POST-SECRETORY DEIODINATION OF IODOTHYRONINES RELEASED FROM NORMAL HUMAN THYROID CELLS IN VITRO

S.P.Bidey, J.Anderson, P.Marsden and C.G.McKerron

Department of Medicine, King's College Hospital Medical School, London SE5 8RX, England.

Received July 7,1976

SUMMARY

The stability of tri-iodothyronine (T3) and thyroxine (T4) in the presence of isolated, cultured normal human thyroid cells has been investigated by radioimmunoassay of the culture medium at intervals to 120 hours.

T3 and T4 were both progressively degraded in the presence of cells, and although no significant deiodination was produced by fresh culture medium, the medium withdrawn from confluent cell cultures at 120 hours was capable of degrading subsequently - added iodothyronines.

These findings provide evidence for the in vitro release of an iodothyronine deiodinase, and this is discussed in terms of the net decrease in medium iodothyronine levels observed in earlier studies of in vitro T3 and T4 release.

INTRODUCTION

The action of bovine thyrotropin in stimulating the release of tri-iodothyronine (T3) and thyroxine (T4) from isolated, cultured normal human thyroid cells has recently been reported (1,2). In these studies, radioimmunoassay of the culture medium for T3 and T4 indicated that net levels of both iodothyronines reached a maximum at 24 hours, then became progressively diminished.

As a direct result of this unexplained decrease in iodothyronine levels, we have attempted to substantiate evidence for the existence of an iodothyronine deiodinase system, by investigating the relative stability of exogenous T3 and T4 in the presence of cells from which the release of endogenous cellular iodothyronines was inhibited, as well as in fresh culture medium, and in cell-free medium withdrawn from confluent cell cultures.

METHODS

Preparation of Cell Cultures

Normal human thyroid tissue was obtained immediately after operation on patients undergoing removal of thyroid cysts, when a wide margin of histologically normal tissue was excised with the cyst. Cell cultures were initiated as described previously (2) in Eagle's BME medium containing 10% (v/v) foetal calf serum and 25mM HEPES buffer. Cells were cultured in 36 x 10mm well-type polystyrene culture plates (Gibco:Biocult Ltd., Paisley, Scotland). Immediately prior to incubation, cells were supplemented with 3mM methimazole (2-Mercapto-1-Methylimidazole, Sigma Chemical Co. Ltd., Kingston, Surrey, U.K.) and 50μM sodium iodide, to inhibit the organification and release, respectively of cellular iodothyronines.

Deiodination of exogenous iodothyronines

After initiation of the cell cultures, the culture plates were randomly assigned to two groups of 10 cultures. Group A received bovine TSH (Sigma) to a final level of 100mU/ml whilst group B received a blank saline solution. Each group was also assigned 10 cell-free cultures which consisted of growth medium alone.

Tri-iodothyronine (Sigma, 5ng/ml, final concentration) or thyroxine (Sigma, 50ng/ml, final concentration) were added to equal numbers of cultures within each of the two groups. All cultures were incubated at 37°C, and aliquots of medium were withdrawn at 0, 24, 48 and 120 hours for quantitation of T3 and T4 levels by radioimmunoassay.

T3 and T4 radioimmunoassays

The T3 content of culture medium was determined after the appropriate incubation period by the method of Marsden & McKerron (3) modified by using a T3 standard curve run in serum-free sodium barbitone buffer (0.08mol/l., pH 8.6). Culture medium samples were diluted to 1:20 or greater, at which dilution the standard curve was parallel to the buffer curve.

T4 levels were similarly determined by a radioimmunoassay (4) which measured T4 directly at high dilutions (1:20). At this dilution, the standard curves in serum, buffer and culture medium were identical.

Cell DNA estimations

After the appropriate in vitro growth period, remaining medium was decanted from the cultures, and 0.06% (w/v) trypsin in Hank's solution was added to the culture wells (1.0ml per well). After incubation for one hour at 37°C, the cell suspensions obtained on gentle dispersion were centrifuged, and the cell pellet resuspended in 2.0ml of 0.05M sodium phosphate buffer, pH 7.4 at 4°C. Two freeze-thaw cycles in an acetone - solid CO_2 mixture released DNA from the cells, and this was subsequently assayed by a diphenylamine colorimetric assay (5).

Iodothyronine deiodination in "cell-exposed" medium

In order to assess whether culture medium, previously used to maintain proliferating thyroid cells to confluency, might be able to deiodinate iodothyronines, T4 was added to membrane - filtered (0.45μ) medium samples withdrawn after 140 hours of exposure to normally growing human thyroid cells, which had been treated initially with 100mU/ml of bovine TSH or had remained untreated. Aliquots of the medium were removed immediately

after addition of the iodothyronines, and subsequently at 24, 48 and 120 hours, for T4 radioimmunoassay.

RESULTS AND DISCUSSION

Although the stability of mono-and di-iodotyrosines has been investigated in cell-free systems (6,7), the post-secretory degradation of T3 and T4 in vitro has not received attention. The results of iodotyrosine stability studies have indicated that mono-iodotyrosine (MIT) and di-iodotyrosine (DIT) were deiodinated in the absence of cells. However, this phenomenon was attributed to the action of oxidising agents within the medium, or to photoactivation of NAD^+ used in the deiodinase assay system, rather than to a specific enzyme system (7). The problem of artefacts arising during assay for deiodinase activity has led to great difficulty in resolving the occurence and role of specific deiodinases within the thyroid. Some evidence for a T4 deiodination system has been reported, however (8) and the mechanism, involving a peroxidase system, was distinct from the iodotyrosine deiodinase system whereby iodide is derived from the intracellular degradation of MIT and DIT.

In our earlier studies (1,2) we observed a progressive decrease in the recovery of medium iodothyronines with time. Therefore the principal objective of the current study was to investigate the possibility of an iodothyronine deiodinase system being responsible for this degradation. Figs. 1 and 2 show the concentrations of exogenous T3 and T4, respectively, remaining in the medium of cultures at progressive time intervals after T3 or T4 addition. In the absence of cells, there was no significant decrease of either iodothyronine between 0 and 120 hours (Fig.1). In cultures originally supplemented with T3, levels of T3 declined, in both TSH - supplemented and control cultures, by 50% within 48 hours of T3 addition. When similar studies of T4 stability were made, an initial rise in T3 was observed between 0 - 24 hours, but a subsequent net fall in T3 was detected which was more rapid in TSH - supplemented cultures than in controls.

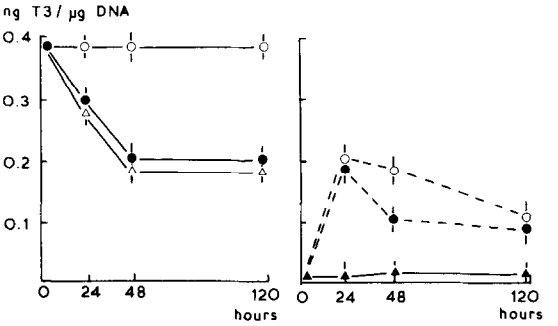

Fig.1 Tri-iodothyronine radioimmunoassay of medium withdrawn from cultures originally supplemented with tri-iodothyronine or thyroxine. All cultures were treated at zero-time (0 hours) with 50µM sodium iodide and 3mM methimazole.

Plotted points are the Mean ± S.D. (n = 5)

o——o , cell-free medium + T3; ●——● , unsupplemented cells + T3; △——△ , TSH-supplemented cells + T3; ▲——▲ , cell-free medium + T4; o- - -o , unsupplemented cells + T4; ●- - -●, TSH-supplemented cells + T4.

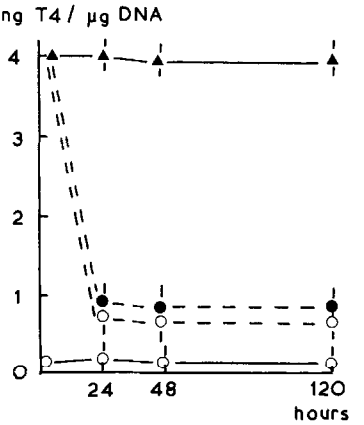

Fig.2 Thyroxine radioimmunoassay of medium withdrawn from cultures originally supplemented with tri-iodothyronine or thyroxine. All cultures were treated at zero-time (0 hours) with 50µM sodium iodide and 3mM methimazole.

Plotted points are the Mean ± S.D. (n = 5)

o——o, cell-free medium + T3; ▲——▲ , cell-free medium + T4; o- - -o, unsupplemented cells + T4; ●- - - ●, TSH-supplemented cells + T4.

Exogenous T4 was also found to be stable in the absence of cells, although levels declined more rapidly in the presence of cells (Fig.2) than did T3 levels (Fig.1).

The relatively slower net generation of T3 from T4 (Fig.1), compared with overall T4 removal (Fig.2) may indicate that further degradation of T3 may occur, i.e. to MIT, DIT and free iodide.

Since no significant decrease in T3 or T4 recovery was found in serum - supplemented culture medium, it was concluded that fresh culture medium contained neither deiodinases nor compounds capable of oxidising exogenous iodothyronines. Since T3 and T4 were both degraded in the presence of cells, either the iodothyronines penetrated the cells prior to being deiodinated by an intracellular process, or the compounds promoting the deiodination were released by the cells into the medium. We have found no evidence for iodothyronine uptake by thyroid cells in studies where 125I-T3 and 125I-T4 were incubated with thyroid cells (unpublished results; free 125I uptake by these cultures was inhibited with 5mM sodium perchlorate, so that any 125I activity detected would not have been in the form of free iodide).

The other alternative, that T3 or T4 degradation was effected by the passage of deiodinases from the cells into the medium, was investigated by adding T4 to medium which had been recovered from normal cell cultures after 120 hours of growth. The complete absence of cells from these medium samples was ensured by membrane filtration prior to iodothyronine addition. Fig.3 shows the levels of T4 present after 0, 24, 48 and 120 hours of such treatment. The pattern of degradation was comparable to that seen in the presence of cells, (Figs.1 and 2), and provides evidence for the cellular origin of the deiodinating activity.

The significance of an iodothyronine deiodination system in vivo has not yet been fully investigated. However, although an increased permeability of the thyroid follicular cell basal membrane may occur in vitro

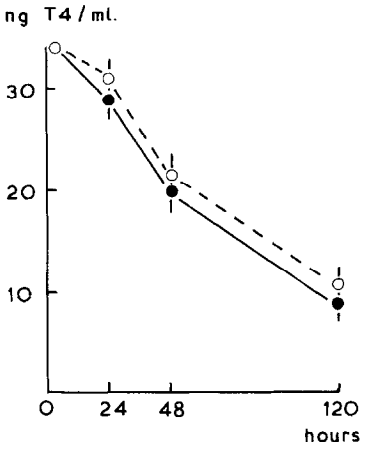

Fig.3 Thyroxine radioimmunoassay of medium withdrawn at intervals following the addition of thyroxine to medium previously exposed for 140 hours to proliferating, normal human thyroid cells.

Plotted points are the Mean ± S.D. (n = 5)

o- - -o, T4 + medium from unsupplemented cell cultures;
•——•, T4 + medium from TSH-supplemented cell cultures.

(9), and allow the leakage of compartmentalised thyroidal enzymes, it seems possible that enzymes may serve within the thyroid to specifically modulate iodothyronine levels, as distinct from those systems controlling iodotyrosine degradation. The possible involvement of TSH as a controlling factor in these systems cannot at this stage be confirmed, and the present evidence suggests that a high basal level of deiodination activity is not dependent on the presence of TSH.

In summary, the available evidence suggests that enzyme systems specifically deiodinating iodothyronines may exist within normal human thyroid tissue, which have not previously been demonstrated.

ACKNOWLEDGEMENTS

We thank Mr Headley Berry for making available the collection of

thyroid tissue, and the Joint Research Committee of King's College Hospital for the financial support of this work.

REFERENCES

1. Bidey, S.P., Marsden, P., McKerron, C.G. & Anderson, J. (1976) Clin. Sci. & Mol. Med.,50, 29P-30P.
2. Bidey, S.P., Marsden, P., McKerron, C.G. & Anderson, J. (1976) Biochem. Biophys. Res. Communs.,70, 418-424.
3. Marsden, P. & McKerron, C.G. (1975) Clin. Endocr., 4, 183-189.
4. Marsden, P., Facer, P., Acosta, M. & Howorth, P.J.N. (1975) J.Clin. Path., 28, 608-612.
5. Leyva, A.Jnr. & Kelley, W.N. (1974) Anal. Biochem.,62, 173-179.
6. Taurog, A. (1963) Endocrinology, 73, 45-46.
7. Morreale de Escobar, G., Llorente, P., Jolin, T. & Escobar del Rey, F. (1963) J.Biol. Chem., 238, 3508-3512.
8. Haibach, H. (1971) Endocrinology, 88, 918-923.
9. Fayet, G., Michel-Bechet, M, & Lissitzky, S. (1971) Europ. J. Biochem., 24, 100-111.