cells in the absence of TSH for up to 8 h.

The release of T₃ in response to TSH by cultured human thyroid cells was investigated at confluence. The medium was removed, cells were rinsed thoroughly with PBS and TSH was added in Medium 199 with 0.1% (w/v) bovine serum albumin followed by an incubation of 16 h at 37°C in 5% CO₂/95% air. All investigations were made in triplicate cultures. At the end of the required incubation period with TSH, the medium was removed and retained at −20°C for the measurement of T₃ by radioimmunoassay using sheep anti-T₃ from the Scottish Antibody Production Unit, Carluke, Lanarkshire. The antibody had low cross-reactivity with T₄ and thyroglobulin. Bound and free T₃ were separated using charcoal and [¹²⁵I]T₃ was counted using an LKB compugamma. The cells were rinsed with PBS and stored at −20°C for protein determinations.

**Protein estimation**

Cells were hydrolysed by incubation with 1 M-NaOH at 37°C for 16 h and the protein content was measured using the method of Lowry, Rosebrough, Farr & Randall (1951).

Results are expressed as pmol T₃ released/mg cell protein (mean ± s.e.m.) for each set of triplicate cultures.

**Iodide organisation**

The ability of monolayer cultures of human thyroid cells to concentrate and organify iodide was determined using an adaptation of the method as described by Ginsberg, Shewring, Howells et al. (1983). Confluent cultures were incubated under the same conditions used for the release of T₃ in the presence of 1³¹I (1 μCi per well) and NaI (10 μmol/l) for an incubation period of 16 h. After this time the medium was
removed by aspiration and counted and the cells were washed with PBS. The cells were detached from the culture dishes using 0.1 M-NaOH and counted. Radioactivity associated with protein was determined by trichloroacetic acid (TCA; 30%, w/v) precipitation of both the cell extract and medium, and control medium which had not been exposed to thyroid cells.

All data are the means (± s.e.m.) of triplicate cultures established from individual thyroids. Each experiment was performed at least three times but due to large variations in the secretion of T₃ by different cultures, data from one representative experiment are shown.

RESULTS

At confluence during a 16-h incubation period human thyroid cells in primary culture isolated from either non-toxic goitres or thyrotoxic tissue released immunoassayable T₃ into the culture medium. This release could be stimulated by TSH in a concentration-dependent manner (Fig. 1a). The release of T₃ under unstimulated conditions was high (250–700 pmol T₃/mg cell protein) and could be increased in the presence of TSH up to 1200–2000 pmol T₃/mg cell protein. The cells showed good sensitivity to TSH with a response to 10 µu. TSH/ml and a median effective dose (ED₅₀) of 30 µu. TSH/ml.

Human thyroid cells which had been subcultured once and consequently in culture for 6–7 days showed reduced responses to TSH when compared with the primary cultures (Fig. 1b). Tri-iodothyronine was detected in the incubation medium in response to 50 µu. TSH/ml and the secretory response had an ED₅₀ for TSH of 0.1 µu. TSH/ml. All subsequent data were obtained using subcultured cells. The sensitivity of cultures from many thyroids to TSH was consistently similar although there was variation in the magnitude of the response (in terms of percentage stimulation over basal T₃ release) from 50 to 400%.

Despite the general reproducibility of sensitivity to TSH in cultures established from most thyroids, a proportion of cultures did not respond to TSH; in these cases no detectable T₃ was released under basal conditions (<7·8 pmol T₃/ml) nor was there an increase in T₃ levels in the incubation medium after stimulation by TSH. Overall, after 6–7 days in culture, eight of 24 thyroid cell cultures were unresponsive to TSH stimulation as measured by the release of T₃; these preparations were unrelated in terms of the clinical conditions of the patients from which the thyroid glands were obtained. Lack of response to TSH in 30% of thyroid cell cultures could not be explained by a reduction in cell viability during the culture period. The measurement of intracellular T₃ after the 16-h incubation period showed undetectable levels in cultures which were unresponsive to TSH whereas cultures which responded to TSH had high levels (400–1000 pmol T₃/mg cell protein).

The effect of chronic exposure of thyroid cell cultures to TSH during the growth period after subculturing on their subsequent response to acute stimulation by TSH was then investigated. The growth of thyroid cells in the presence of TSH caused reorganization of cells into two-dimensional structures resembling thyroid follicles. There was a concentration-dependent increase in the amount of T₃ released into the medium in cultures of cells grown with TSH but the subsequent addition of TSH over a 16-h period did not increase T₃ secretion further as was seen from control cells grown in the absence of TSH (Fig. 2). However, after growth over 2–3 days with TSH followed by a recovery period of 8 h in TSH-free medium, unstimulated levels of T₃ secretion had fallen to a level comparable to unstimulated secretion from
cells grown in the absence of TSH and there was an increase in T₃ release to high TSH concentrations of 10–100 mu. TSH/ml only.

The time-course of release of T₃ in response to TSH was investigated over incubation periods lasting up to 48 h. The time-courses appeared to be dependent on the concentration of TSH to which the cells were exposed (Fig. 3). Changes in T₃ levels in the medium were detected within an incubation period of 2 h with a maximal stimulating concentration of TSH of 10 mu. TSH/ml but lower TSH concentrations (500 mu./ml) required longer incubation periods (of greater than 8 h) before T₃ was released.

The uptake of inorganic iodide and its organification into TCA-precipitable material was investigated during a 16-h incubation period (data not shown). There was a very low level of iodide uptake (<0.3% of radioactivity added) which was all in the form of free iodide. The percentage of radioactivity which could be precipitated with TCA in the incubation medium (4% of radioactivity added) at the end of the 16-h period was found to be associated in a non-specific manner with other proteins rather than in the form of secreted thyroid hormones, as control medium which had not been exposed to thyroid cells showed the same proportion of TCA-precipitable radioactivity.

**DISCUSSION**

This study demonstrates the release of T₃ by mono-layer cultures of human thyroid cells isolated from either thyrotoxic tissue or non-toxic goitres. For the first time we have shown that the secretion of T₃ can be stimulated by TSH in a concentration-dependent manner from thyroid cells in culture. The quantitative response to TSH stimulation could be related to the age of the culture as primary cultures of human thyroid cells released far higher levels of T₃ than cells which had been subcultured and consequently maintained in culture for a longer period. However, the need to increase cell number and to eliminate the possibility of intact follicles in the primary cultures dictated the use of cells which had been subcultured once and consequently had been in culture for a longer period. The
presence of TSH during the growth period resulted in the loss of response to subsequent acute stimulation with TSH. This may be due to desensitization to TSH caused by prolonged exposure to the hormone, which has been described by others (Kaneko, 1976; Rapoport & Adams, 1976) and shown to be reversible (Kaneko, 1976). Consequently a recovery of 8 h allowed the return of a diminished, less sensitive response to TSH. Time-course studies showed a dependence on the concentrations of TSH used as low concentrations of TSH required longer incubation periods with thyroid cells before an increase in $T_3$ levels in the incubation medium was detected. After 7 days in culture human thyroid cells lost the ability to concentrate and organize iodide suggesting that $T_3$ secretion under these conditions did not rely on de-novo $T_3$ synthesis after stimulation with TSH but corresponded to the release of preformed $T_3$ from intracellular stores.

It has been postulated that the follicular association of thyroid cells produced in response to growth with TSH is required to allow the cells to preserve their differentiated function (Lissitzky et al. 1971). Porcine thyroid cells lose the ability to concentrate iodide between days 1 and 2 of culturing in monolayer and from days 3 to 13 neither iodide trapping nor organization can be detected (Lissitzky et al. 1971). We have now shown that human thyroid cells also lose the ability to concentrate or organize iodide. The same cultures, however, released radiomimoassayable $T_3$ into the incubation medium, a process which could be stimulated by TSH. This would suggest the secretion of preformed $T_3$ present in an intracellular store of the thyroid follicular cell at the time of operation. Intracellular stores of $T_4$ have been demonstrated in freshly isolated swine thyroid epithelial cells (Kawada, Nishida, Yoshimura & Yamamoto, 1982) and $T_4$ has been shown to be secreted by hog cells, a process which can be stimulated by TSH (Rousset et al. 1976).

Previous studies, however, have used freshly isolated cells in the demonstration of intracellular stores of thyroid hormones. Our results are consistent with the maintenance of intracellular stores of $T_3$ within human thyroid monolayers for a culture period of approximately 7 days. It may be that the substantial proportion of cultures (30%) which did not release assayable $T_3$ after incubation with TSH resulted from low intracellular stores of $T_3$ in the thyroid at the time of removal as indicated by the fact that intracellular $T_3$ was undetectable at the end of the incubation period.

The mechanisms involved in TSH-stimulated $T_3$ release from monolayer cultures of human thyroid cells and the relevance of deiodination in the medium in influencing the levels of $T_3$ detected are unknown. However, post-secretory deiodination of iodothyronines released from human thyroid cells has been reported (Bidey, Anderson, Marsden & McKerron, 1976).

Our investigations indicate that some of the biochemical events involved in thyroid hormone secretion can be separated from each other in vitro. In conjunction with studies using thyroid cells grown in the presence of TSH it should now be possible to investigate in detail processes involved in thyroid hormone synthesis and storage and processes involved in the final secretory pathway.

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